Mohd Sayeed Akhtar
Mallappa Kumara Swamy *Editors*

Anticancer Plants: Properties and Application

Volume 1

Springer

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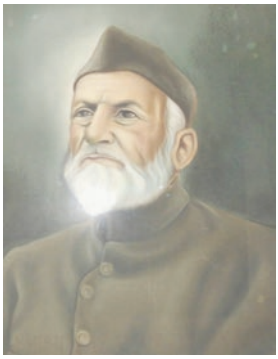

Khan Bahadur Mohd. Fazl-ur-Rahman Khan (1893–1971)

A great visionary, social reformer, statesman of the 20th century and founder of Gandhi Faiz-e-Aam College, Shahjahanpur, Uttar Pradesh, India

Foreword

The report published by Ferlay et al. in GLOBOCAN 2012 volume 2 under IARC Cancer Base No. 11,2014, Lyon, France, and available from: http://globocan.iarc.fr, reveals that the cancer incidence and mortality lies around 182 per 100,000, with higher values for males (205 per 100,000) as compared to the females (165 per 100,000). The highest values have been recorded from Denmark with 338 people per 100,000 being diagnosed in 2012. The age-standardized rate is around 300 per 100,000 for Denmark, France, Australia, Belgium, Norway, United States of America, Ireland, Republic of Korea, and the Netherlands. A recent report for cancer published by the WHO mentions that the second leading cause of death on global basis is cancer, which was responsible for 8.8 million deaths in 2015, and over 200,000 children are diagnosed with different cancer types almost every year.

"Cancer" is the abnormal growth of cells due to imbalance in our bodies, which invade and destroy our normal cells. Billions of dollars are spent on research in this connection. The deaths arising from cancer comprise nearly 3% of the annual global deaths and major causes for these are smoking, dietary imbalances, hormones, and chronic infections leading to chronic inflammation. Many chemopreventive agents are used to treat this disease, but they cause toxicity and similarly chemo- and radiation therapy produce serious side effects; therefore, many cancer patients seek complementary treatment methods. Any drug effective against the malignant or cancerous disease is listed as an anticancer drug or antineoplastic drug. The term anticancer is a broad one and can be divided as antitumor, i.e., toxic to tumors in animal studies, cytotoxic, i.e., used to fight tumors in the laboratory cell cultures (in vitro), and anticancer accepted to fight tumors in humans. Some of the drugs used for the treatment are alkylating agents, antimetabolites, natural products, and hormones. There are also plant-derived anticancer agents such as the vinca alkaloids (vinblastine, vincristine, and vindesine), the epipodophyllotoxins (etoposide and teniposide), the taxanes (paclitaxel and docetaxel), and the camptothecin derivatives (camptotecin and irinotecan).

Nearly 50% of modern drugs in clinical use are of natural products, many with an ability to control cancer cells. The latest published record reveals that a higher number of cancer patients use vitamins or herbs as therapy. There are several medic-

inal plants all over the world, which are used traditionally for the prevention and treatment of cancer, but only few have attracted the interest of scientists to investigate the remedy for neoplasm. Some of these possess a good immunomodulatry and antioxidant properties leading to anticancer activity. Most commonly used and referred herbs to fight cancer are *Podophyllum peltatum, Sanguinaria canadensis, Ruscus aculeatus, Uncaria tomentosa, Larrea mexicana, Codonopsis pilosula, Bulpleurum scorzoneraefolium, Scutellaria barbata, Artemisia annua, Glycyrrhiza glabra, Aloe vera, Allium cepa, Camellia sinensis, Crocus sativus, Curcuma longa, Viscum album, Silybum marianum, Trifolium pratense, Astragalus, Vitis vinifera, Zingiber officinale, Syzygium aromaticum, Tanacetum parthenium, Hydrastis canadensis, Tabebuia impetiginosa, Rumex acetosella, Sutherlandia frutescens, Thinopyrum intermedium, Arctium lappa, Sanguinaria canadensis, Berberis vulgaris, Taraxacum officinale, Digitalis purpurea,* and *Guanabanus muricatus*. Interestingly, *Silybum marianum* has been found to kill only cancer cells.

The present volume to be published by the "Springer" on Anticancer Plants: Properties and Application, Volume 1 , includes 24 chapters from different parts of the world. The first chapter by Italian authors presents information on the current practices and awareness of anticancer plants in the traditional healthcare system. Brasilian researchers in the second chapter have discussed the phytochemical and biological properties of *Lippia gracilis.* The third and fourth chapters by Indian colleagues give information on the use of Indian indigenous fruits in cancer prevention and treatment and potentiality of Northeast Indian anticancer plants. The chapter five from Malaysia highlights the role of emerging plant-derived compounds with anticancer activities and also focuses on the underlying mode of action involved in the pharmacological effects of phytochemicals. In the chapter six, the role of anticancer plant molecules for improvement of immune system has been discussed. The chapter seven is a joint effort made by researchers from Canada, Pakistan, and Jordan, who are discussing the fermented food-derived bioactives compounds with anticarcinogenic properties with a case study on fermented royal jelly as a novel source. Another group of Indian researchers have compiled the data in chapter eight on mass spectrometry based techniques for assessment of pharmacological responses of ayurvedic drugs. It is followed by another group of Indian workers presenting an assessment of anticancer properties of *Piper betelvine* leaves. An analysis of the patents filed for the herbal therapeutics against cancer by Indian researchers has been presented in the chapter ten. An appraisal of medicinal plants with anticancer properties from South America has been prepared by an enthusiatic group from Venezuela under chapter eleven, followed by scientific validation of the usefulness of *Withania somnifera* in the prevention and treatment of cancer from India in the chapter twelve. The chapter thirteen discussed the anticancer potential of mangrove plants of marine ecosystem by joint effort of Indian, Korean, and Malysian authors. The institute of oncology, India, has made an important contribution in chapter fourteen on *Piper betel* in cancer: past, present, and future. The chapter fifteen from America discusses the anticancer properties of curcumin and its efficacy for treating central nervous system neoplasms. The Malaysian workers have summarized the findings on Vitamin E: the nature's gift to fight cancer in the

chapter sixteen. In the chapter seventeen, the researchers from the Kingdom of Saudi Arabia have presented data on the use of plant secondary metabolites as nutraceuticals for treatment and management of cancer with approaches and challenges. In chapters eighteen and nineteen, the Indian scientists have summarized the information on the usefulness of *Ocimum sanctum* in cancer prevention and the phytochemicals with anticancer potential: methods of extraction, basic structure, and chemotherapeutic action, whereas anticancer plants and their conservation strategies in the chapter twenty, followed by a discussion on the anticancer plants: phytochemistry, pharmacology, and potential applications from India in chapter twenty one. The chapters twenty two and twenty three provided the informations on the botany, chemistry, and pharmaceutical significance of *Sida cordifolia* and anticancer properties of natural compounds on prostate cancer. The last chapter by the Malaysian researchers gives information on phytochemicals against cancer stem cells.

The chapters included in this volume show that all herbs presented here against cancers work in a different manner. Some are "immunomodulators," stimulating the patient's immune system to fight cancer cells, while others are cytotoxic and kill the cancerous cells; however, they can also damage the healthy normal cells, thus need to be used under the supervision of a specialist. The information presented in this volume of course includes well appreciated and most desirable herbs. However, nobody can say that these herbs are without any side effects. Equally, they may conflict with some drugs. Eventhough many people have accepted herbs for cancer therapy, still many are against their use. A huge amount of research has already been completed; the information presented in this volume will definitely fill the gap to great extent. Undoubtedly, cancer takes time to develop, so prevention is more preferable to any treatment. We must avoid all known carcinogens such as tobacco, excessive alcohol, processed foods, and exposure to chemicals. A plant-based diet can help to protect us from cancers as plants are rich in antioxidant and anti-inflammatory compounds, both of which are powerful cancer fighters. I congratulate the editors Dr. Mohd. Sayeed Akhtar and Dr. Mallappa Kumara Swamy for their noble academic efforts for bringing the updated and recent informations about the properties and applications of anticancer plants in the form of first book of such kind in the world.

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Preface

Cancer is one of the leading causes of death of human population increasingly seen in recent times. Plants have been used for medicinal purposes since times immemorial. Though several synthetic medicines are useful in treating cancer, they are inefficient and unsafe. However, plants have proved to be useful in cancer cure. Moreover, natural compounds from plants and their derivatives are safe and effective in treatment and management of several cancer types. The anticancer plants such as *Catharanthus roseus, Podophyllum peltatum, Taxus brevifolia, Camptotheca acuminata, Andrographis paniculata*, *Crateva nurvala*, *Croton tonkinensis*, *Oplopanax horridus,* etc., are important source of chemotherapeutic compounds. These plants have proven their significance in the treatment of cancer and various other infectious diseases. Nowadays, several well-known anticancer compounds such as taxol, podophyllotoxins, camptothecin, vinblastine, vincristine, homoharringtonine, etc., have been isolated and purified from these medicinal plants. Many of them are used effectively to combat cancer and other related diseases. The herbal medicine and their products are the most suitable and safe to be used as an alternative medicine. Based on their traditional uses and experimental evidences, the anticancer products or compounds are isolated or extracted from the medicinally important plants. Many of these anticancer plants have become endangered due to ruthless harvesting in nature. Hence, there is a need to conserve these species and to propagate them in large scale using plant tissue culture. Alternatively, plant cell tissue and organ culture biotechnology can be adopted to produce these anticancer compounds without cultivation. The proper knowledge and exploration of these isolated molecules or products could provide an alternative source to reduce cancer risk, anti-tumorigenic properties, and suppression of carcinogen activities.

Anticancer Plants: Properties and Application: Volume 1 is a very timely effort in this direction. This book volume with 24 contributions from Brazil, Canada, India, Italy, Jordan, Malaysia, Nigeria, Pakistan, Saudi Arabia, Venezuela, and USA discusses about the various types of anticancer plants as a source of cancer curative agent, their pharmacological and neutraceutical properties, cryo-preservations, and recent trends to understand the basic cause and consequences involved in disease diagnosis. We hope that this book will be helpful for graduate students, teachers,

researchers, and industry persons who are functioning in the fields of cancer biology, natural product chemistry, pharmacology, health care, and medicinal plant research.

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

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

Contents

About the Book

This book provides wide-ranging information on cancers and their safe and effective treatments using medicinal plants and their isolated chemotherapeutic compounds. It covers topics related to ethnobotany, phytochemistry, and pharmacological effects highlighting on the emerging role of plant-derived compounds and their anticancer properties. It emphasizes on various traditional medicinal plants and their application in the modern medicines, toxicological effects, clinical investigations, and advanced tactics of drug delivery, extraction methods, and analytical techniques to authenticate phytochemicals. *Anticancer Plants: Properties and Application: Volume 1* provides the information about the various types of anticancer plants as a source of cancer curative agent, their pharmacological and neutraceutical properties, cryo-preservations, and recent trends to understand the basic cause and consequences involved in the disease diagnosis and drug discovery aspects. This book provides an insight into the development of novel anticancer agents from diversified flora, their properties, and applications. The book will be more useful to graduate students, teachers, researchers, and industry persons, who are functioning in the fields of cancer biology, natural product chemistry, pharmacology, health care, and medicinal plant research.

About the Editors

Mohd. Sayeed Akhtar is working as an Assistant Professor in Gandhi Faiz-e-Aam College, Shahjahanpur, U.P., India. He has received his Ph.D. degree from Aligarh Muslim University (AMU), India, in 2008. He has conducted his postdoctoral research at the Botanical Institute, University of Basel (BIB), Switzerland (2008–2010), and Chonbuk National University (CBNU), Republic of Korea, in 2011, respectively, and also worked as Assistant Professor, Department of Biology, College of Natural Sciences, Jimma University, Jimma, Ethiopia, form 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 nano-biotechnology. 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 nano-biotechnology. 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 5 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.

Mallappa Kumara Swamy is presently a Postdoctoral Researcher at the Department of Crop Science, Faculty of Agriculture, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia. Dr. Swamy has obtained his Ph.D. (Biotechnology) from Acharya Nagarjuna University, Guntur, India, in 2013. He has more than 14 years of teaching and research experience in the fields of plant biotechnology, secondary metabolites production, phytochemistry, and bioactive

studies. To his credit, Dr. Swamy has published more than 50 research publications in peer-reviewed journals. Dr. Swamy is also serving as the editorial board member and reviewer of several high impact international journals. Dr. Swamy is presently engaged in cell and tissue culture technology for bioactive compounds production and their bioactivities studies. Also, his research focuses on nanotechnology for medical applications.

Chapter 1
Current Practices and Awareness of Anticancer Plants in the Traditional Healthcare System

Paola De Cicco, Elisabetta Panza, Chiara Armogida, Giuseppe Ercolano, Giuseppe Cirino, and Angela Ianaro

1.1 Introduction

Cancer is the second leading cause of death worldwide accounting for 8.8 million deaths in 2015 (WHO 2016). It is caused by dynamic abnormalities arising in the genome of cancer cells during cellular divisions altering the expressions of proteins involved in targeting tumor suppressor proteins, growth factors, and transcription factors. These genetic alterations lead to the transformation of normal cells into malignant cancer cells that are able to evade apoptosis, avoid immunity detection, and replicate limitlessly (Cavallo et al. 2011; Hanahan and Weinberg 2011). Even though huge efforts have been devoted to the development of a cure for this grave disease, the number of cancer patients has been increasing constantly with millions of new cases arising each year (Ashkenazi 2008). The increased resistance of cancer cells to apoptosis and to effective chemotherapeutic agents are some of the major obstacles that the oncologists have to overcome in their clinical practice. Thus, there is an urgency to discover novel adjuvant therapies (Morin 2003). Herbal medicine constitutes the largest and most valuable source of natural agents that are proven to have potent effects against many varieties of maladies, including cancer (Yuan et al. 2016). In fact, more than 70% of anticancer agents have their origin in natural sources (Newman and Cragg 2016). Ample evidences confirm that the biological activities of these plants are mainly because of their secondary metabolites including the promising alkaloids. The majority of studies focus on the induced cytotoxicity of well-known alkaloids such as vinblastine, topotecan, taxol, vincristine, and vinflunine that are used clinically in cancer therapy worldwide (Habli et al. 2017). The growing interest in the use of complementary and alternative medicines is mainly due to the disadvantages associated with the conventional cancer chemotherapies and the supposed advantages of more natural treatment options (Molassiotis

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et al. 2005). On the other hand, we cannot avoid considering the disadvantages of natural products and traditional medicines including variation in preparation methods and also chemical composition, dosage determination and adjustment, and the suitable route of administration. Although much research on compounds of natural origin to produce new drug substances occurs, research, specifically aimed at naturally derived medicines to optimize dosages for the intended route of administration and to design the most effective dosage forms, has become essential. Thus, the aim of the present chapter is to highlight the plant-derived natural products and their analogues established as anticancer agents in the traditional and current medicinal practices, their toxicological effects, and clinical exercises along with the new technologies applied to the delivering natural anticancer drugs.

1.2 The Vinca Alkaloids

Vinca alkaloids are a subset of drugs mainly extracted from the Madagascar periwinkle plant *Catharanthus roseus* (*Vinca rosea* Linn., belonging to the family Apocynaceae), discovered in the 1950s by Robert Noble and Charles Beer of Canada. Although they have been used to treat diabetes and high blood pressure or as disinfectants, their importance is due to the cancer-fighting ability, in fact they have become part of the standard of care for more than 30 years. The four major vinca alkaloids having medicinal properties are vinblastine (VBL), vincristine (VCR), vinorelbine (VRL), and vindesine (VDS). Vinflunine (VLF) is a new synthetic vinca alkaloid, which has been approved in Europe for second-line treatment of metastatic and advanced urothelial cancer after failure of platin-containing therapy (Gerullis et al. 2017). The naturally occurring vinca alkaloids (VBL and VCR) have been used in the treatment of a wide range of malignancies, primarily hematological cancers such as leukemia and lymphoma, but also testicular cancer. The semisynthetics (VRL and VDS) have exhibited clinical activity against lung, ovarian, and breast malignancies. The vinca alkaloids have closely related structures (Fig. 1.1), containing both an indole nucleus (catharanthine portion) and a dihydroindole nucleus (vindoline portion) linked by a carbon-carbon bridge. Modifications to the catharanthine moiety have a negative effect on the activity, while changes to the vindoline portion have a minor impact. Moreover, relatively small modification to the complex vinca skeleton can result in significant changes in the spectrum of activity and toxicity.

1.2.1 Mechanism of Action and Resistance of Vinca Alkaloids

The principal mechanism of action of the vinca alkaloids is the disruption of microtubule function, particularly of microtubules comprising the mitotic spindle apparatus through the interaction with tubulin, directly causing metaphase arrest

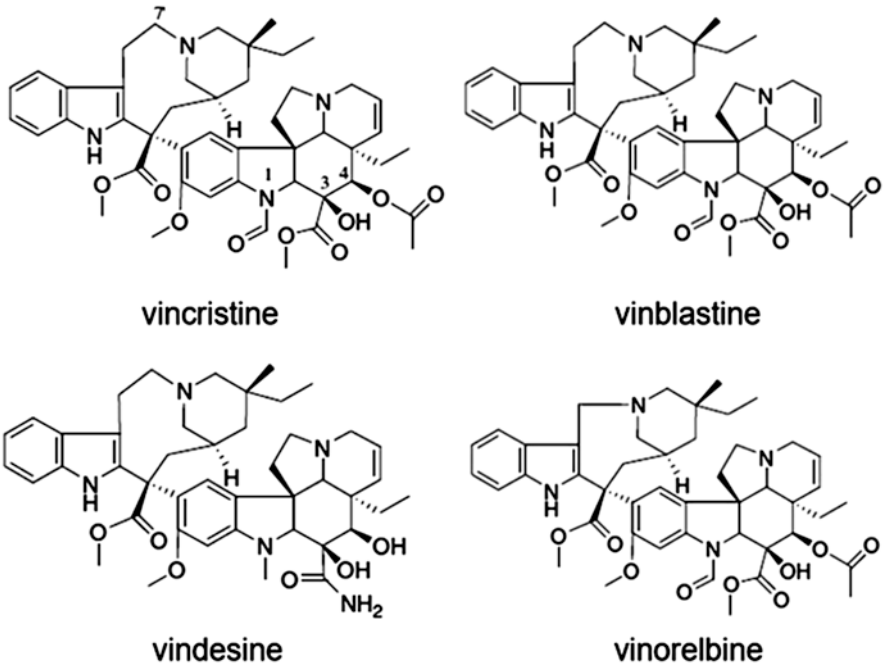

Fig. 1.1 Chemical structure of vinca alkaloids

(Himes 1991). Tubulin is a heterodimeric protein of α-tubulin and β-tubulin each with a molecular weight of 55 kDa. Under normal physiological conditions, the heterodimers polymerize to form microtubules which are essential for such cellular processes such as mitosis, meiosis, secretion, and axonal transport. The assembly and disassembly of the microtubule polymers are regulated by the binding of tubulin and guanosine 5-triphosphate (GTP) (Mitchison 1993). All the vinca alkaloids have a high affinity for tubulin, and, by binding to the protein, they both prevent polymerization and promote depolymerization of the microtubules. The interaction of the vinca alkaloids with the microtubules of the spindle apparatus disrupts the spindle apparatus and leads to metaphase arrest. VRL, VCR, and VBL have all been shown to possess approximately equal tubulin-binding constants and cause metaphase block at roughly the same concentrations. The differences in the relative potencies of these agents may not be due to their binding efficiencies, but rather to differences in their intracellular retention times or the stability of the drug-tubulin complexes. Furthermore, VCR is a much more potent inhibitor of axonal microtubule formation. Vinca alkaloids are also capable of many other biochemical activities that may or may not be related to their effects on microtubules, including inhibiting the synthesis of proteins and nucleic acids, altering lipid metabolism and membrane lipids, elevating oxidized glutathione, elevating cyclic adenosine monophosphate (cAMP), and inhibiting calcium-calmodulin-regulated cAMP

phosphodiesterase. Many of the effects that do not include microtubule disruption happen only after the treatment of cells with clinically irrelevant doses of the vinca alkaloids. While the disruption of microtubules assembly and function results in metaphase blockade, the terminal event in the cytotoxic pathway is the death of the cell through activation of apoptotic pathways (Harmon et al. 1992; Tsukidate et al. 1993). In vitro experiments carried out in multiple cell lines have identified vinca alkaloids as the potent inducers of apoptosis through both p53-dependent and p53-independent pathways. Tumor cells that have been exposed to these agents show characteristic morphological and molecular changes that are associated with the induction of apoptosis in a dose- and time-dependent fashion. Resistance to the vinca alkaloids develops rapidly and is associated with decreased drug accumulation and retention within target tumor cells. The most widely documented mechanism typifies the "classic" pleiotropic or multidrug-resistant (MDR) phenotype that can be either innate (primary) or acquired. Although many proteins that mediate MDR have been characterized, the most well known is the ATP-binding cassette (ABC) transporter family, a huge gene family of transmembrane transporters which efflux large endobiotic and xenobiotic compounds from cells in an ATP-dependent fashion. The best characterized ABC transporters, in regard to vinca alkaloid resistance, are the permeability glycoprotein (P-gp)/MDR1 encoded gene product (ABC subfamily B1; ABCB1) and the multidrug resistance protein MRP (ABC subfamily C2; ABCB1) (Lautier et al. 1996; Sikic et al. 1997). MDR1 is a 170-kD P-gp transmembrane pump that regulates the efflux of a wide range of bulky hydrophobic substances in an energy-dependent fashion. The protein is constitutively overexpressed in various normal tissues, such as endothelium and epithelial tissue, especially renal epithelium and colonic endothelium, as well as tumors derived from these tissues. The expression of MDR1/P-gp is highly regulated. Drugs and modulators that are P-gp substrates or inhibitors, as well as a multitude of DNA-damaging agents that are commonly used against cancer, may also induce expression of MDR1/P-gp. Moreover, modulation of the cell cycle checkpoint protein p53 affects MDR1 expression and, therefore, may regulate the expression of the MDR phenotype. MRP, a 190-kD membrane-spanning protein that shares 15% homology with MDR1, has been shown to mediate vinca alkaloid resistance as well as resistance to other chemotherapeutic agents such as methotrexate. MRP expression has been found in many types of tumors and has been associated with the MDR phenotype in lung, colon, breast, bladder, and prostate cancer, as well as leukemia. Other mechanisms of resistance to the vinca alkaloids have also been identified, although in preclinical models. Each of these represents a different modification in the mechanism of vinca alkaloid action or of downstream signaling to allow the tumor cell to escape apoptosis. For instance, structural and functional alterations in α- and β-tubulins, resulting from either genetic mutations, posttranslational modifications, or differential expression of tubulin isotypes, have also been identified in tumor cells with acquired resistance to the vinca alkaloids (Hari et al. 2003).

1.2.2 Vincristine

1.2.2.1 Clinical Use

Vincristine (VCR) is considered as an elective treatment for childhood and adult acute lymphocytic leukemia. It is used in pediatric oncology because of its greater sensitivity for pediatric targets than other vinca alkaloids, and lower toxicity of therapeutic doses in children. VCR is also generally used in combination with other antineoplastic agents as part of the chemotherapy regimen CHOP, cytoxan, hydroxyrubicin (Adriamycin), Oncovin (vincristine), and prednisone, for non-Hodgkin's lymphoma treatment (Nachman 1990) and as a part of MOPP, nitrogen mustard, Oncovin (vincristine), procarbazine, and prednisone, or COPP for the treatment of Hodgkin's lymphoma. VCR is also commonly used in the treatment of multiple myeloma, chronic lymphocytic leukemia, lymphoblastic crisis of chronic myelogenous leukemia, sarcomas, and small-cell lung carcinoma.

1.2.2.2 Schedule of Administration

A significant interpatient variability exists, and some patients can tolerate much higher doses with limited toxicity (Peltier and Russell 2002). VCR may be given to children weighing more than 10 kg (body surface area ≥ 1 m^2) as a bolus intravenous injection at a dose of 1.5–2.0 mg/m^2 weekly, although 0.05–0.65 mg/kg weekly is conventionally used in smaller children (less than 10 kg, body surface area <1 m^2). However, for adults, the common weekly dose is 1.4–2.0 mg/m^2. There have been efforts to create a prolonged infusion scheme as a result of some evidence that the duration of exposure above a critical concentration is important for cytotoxicity (Van den Berg et al. 1982; Rowinsky and Donehower 1991). A restriction of the whole single dose of VCR from 2.0 to 2.5 mg in children and 2.0 mg in adults has been generally used due to early reports of substantial toxicity in the gastrointestinal and nervous system at higher doses. However, there is very little pharmacologic or toxicologic evidence to support this practice, and available evidence suggests that it should be reconsidered, particularly in light of the wide interpatient variability in pharmacokinetic behavior and tolerance. VCR dosage modification should be based on the appearance of toxicity such as the appearance of peripheral or autonomic neuropathy (Johnson et al. 1963). In clearly palliative situations, dose reductions, lengthening dosing intervals, or selecting an alternative agent may be justified in the event of moderate toxicity. Due to the hepatobiliary elimination of vincristine, a 50% dose reduction is indicated for patients with plasma total bilirubin levels of 1.5–3.0 mg/dl and a 75% dose reduction for patients with a serum total bilirubin >3.0 mg/ml. There is no dosage reduction indicated for renal dysfunction (Jackson et al. 1984, 1986). A routine prophylactic regimen to prevent the serious consequences of severe autonomic toxicity, particularly severe constipation, is also recommended.

1.2.2.3 Pharmacokinetics

The characteristics of the vinca alkaloids include:

- Large volumes of distribution, in fact VCR is rapidly distributed to the peripheral compartment following intravenous administration.
- Long terminal half-lives in the range of 23–85 h.
- Significant hepatic metabolism, predominantly by hepatic cytochrome P450 CYP3A. VCR is metabolized and excreted primarily by the hepatobiliary system. Seventy-two hours after the administration of radiolabeled VCR, approximately 12% of the radioactivity is recovered in the urine (50% of which consists of metabolites), and approximately 70% is recovered from feces (40% of which consists of metabolites).
- Extensive binding to proteins: VCR bind strongly to both plasma proteins and formed blood elements, particularly platelets, which contain high concentrations of tubulin. This served as the rationale for using VCR-loaded platelets for treating disorders of platelet consumption such as idiopathic thrombocytopenia purpura. The platelet count inversely influences drug exposure.
- Poor blood-brain barrier penetration, probably because of the molecules large size and it is an avoid substrate for multidrug transporter pumps that maintain the integrity of the blood-brain barrier.
- Total clearance is slow, which reflects avid tissue binding and slow release.

1.2.2.4 Toxicity

Significant side effects are due to the fact that in addition to inhibiting replication of cancer cells, VCR also blocks replication of healthy cells, particularly in hematopoietic tissues. This effect can lead to lack of corpuscular elements of blood such as anemia and decreased immune response to blocking lymphoid and myeloid precursors of immune cells. The VCR, compared to the other vinca alkaloids, demonstrates a particular neurotoxicity. The toxicologic profile is manifested by the appearance of paresthesias, decreased reflexes and a load sensitivity of peripheral nerves, and autonomic nervous system disorders (e.g., constipation, which can reach up paralytic ileus). Gastrointestinal toxicities, aside from those caused by autonomic dysfunction, may be caused by all the vinca alkaloids. Mild and reversible alopecia occurs in approximately 20% of patients treated with VCR.

1.2.3 Vinblastine

1.2.3.1 Clinical Use

Vinblastine (VBL) is used in combination with other agents as a frontline therapy for the treatment of Hodgkin's disease and testicular cancer. For Hodgkin's lymphoma, VBL is often used in combination with doxorubicin, bleomycin, and dacarbazine (ABVD). This regimen is either administered alone or alternated with MOPP, which is non-cross-resistant to ABVD. It is also approved for use as a single agent or in combination with cisplatin for the treatment of non-small-cell lung carcinoma (NSCLC) and advanced breast cancer (Rowinsky and Donehower 1991; Joel 1996). Antineoplastic activity is also observed with VBL as a single agent or in combination with other antineoplastic drugs in carcinomas of the bladder, Kaposi's sarcoma, choriocarcinoma, terminal phase of chronic myelogenous leukemia, mycosis fungoides, Letterer-Siwe disease (histiocytosis X), and choriocarcinomas that are resistant to other chemotherapy agents.

1.2.3.2 Schedule of Administration

VBL may be given to children on a weekly schedule starting at 2.5 mg/m^2 followed by dose escalation of 1.25 mg/m^2 each week. Initial dose recommendation is 3.7 mg/m^2 weekly for adults with gradual dose escalation of 1.8 mg/m^2 each week. For both children and adult, dose escalation is based on hematological tolerance of the drug. It is not recommended to use a dose higher than 12.5 mg and 18.5 mg in pediatric and adult patients, respectively, although most patients have myelosuppression at submaximal doses regardless. VBL is also commonly used as a bolus injection of 6 mg/m^2 in cyclic combination chemotherapy regimens.

1.2.3.3 Pharmacokinetics

The pharmacokinetic profile of VBL is similar to that of VCR. Following rapid intravenous injection of VBL at standard doses, peak plasma drug concentrations are approximately 0.4 μmol/L. Like VCR, binding of VBL to plasma proteins and formed elements of blood is extensive, distribution is rapid, and terminal half-life values in the range of 20–24 h have been reported. Like VCR, VBL disposition is principally through the hepatobiliary system. Fecal excretion of the parent compound is low, indicating substantial metabolism, and the cytochrome P450 CYP3A isoform appears to be principally responsible for its biotransformation.

1.2.3.4 Toxicity

The manifestations of neurotoxicity are similar for all vinca alkaloids; however, severe neurotoxicity is observed less frequently with VBL as compared to VCR. Neutropenia is the principal dose-limiting toxicity of VBL; thrombocytopenia and anemia are usually less common and less severe. The onset of neutropenia is usually 7–11 days after treatment, and recovery is generally by days 14–21. Mucositis occurs more frequently with VBL than the other vinca alkaloids.

1.2.4 Vindesine

1.2.4.1 Clinical Use

Vindesine (VDS) is used in combination with other agents, such as mitomycin C and/or platinating agents, in the treatment of NSCLC (Dancey and Steward 1995). In addition, antineoplastic activity has been seen in acute lymphocytic leukemia, blast crisis of chronic myeloid leukemia, malignant melanoma, pediatric solid tumors, and metastatic renal, breast, esophageal, and colorectal carcinomas. However, a unique role of VDS in oncology remains to be defined.

1.2.4.2 Schedule of Administration

VDS is most commonly administered as a single intravenous bolus of 2–4 mg/m^2 weekly to biweekly which is associated with antitumor activity and a tolerable toxicity profile (Rowinsky and Donehower 1991). Intermittent or continuous schedules usually infuse 1–2 mg/m^2 per day for 1–2 days or 1.2 mg/m^2 for 5 days every 3–4 weeks (Rowinsky and Donehower 1991; Rahmani et al. 1987). As with the other vinca alkaloids, a dose reduction is warranted if the patient has hepatic dysfunction.

1.2.4.3 Pharmacokinetics

VDS is rapidly distributed to tissues; terminal half-life values range from 20 to 24 h. Its large volume of distribution, low renal clearance, and long terminal half-life suggest that VDS undergoes extensive tissue binding and delayed elimination. Similar to the other vinca alkaloids, VDS disposition is primarily by hepatic metabolism and biliary clearance, and the cytochrome P450 isoform CYP3A plays a major role in drug metabolism. Renal clearance is negligible, accounting for 1–12% of drug disposition.

1.2.4.4 Toxicity

The manifestations of toxicity are similar to VBL. Also for VDS, as VBL, neutropenia is the principal dose-limiting toxicity, and mucositis occurs frequently; nausea, vomiting, and diarrhea may also occur to a lesser extent.

1.2.5 Vinorelbine

1.2.5.1 Clinical Use

Vinorelbine (VRL) is the only vinca alkaloids which can be administered orally, resistance to VRL develops more slowly and it is less cross-resistant than VCR and VBL. VRL is approved in the USA as either alone or in combination with cisplatin for the initial treatment of patients with unresectable, advanced NSCLC. It has also demonstrated prominent antitumor activity in patients with advanced or metastatic breast cancer recurring following initial treatment and as a component of first-line regimens consisting of other non-cross-resistant chemotherapeutic agents and/or trastuzumab. In addition, VRL has also demonstrated prominent activity in patients with advanced Hodgkin's and non-Hodgkin's lymphomas and ovarian carcinoma, and its role in the treatment of these and other malignancies is currently being evaluated. VRL activity has also been noted in cervical and prostate carcinoma.

1.2.5.2 Schedule of Administration

VRL is commonly given intravenously at dose of 30 mg/m^2 as an injection through a sidearm port into a running infusion. Alternatively, VRL may be given as a slow bolus injection followed by flushing the vein with 5% dextrose or 0.9% sodium chloride solutions or a short infusion over 20 min. It appears that the shorter infusions are associated with a decrease in local venous toxicity (Zeffren et al. 1984). Oral doses of 80–100 mg/m^2 given weekly are generally well tolerated, but an acceptable oral formulation has not yet been approved. Patients with hepatic dysfunction should be given a lower dose (Robieux et al. 1996). As with the other vinca alkaloids, dosage reductions are not indicated in patients with renal insufficiency.

1.2.5.3 Pharmacokinetics

The pharmacokinetic profile of VRL is similar to those of the other vinca alkaloids. After intravenous administration, there will be a rapid decay of VRL concentrations followed by much slower elimination phases; terminal half-life range is 18–49 h. Plasma protein binding has been reported to range from 80% to 91%. VRL is widely distributed with extensive sequestration in virtually all tissues, except brain. The

drug is also extensively bound to platelets. The wide distribution of VRL probably reflects the agent's lipophilicity, which is among the highest of the vinca alkaloids. Hepatic metabolism is the predominant mode of drug disposition. Approximately 33–80% of the administered dose of VRL is excreted into the feces, whereas urinary excretion represents only 16–30% of total drug disposition, the majority of which is unmetabolized VRL. The cytochrome P450 CYP3A is principally involved in biotransformation. VRL is bioavailable following oral administration; however, the development of a suitable oral formulation is still ongoing.

1.2.5.4 Toxicity

Severe neurotoxicity is observed less frequently with VRL as compared to all other vinca alkaloids because VRL has a lower affinity for axonal microtubules than either VCR or VBL. Again, neutropenia is the principal dose-limiting toxicity, and pancreatitis has also been reported with VRL. Mild and reversible alopecia occurs in approximately 10% of patients treated with VRL.

1.3 Podophyllotoxins

The podophyllotoxins are an important class of anticancer agents including the semisynthetic compounds, teniposide (VM-26) and etoposide (VP-16) that exert cytotoxic activity by inhibiting topoisomerase II (TOP II). They are currently used in chemotherapy for the treatment of many cancers. Podophyllotoxin is a natural lignan isolated from podophyllin, a resin produced by roots and rhizomes of different plants of the genus, *Podophyllum* (Berberidaceae family), and it is particularly abundant in American *Podophyllum*, called *Podophyllum peltatum*, and Indian *Podophyllum*, usually named *Podophyllum emodi* (Imbert 1998). The podophyllotoxins have a long therapeutic history as they have been extensively used for over 1000 years in traditional medicine of diverse cultures as antiemetic, cathartic, suicidal agents, antidote for snake venom, anthelmintic, cholagogue, and expectorant. Their anticancer activity was well known for many years as it was already recorded in 900 in the early medical English book, *Bald's Leechbook*. In addition to the antitumor activity, podophyllotoxins also exhibit significant antiviral properties. In fact, they are included in many pharmacopoeias and used in the treatment of condyloma acuminatum caused by human papilloma virus (HPV) and other venereal and perianal warts (Kelly and Hartwell 1954).

Podophyllotoxins are the potent inhibitors of microtubule assembly which are crucial for cell division. More specifically, they bind to the tubulin, the major protein component of microtubules, and inhibit its polymerization which results in impediment for microtubule cytoskeletal structure formation and in block of cell cycle in metaphase stage (Desbene and Giorgi-Renault 2002). Since the discovery

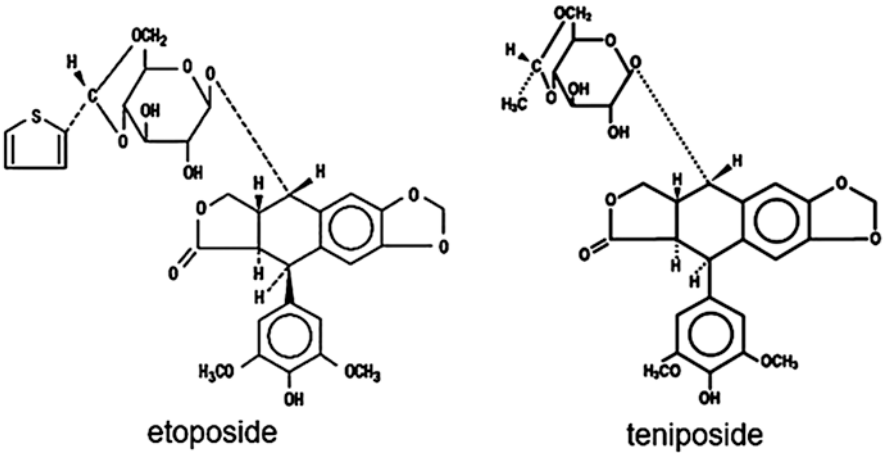

Fig. 1.2 Chemical structure of podophyllotoxins

of the anticancer properties of podophyllotoxin, many efforts have been made in the development of new potent drugs with better therapeutic index based on structural modifications of the lead compound podophyllotoxin. In the 1950s, Sandoz Pharmaceuticals begun a research program direct to study podophyllotoxin glycosides obtained from a series of aldehyde condensation products of the non-purified root of the Indian *Podophyllum* plant, which resin is richer in podophyllotoxin compared to the resin of the American one. Several attempts that were made to reduce its toxicity while retaining antineoplastic activity resulted in the development of two structurally modified derivatives, namely, C-4β-epipodophyllotoxin (EPPT) or etoposide and 4′-demethyl epipodophyllotoxin (DEPPT) or teniposide. These compounds showed the best antitumor activity against L1210 leukemia and were selected for clinical trials. The study begun in 1971 and first reported in 1973–1974 demonstrated antineoplastic activity for etoposide and teniposide in acute myeloid leukemia, Hodgkin's disease, non-Hodgkin's lymphoma, lung cancer (both small-cell and non-small-cell), gastric cancer, breast cancer, and ovarian cancer. In 1978 Sandoz Pharmaceuticals licensed both etoposide and teniposide to Bristol-Myers Squibb. In 1983, etoposide received the approval from FDA for the treatment of testicular cancer, while teniposide had to wait almost 10 years to be approved by FDA as an anticancer drug.

The characteristic structure of podophyllotoxin derivatives consists in four almost planar fused rings containing a dioxole ring, a lactone ring, and an aromatic ring with α configuration that reduces its free rotation. The lactone ring is required for the inhibition of the polymerization of tubulin as it represents the region that interacts with the protein. The introduction of a β-glycosidic moiety (Fig. 1.2) that occurred in the new derivatives (etoposide and teniposide) converts these podophyllotoxins from inhibitors of polymerization of tubulin into potent irreversible inhibitors of DNA TOP II (Damayanthi and Lown 1998; Canel et al. 2000; Gordaliza et al. 2000).

1.3.1 Mechanism of Action and Resistance of Podophyllotoxins

Etoposide, teniposide, and their derivatives act as DNA TOP II inhibitors causing cell cycle arrest in the late S or early G2 phase leading to cell death. DNA topoisomerases are essentially ubiquitous enzymes that resolve the topological entanglements, which arise during fundamental biological processes, such as DNA replication, transcription, DNA repair, and chromatin remodeling, by introducing transient breaks in the sugar-phosphate backbone of DNA through two transesterification reactions. According to their structure and mechanism of action, these enzymes are classified as type I (TOP I) and type II (TOP II) enzymes. TOP I acts by making a single-strand break, while TOP II generates double-strand break in the DNA backbone. TOP II cleaves the DNA on both opposite strands of the double helix and forms a covalent phosphodiester bond between its tyrosine moiety in the active site and a 5′ phosphate at the end of each broken DNA. This covalent intermediate is referred as the "cleavage complex" that under normal circumstances is reversible and has short life. The final step catalyzed by TOP II is the DNA relegation that takes place by a reversal of the transesterification reaction and requires ATPs as a cofactor. ATP hydrolysis is essential to induce a conformational change in TOP II that triggers the passage of the intact helix through the break created in the DNA backbone to restore the integrity of the double helix. By this strand-passing mechanism, TOP II can relax supercoiled DNA and removes nucleic acid knots and tangles (Chen et al. 2013).

TOP II is expressed in all proliferating cells, and it is essential for cellular growth and survival. As a result of elevated enzyme levels and activity in transformed cells, TOP II represents an ideal target for many chemotherapeutic drugs. The existence of a direct correlation between enzyme concentration and levels of DNA damage and hence toxicity makes this drug highly selective for cancer cells. Etoposide and teniposide strongly inhibit enzyme-mediated DNA relegation by stabilizing the covalent transient "cleavable complex." The accumulation of this complex in cells is potentially toxic since it promotes mutation, permanent double-strand breaks, and illegitimate recombinations that ultimately culminate in cell death by apoptosis.

Compared with other TOP II inhibitors, as anthracyclines or rebeccamycin analogues, etoposide and teniposide show low affinity for free DNA, and they are not able to intercalate sterically within DNA strands. They, in fact, bind directly to TOP II in a saturable fashion and with high affinity. Interactions with specific amino acids of the enzyme are critical for etoposide and teniposide to enter the TOP II-DNA complex. In particular, they display specificity for the complexes containing a cytosine located one position (−1) 5′ at the cleavage site (Fortune and Osheroff 2000).

Many physiological circumstances can influence the sensitivity of cells towards etoposide and teniposide. The proliferative state of the cell, changes in drug uptake or efflux, and DNA repair or recombination processes represent all possible drug resistance mechanisms that cells can develop to TOP II poisons. Several cell lines

have been shown to acquire resistance to etoposide or teniposide through membrane transporter changes. Podophyllotoxins are substrates for P-gp, an ATP-dependent efflux pump for a wide variety of natural products, associated with multidrug resistance. Elevated levels of this protein in cells, due to gene amplification or overexpression of the human *mdr1* gene that codes for P-gp, enhance the transport of the drug out of the cells causing decrease in the drug intracellular concentration.

Other forms of resistance to podophyllotoxins are directly related to TOP II enzyme. First, reduced concentration or activity of TOP II within the cell, induced by mutations in TOP II genes or altered mRNA transcription, leads to drug resistance. Second, exclusion of TOP II from the nucleus caused by mutation in nuclear localization sequences restricts the access to DNA substrate. Alteration in the binding affinity or in ATP hydrolysis also contributes to decreased drug sensitivity of TOP II (Long et al. 1991; Fortune and Osheroff 2000). Finally, it has been found that a single nucleotide polymorphism SNP309 (T/G) in the gene encoding for double minute 2 (MDM2), an ubiquitin ligase responsible for proteasome-mediated TOP II degradation, induces upregulation and results in lower response (tenfold) to etoposide and teniposide. Given the frequency of SNP309 in the general population (40% of T/G heterozygosity and 12% of G/G homozygosity), this polymorphism may represent a relatively common determinant of drug sensitivity (Nayak et al. 2007).

1.3.2 Etoposide

1.3.2.1 Clinical Use

Etoposide (4′-demethylepipodophyllotoxin 9-[4,6-O-(R)-ethylidene-β-D-glucopyranoside]), also commonly known as VP-16, is a semisynthetic β-D-glycoside derivative of podophyllotoxin obtained with removal of the methyl group at the 4 position of the aromatic ring. It is a cell-cycle, phase-specific agent. Its cytotoxic activity is achieved either by causing DNA breaks through an interaction with DNA TOP II or by forming free radicals. It was synthesized in 1966 by Sandoz Pharmaceutical Corporation and approved in 1983 by the FDA for the treatment of certain neoplastic diseases. A license for the products was issued to the American company Bristol-Myers Squibb that successfully introduced VP-16 onto the American market as VePesid. Etoposide has a wide range of anticancer activity and is relatively well tolerated. It has demonstrated to be, either alone or in combination with other treatments, effective in numerous human malignancies, including small-cell lung cancer (SCLC), germinal tumors, Kaposi's sarcoma, Hodgkin's disease, non-Hodgkin's lymphomas, ovarian carcinoma, and neuroblastoma. In association with vincristine and prednisone, it has been used for refractory acute lymphoblastic leukemia. In 1987 FDA also granted the approval for soft gelatin capsule formulation (Hande 1992). A strong disadvantage of etoposide is its insolubility in water that makes necessary to administrate significant volumes by slow infusion. Therefore, to overcome solubility problems, etoposide phosphate (Etopophos,

Bristol-Myers Squibb Co., Princeton, New Jersey, USA), a water-soluble ester of etoposide, was developed as a prodrug. It was approved for intravenous use by the US FDA in 1996 with the same therapeutic indications of VePesid. Etoposide phosphate appears to have equivalent antineoplastic activity to etoposide. Following intravenous administration, etoposide phosphate is rapidly (within 15 min) and completely converted to etoposide in plasma by the action of alkaline phosphatases. It can be safely administered over short (30 ± 5 min) time periods. Pharmacokinetics and pharmacodynamics properties of etoposide phosphate are equivalent to etoposide, and also the toxicities are identical to those observed with etoposide (Brooks et al. 1995). VePesid, as oral or intravenous formulations, is indicated in the first-line therapy, in combination with other approved chemotherapeutic, in patients with refractory testicular tumors who have already received appropriate surgical, chemotherapeutic, and radiotherapeutic therapy and for the treatment of small-cell anaplastic lung tumors (Hande 1992).

1.3.2.2 Schedule of Administration

Etoposide or Etopophos is administered as intravenous infusion in patients with refractory testicular tumors at the dose ranging from 50 to 100 mg/m^2/day on day 1 through 5 to 100 mg/m^2/day on days 1, 3, and 5 repeated at 3- to 4-week intervals. Patients with SCLC receive etoposide or Etopophos 500 mg/m^2 as an intravenous infusion over 24 h or 100 mg/m^2 as a daily 2 h infusion for 5 days repeated at 3- to 4-week intervals. Alternatively, the dosage for oral etoposide as gelatin capsules is 70 mg/m^2 once a day for 4 days to 100 mg/m^2 orally once a day for 5 days.

1.3.2.3 Clinical Pharmacokinetics

All pharmacokinetics parameters are similar for both oral and intravenous administration. After intravenous administration of etoposide, the peak plasma concentration is 20 μg/mL, and terminal elimination half-life ranges from 4 to 11 h. The maximum plasma concentration values increase linearly with dose. Total body clearance has been determined to range from 16 to 36 mL/min/m^2, and it is independent of dose over a range of 100–600 mg/m^2. In adults, the total body clearance of etoposide is correlated with creatinine clearance, serum albumin concentration, and nonrenal clearance.

Etoposide does not accumulate in the plasma following daily administration of 100 mg/m^2 for 4–5 days. Etoposide's steady-state volume of distribution ranges from 5 to 17 L/m^2. Etoposide is highly bound (97%) to human plasma proteins, and in fact, the ratio between the amount of bound drug and the amount of free drug is directly related to the serum albumin concentration. Since free drug is biologically active, an inverse relationship between plasma albumin levels and unbound etoposide has been found. Therefore, a reduction in protein binding increases the pharmacological activity of a given dose of etoposide, but increases the toxicity too.

Approximately 30–40% of etoposide is excreted in the urine as unchanged drug, and a small percentage, less than 5%, undergoes biliary excretion. The mean renal clearance of etoposide is 35% of the total body clearance, so other nonrenal processes occur, like metabolism and biliary excretion. Hepatic metabolism accounts for one-third of drug. The main etoposide biotransformation is primarily mediated by CYP3A4, and less so by CYP3A5, and results in the formation of a catechol metabolite (3-hydroxyetoposide) via O-demethylation, which is then converted, by opening the lactone ring, in the corresponding semiquinone and quinone moieties that are subject to urinary excretion. It is still not clear whether or not the catechol metabolite may contribute to the late adverse effects of etoposide, particularly its leukemogenic activity. Further metabolic pathways of minor quantitative importance include glucuronidation and sulfate conjugation. Total etoposide clearance is modestly decreased in patients with renal failure, but not in patients with hepatic obstruction. After oral administration, the bioavailability is approximately 50% and decreases as drug dose increases. The half-life is 0.4 h with peak concentrations occurring between 0.5 and 4 h. Given the existence of a significant interpatient and intrapatient variability in the pharmacokinetics of oral etoposide, unsuspected underdosing and overdosing are possible in schedules extending for long period. The benefit of oral etoposide seems to be only the major compliance of patients with long-duration of herapy that can avoid hospitalization. Indeed, once absorbed, there is no pharmacological difference between oral and intravenous etoposide with respect to the mechanism of action, half-life, metabolism, excretion, and type of toxicity.

1.3.2.4 Toxicity

The most common adverse effect associated with etoposide and etoposide phosphate is dose-limiting myelosuppression. Anorexia, alopecia, nausea, and vomiting are generally of mild severity, but mucositis is more severe after intravenous etoposide administration. Hypersensitivity reactions characterized by bronchospasms, facial flushing, rashes, dyspnea, fever, chills, tachycardia, chest tightness, cyanosis, and changes in blood pressure (hypotension and hypertension) also occur in patients treated with the parental etoposide formulations. Neurotoxic symptoms including headache, transient mental confusion, and vertigo are rarely observed (Hande 1998).

Etoposide and teniposide can be classified as potential carcinogens, and they may represent important risk factors for the development of secondary acute myelocytic leukemia (AML). Etoposide-induced AML is frequently associated with translocations of the *mixed lineage leukemia* (*mll*) gene at human chromosome 11q23. This locus is particularly susceptible to etoposide-mediated cleavage, and subsequent illegitimate repair events lead to a growth advantage, and ultimately tumorigenesis, in a critical hematopoietic stem-cell subpopulation (Ezoe 2012).

1.3.3 Teniposide

1.3.3.1 Clinical Use

Teniposide (4′-demethylepipodophyllotoxin 9-[4,6-O-(R)-2-thenylidene-β-D-glucopyranoside) also known as VM26 is a semisynthetic β-D-glycoside derivative of podophyllotoxin. Teniposide is an analogue of etoposide, and it solely differs by the substitution of a thenylidene group on the glucopyranoside ring. Although it was isolated and tested in patients before etoposide, FDA approval was granted in the USA only in 1993, 10 years after etoposide because of the early concerns about hypersensitivity reactions induced by teniposide administration. Teniposide is a phase-specific cytotoxic drug, acting in the late S or early G2 phase, thus inducing a premitotic blockade of the cell cycle. It also causes DNA damage through activating oxidation-reduction reactions to produce derivatives that bind directly to DNA. Under in vitro conditions, teniposide is approximately ten times more potent than etoposide in terms of cytotoxicity against human cell lines and has the ability to create DNA strand breaks. Indeed, its cytotoxic effect is related to the relative number of double-strand DNA breaks produced in cells. In particular, the frequency of double-strand breaks observed with teniposide is five times higher compared to an equal dose of etoposide, which is a reflection of the greater potency of teniposide than etoposide. Under in vivo conditions, teniposide is only two to three times more potent than etoposide because of differences in lipophilicity, protein binding, hepatic metabolism, and related pharmacokinetics characteristics. Teniposide has a broad spectrum of good antitumor activity against different types of cancer, including hematologic malignancies and various solid tumors. It is marketed by Bristol-Myers Squibb as Vumon, and, actually, its pivotal indication is in combination with other approved anticancer agents, for the treatment of refractory childhood acute lymphoblastic leukemia (ALL).

1.3.3.2 Schedule of Administration

Several clinical trials have suggested that teniposide has the modest activity as a single agent. An intermittent twice-weekly schedule dose appears to have superior antitumor effect. Teniposide is only available for intravenous infusion. Childhood ALL (acute lymphoblastic leukemia) patients refractory to cytarabine are treated with teniposide 165 mg/m^2 in combination with cytarabine 300 mg/m^2 intravenously, twice weekly for eight to nine doses. Childhood ALL patients refractory to vincristine-/prednisone-containing regimens receive the combination of teniposide 250 mg/m^2 and vincristine 1.5 mg/m^2 intravenously, weekly for 4–8 weeks, and prednisone 40 mg/m^2 orally for 28 days.

1.3.3.3 Clinical Pharmacokinetic

The pharmacokinetic characteristics of teniposide differ from those of etoposide. Teniposide exhibits more affinity for plasma protein and has a greater cellular uptake. About 99% of the drug is protein bound resulting in a limited distribution within the body. The fraction of free teniposide inversely correlates to the serum albumin concentration. Mean steady-state volumes of distribution range from 3 to 11 L/m^2 for children and increase with a decrease in plasma albumin levels. Therefore, a careful monitoring of children with hypoalbuminemia is indicated during the therapy. Levels of teniposide in saliva, cerebrospinal fluid, and malignant ascites fluid are low relative to those measured in plasma. Plasma drug levels declined biexponentially following intravenous infusion. In pediatric patients, maximum plasma concentrations after infusion of 165 mg/m^2 over 30–60 min are 30 μg/ml. By 20–24 h, after infusion, plasma levels are generally <2 μg/mL. Teniposide also has a lower total body clearance (mean 10.3 ml/min/m^2) and a longer terminal half-life (mean, 5 h). Renal clearance of parent teniposide represents only 10% of total body clearance. After intravenous administration, teniposide is in fact extensively metabolized. Thus, from 4% to 12% of a dose is excreted in urine as parent drug and almost 80% as metabolite mainly hydroxy acid, formed by opening of the lactone ring. Less than 10% is excreted in feces. Teniposide, as etoposide, is a substrate of CYP3A4 responsible for its O-demethylation. Therefore, its clearance is increased by inducers of this enzyme, such as carbamazepine, phenobarbital, phenytoin, and rifampicin, while reduced by competitive inhibitors.

1.3.3.4 Toxicity

Teniposide toxicities are identical to those of etoposide. The main dose-limiting toxic effect is myelosuppression, manifest mainly as leukopenia and thrombocytopenia (65% and 80%, respectively). Less severe effects, including nausea and vomiting, diarrhea, and alopecia, are also common. Rare effects include transient increases in liver enzyme activity and hypertension. Hypersensitivity reactions to teniposide are more frequently than with etoposide. Embryotoxicity and teratogenicity, especially in the heart and central nervous system, have been observed. Teniposide may cause fetal harm when administered to a pregnant woman (Hartmann and Lipp 2006). Increased risks of secondary AML associated with 11q23 chromosomal abnormalities, similar to cases seen with etoposide develop following the teniposide use (Ezoe 2012).

1.4 Camptothecins

The camptothecins are a small class of natural anticancer drugs, which include topotecan and irinotecan, acting as TOP I inhibitors. The lead molecule of this family is 20-(S)-camptothecin (CPT), a pentacyclic alkaloid isolated from the bark of *Camptotheca acuminata*, commonly called as Xi Shu (translated as "Happy Tree"), a native tree of China and Tibet which has been extensively used in the traditional Chinese medicine (Perdue et al. 1970). However, due to the problematic recovery of CPT from plants and low yield, there is an increased demand worldwide from pharmaceutical industries. In this regard, many efforts have been successfully made to develop a competitive chemical synthesis (Dallavalle et al. 2006). CPT was first discovered by Wall during a great research program carried out from 1950 to 1958 at the Eastern Regional Research Laboratory (United States Department of Agriculture, Philadelphia) which involved a screening study of thousands of plants for their antibiotic, antitumor, and antiviral activity. Among those compounds, the *Camptotheca* extracts were the only to show the highest antitumor activity as demonstrated by in vitro test on KB (oral epidermoid human carcinoma) cells and in vivo on different tumor mouse models like S-180 (a sarcoma model), CA-755 (an adenocarcinoma model), and L1210 (a lymphoid leukemia model). In 1966 Wall and Wani, finally, isolated the pure active compound that was termed as CPT, and its structure was determined. In 1970 the National Cancer Institute (NCI) decided to start the clinical trial, at the Baltimore Cancer Research Center, with the water-soluble sodium salt form of CPT in patients with various advanced solid tumors (i.e., colorectal, gastric, non-small-cell lung carcinoma, and malignant melanoma) (Cragg and Newman 2004). In the next 10 years, many pharmacological and chemical studies resulted in the design of new semisynthetic, water-soluble derivatives with higher stability and increased therapeutic index. Two of them, topotecan (Hycamptin) and irinotecan (Camptosar), have been approved by FDA as anticancer drugs for the treatment of several tumors (ovarian cancer, SCLC, and cervical cancer). Numerous new molecules are currently in clinical trials at various stages (Rowinsky et al. 1992a; Saltz et al. 2000; Vanhoefer et al. 2001).

The characteristic structure of CPT consists in a planar pentacyclic system comprised of a quinolinic group fused through two interposed rings, a pyrrole ring and a lactam ring, to a terminal α-hydroxyl-lactone ring with a chiral center at position C-20 (Fig. 1.3). From the structure-activity relationship studies, it has become clear that two structural moieties are absolutely required for successful interaction with the TOP I target and antitumor potency in vivo: the α-hydroxyl-lactone and chiral carbon at 20 position in the S enantiomeric form (Wall et al. 1993). The α-hydroxyl-lactone is an important pharmacophore. However, it is also susceptible to spontaneous reversible hydrolysis forming an opened-ring inactive and water-soluble carboxylate species. This reaction is influenced by pH and solution composition (Hertzberg et al. 1989). At physiological pH, the predominant form is the carboxylate CPT which binds to human serum albumin with 200-fold greater affinity than the lactone ring resulting in reduction of the circulating concentration of CPT. The less

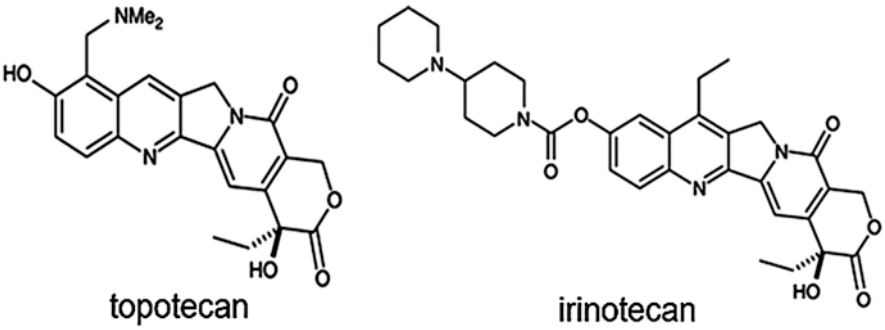

Fig. 1.3 Chemical structure of camptothecins

cytotoxic activity of the carboxylate form has to be ascribed to decreased cell membrane binding, limited diffusibility through cell lipid bilayer and reduced intrinsic potency against the TOP I (Burke and Mi 1994).

1.4.1 Mechanism of Action and of Resistance of Camptothecins

Camptothecins are a potent inhibitor of TOP I. TOP I promotes relaxation of supercoiled DNA by breaking and resealing phosphodiester bonds. It participates in important cellular processes that require the separation of the two strands, such as replication and transcription. Thus, TOP I binds to DNA and facilitates a single-strand formation that serves as a template for transcription and replication factors by inducing reversible single-strand breaks of duplex DNA. Then, TOP 1 resolves over-wounding or under-wounding in the DNA that occurs at the replication or transcription forks, by rotating the broken strand around the intact one and subsequent religation of the cleaved DNA strands to restore the integrity of the double helix (Wang 2002). In detail, the cleavage on the single strand is mediated by reversible transesterification, in which the hydroxyl group of the catalytic tyrosine (Y723 in human) in the active site of TOP I attacks the 3′ phosphate at DNA terminus to form a covalent phosphodiester bond between the enzyme and DNA. The resulting intermediate, the "cleavable complex" drives the rotation of the 5′ end of the nicked DNA strand around the unbroken strand and lastly rejoins the 5′ end of the nicked DNA with the corresponding 3′ restoring the phosphodiester bond with concomitant release of the enzyme (Stewart et al. 1998).

TOP I is constitutively expressed in all mammalian cells, and it is even hyperexpressed in some malignant tissues (particularly colon and cervical cancers) where its role is critical for cancer cell replication. Indeed, the sensitivity of cells to the CTP cytotoxic effect directly correlates with TOP I levels (Hsiang and Liu 1988).

The anticancer activity of CPT and its analogues is based on the formation of a non-covalent and reversible bond with the cleavable complex TOP I-DNA. CPT has low affinity for DNA or TOP I alone, so it only binds them when they are linked together by forming a ternary complex resulting in the inhibition of the religation step. Thus, the DNA-TOP I complex stabilized by CPT acts as physical barriers to DNA synthesis and transcription. The main models proposed to explain CPT activity are in fact represented by the "replication fork collision model" and the "RNA polymerase collision model." According to these hypotheses, in the presence of the drug, replication and transcription complexes "catch up" and "collide" with the TOP I-DNA complexes generating irreversible TOP I covalent complexes that produce double-strand break that ultimately leads to cell death. In highly proliferative cancer cells, the replication fork collision seems to be the primary cytotoxic mechanism of CPT. Camptothecins are, in fact, S phase-specific drugs because ongoing DNA synthesis is a necessary condition to induce cytotoxicity. However, transcription-dependent DNA damage can induce death in cancer cells promoting proteasomal degradation of both TOP I and RNA polymerase II or accumulation of negative supercoiling downstream of the transcription block, which could favor the formation of R-loop structures (DNA:RNA hybrids) that are known to be cytotoxic and mutagenic (Pommier 2006).

The interaction between CPTs and the cleavable complex results from the intercalation of the active lactone ring between the DNA base pairs inside the cleavage site, referred as −1, the thymine covalently linked to the TOP I catalytic tyrosine, and +1, the guanine at the 5′ end of the cleaved strand. Thus, CPT trapping TOP I-DNA complex precludes the relegation step and the duplex regeneration (Thomas et al. 2004). Numerous phenomena of resistance to CPTs have been described in several families of tumors. The classical mechanisms of resistance include those processes that reduce cellular accumulation and subcellular distribution of the drug, such as interaction with blood proteins, mutations of membrane receptors and transporters, or structural modifications and underexpression of TOP I. Multidrug efflux proteins that belong to the ABC superfamily have been implicated in the resistance of cancer cells to the CPTs. In particular, ABCG2 seems to be involved in topotecan resistance in human ovarian cell lines and in lung cancer resistance to irinotecan. Drug metabolism also contributes to the resistance of tumors to the prodrug irinotecan that needs to be converted to the active form by carboxylesterase. Reduced activity of this enzyme may have an important role in determining clinical sensitivity to this agent. Tumor cells have developed novel mechanisms of protection that occur downstream from the generation of DNA damage and that could interfere with the apoptotic process and cell death, such as enhanced DNA repair activity; upregulation of NFκB, the main regulator of anti-apoptotic gene transcription; and possible deregulation of miRNA during early phases of cellular degeneration (Wang et al. 1999; Beretta et al. 2013).

1.4.2 Topotecan

1.4.2.1 Clinical Use

Topotecan ((S)-9-N, N-dimethylaminoethyl-10-hydroxycamptothecin) is the first water-soluble CPT semisynthetic derivative approved in the USA for clinical use. It received FDA approval in 1996 for ovarian cancer treatment, in 2006 for cervical cancer, and lastly in 2007 for SCLC. It was synthesized by Kingsbury et al. by introducing an N, N-dimethylaminomethyl group at C-9 position into dioxole ring to increase water solubility keeping the lactone ring. It is marketed by Smith-Kline Beecham Pharmaceuticals (Philadelphia, PA) as Hycamtin. The primary indication of topotecan is in combination therapy with cisplatin for the treatment of stage IV-B, recurrent, or persistent carcinoma of the cervix for which is not amenable to curative treatment with surgery and/or radiation therapy. It was also granted FDA approval in second-line therapy against metastatic ovarian carcinoma in patients who have failed previous treatment with platinum compounds or paclitaxel-containing chemotherapy regimens and for the treatment of relapsed SCLC-sensitive disease. In addition, topotecan has shown some interesting activity against hematological malignancies.

1.4.2.2 Schedule of Administration

The approved recommended dose of topotecan in the second-line treatment of advanced ovarian cancer and SCLC is 1.5 mg/m^2 by intravenous infusion over 30 min daily for 5 consecutive days, repeated every 3 weeks. Topotecan 0.75 mg/m^2 may also be given in combination with cisplatin (50 mg/m^2/die) by intravenous infusion over 30 min daily for 3 days every 3 weeks for the treatment of cervical cancer. Administration of topotecan by the oral route has also been assessed using once daily dosing for 5 or 10 days every 3 weeks.

1.4.2.3 Clinical Pharmacokinetics

Topotecan undergoes a reversible pH-dependent hydrolysis of its lactone moiety. Thus, following intravenous administration, a considerable amount of topotecan is rapidly converted in plasma to the open ring inactive carboxylate form. To maintain essentially all drugs in the lactone form, tartaric acid has been introduced in the parenteral formulation to provide a sufficiently low pH. Plasma levels of topotecan lactone and total drug decline in a biexponential manner after intravenous infusion with a serum half-life of approximately 2–4 h. As a consequence, drug accumulation does not occur after five daily doses. Furthermore, there are no significant changes in its pharmacokinetics on repeated daily dosing. The total body clearance of topotecan averages 27.4 l/h/m^2 for the lactone form and 13.4 l/h/m^2 for

the total drug. Topotecan has high volume of distribution about 160 L consistent with the hydrophilic character of the compound. Plasma protein binding of topotecan has been reported to be quite low as ranging from 7% to 35%. Topotecan is only in part metabolized by the CYP450 enzyme system to N-desmethyl-topotecan which peak concentration in plasma is 0.7% of parent drug. Other metabolites identified in plasma, urine, and bile at low concentrations are topotecan-O-glucuronide and N-desmethyl-topotecan-O-glucuronide. Hepatic metabolism represents only 10% of topotecan elimination, whereas most of the agent, about 40%, is excreted by the kidneys, and it appears as unchanged drug in the urine within the first 24 h of treatment. Thus, dose adjustment is necessary when renal function is impaired but in patients with limited hepatic function. When topotecan is administrated by oral route, its bioavailability ranges from 30% to 40%, and the peak plasma concentrations are achieved within 1 h after ingestion. Co-administration of topotecan with food results in a small level decrease in the absorption rate, but does not affect the extent of absorption.

1.4.2.4 Toxicity

Topotecan is cytotoxic to the dividing cells. Mammalian cells that are unable to repair the double-strand breaks produced by the ternary complex die. Its primary toxicity is myelosuppression manifested as dose-limiting leukopenia and neutropenia, but thrombocytopenia and anemia occur as well. Alopecia and gastrointestinal adverse effects, like diarrhea, nausea, and vomiting, are frequently observed as well. Topotecan is a potent mutagen and clastogen, too. Topotecan has a pregnancy Category D designation. In rats, it diminishes fertility and causes fetal resorption, numerous malformations, microphthalmia, pre-implant loss, with only mild maternal toxicity. It is also able to penetrate the intact blood-brain barrier (Gerrits et al. 1999; von Pawel et al. 1999; Garcia-Carbonero and Supko 2002; Martino et al. 2017).

1.4.3 Irinotecan

1.4.3.1 Clinical Use

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyl oxy camptothecin) is a water-soluble derivative of CPT synthetized by Sawada et al. bonding the phenolic hydroxyl group of 7-ethyl-10-hydroxy camptothecin with diamines through a monocarbamate linkage. It was the first water-soluble semisynthetic derivative of CPT to enter in clinical trials. In 1994, it became commercially available in Japan for the treatment of the lung (small-cell and non-small-cell), cervix, and ovary cancer. Thereafter, it was approved in Europe in 1995 and in the USA in 1996 for treatment of advanced colorectal cancer. It is actually marketed by

Pfizer as Camptosar, and its indication is the first-line therapy in combination with 5-fluorouracil (5-FU) and leucovorin (LV) for patients with metastatic carcinoma of the colon or rectum. Camptosar is also indicated as second-line therapy for patients with metastatic colorectal cancer refractory to 5-FU. Promising antitumor activity has also been observed against several other types of solid tumors, including SCLC and NSCLC, cervical cancer, ovarian cancer, gastric cancer, and malignant gliomas.

1.4.3.2 Schedule of Administration

The approved recommended regimens of irinotecan for the treatment of colorectal cancer is 125 mg/m^2 given as 90 min intravenous infusion once a week for 4 weeks or 350 mg/m^2 intravenous infusion over 60 min once every 3 weeks. Irinotecan/5-FU/LV combination results more effective in terms of tumor response rates, progression-free survival, and overall survival when compared with 5-FU/LV alone. The recommended dose of irinotecan when given in combination therapy is 125 mg/m^2 with LV 20 mg/m^2 and 5-FU 500 mg/m^2 or 180 mg/m^2 with LV 200 mg/m^2 and 5-FU 400 mg/m^2. In both regimens irinotecan is administered as intravenous infusion over 90 min immediately followed by intravenous bolus infusion of LV first and 5-FU afterward.

1.4.3.3 Clinical Pharmacokinetics

Irinotecan is a prodrug that is converted to the biologically active metabolite SN-38 by carboxylesterase enzymes in the liver through cleavage of the ester bond at C10. SN-38 is approximately 1000 times more potent than irinotecan as an inhibitor of TOP I. Both irinotecan and SN-38 are converted to carboxylate forms by pH-dependent hydrolysis. A pH-dependent equilibrium exists between the two forms; thus, to avoid this transformation and stabilize the active lactone, the pH of the solution has been adjusted to 3.5. One hour after the end of drug infusion, the active metabolite SN-38 reaches the maximum concentrations in plasma, while irinotecan plasma concentrations decline in a multiexponential manner. The half-life of irinotecan is in fact in the range 5.2–9.3 h. Besides the active form SN-38 represents only 4% of the total irinotecan plasma concentrations; its half-life is about 11.5 h, much longer than topotecan. The long-lasting presence of SN-38 in the plasma of patients is attributable to the preferential binding of the lactone form to human plasma proteins (approximately 95% bound), especially to serum albumin. Irinotecan plasma protein binding is less high (30–43% bound). Irinotecan has a moderate apparent volume of distribution about 142 l/m^2. The total body clearance of irinotecan lactone is 53.5 l/h/m^2, two times greater than that of topotecan. The major route of irinotecan elimination is the biliary excretion, while the urinary excretion accounts for only 26% of the administered dose in patients. In the liver, irinotecan, in addition to being converted in the active form SN-38, is also subject to biotransformation in two oxidative metabolites, namely, 7-ethyl-10-[4-N-(5-aminopentanoic acid)-1-piperidino]

carbonyloxycamptothecin and 7-ethyl-10-(4-amino-1piperidino)-carbonyloxycamptothecin by CYP3A4 enzyme. Both metabolites are poor inhibitors of TOP I, and they are not significantly converted to SN-38. Because this enzyme is involved in the metabolism of many commonly used drugs, several drug-drug interactions occur. Thus, anticonvulsants (phenytoin, phenobarbital, or carbamazepine) and glucocorticoids that are CYP3A4 enzyme-inducing increase irinotecan clearance. Ketoconazole, which is a strong inhibitor of CYP3A4 enzymes, causes higher plasma accumulation of irinotecan and its active metabolite SN-38 instead. No relevant pharmacokinetic interactions have been apparent with other cytotoxic agents, such as 5-FU, etoposide, or oxaliplatin. SN-38 itself is further metabolized in the liver by glucuronidation to an inactive compound, SN-38G. In particular, SN-38 is conjugated by the enzyme UDP-glucuronosyl transferase 1A1 (UGT1A1) to form a glucuronide metabolite and then is eliminated through bile by the canalicular multispecific organic anion transporter 1 (cMOAT). UGT1A1 activity is reduced in individuals with genetic polymorphisms that lead to reduced enzyme activity such as the UGT1A1*28 polymorphism. P-gp may also be involved in the biliary transport of the carboxylate form of irinotecan. Renal excretion of unchanged SN-38 represents only 0.26% of the irinotecan dose.

1.4.3.4 Toxicity

Generally, the treatment with irinotecan is well tolerated. The most frequently observed toxicity is myelosuppression manifested as neutropenia, lymphocytopenia, anemia, and thrombocytopenia. Gastrointestinal toxicity (nausea, vomiting, abdominal pain, and diarrhea) could be related to the presence of glucuronidase activity in the bacterial microflora of the intestinal tract. Other common adverse reactions (≥30%) observed both in monotherapy and in combination therapy are pulmonary toxicity asthenia, pain, fever, infection, abnormal bilirubin, and alopecia. No toxicity was observed for the urinary bladder, kidney, or liver, and no allergic reactions to irinotecan were reported (Gupta et al. 1997; Garcia-Carbonero and Supko 2002; Kudoh et al. 1998; Martino et al. 2017).

1.5 Taxanes

Taxanes constitute a model of anticancer drugs that has changed the treatment of numerous solid tumors including breast, ovarian, lung, head, and neck cancer. Taxanes' history started in 1962 when the United States Department of Agriculture (USDA) and the NCI Plant Program established an extensive screening program in order to identify promising anticancer drugs from extracts and substances of various types of plants, including *Taxus brevifolia* in Washington state (Rowinsky et al. 1992b). In 1967, Monroe E. Wall and Mansukh C. Wani obtained a mitotic inhibitor from the bark of the *T. brevifolia* named "Taxol" and then marketed it under the

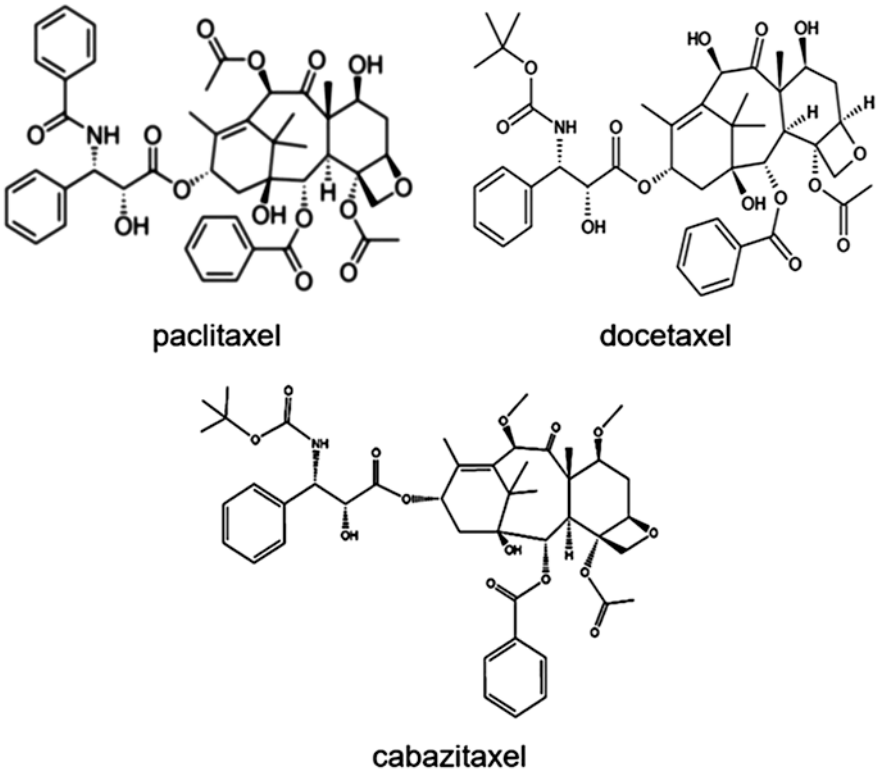

Fig. 1.4 Chemical structure of taxanes

name of paclitaxel (PTX). Unfortunately, the clinical application of PTX has been restricted because of its poor solubility and availability for the slow growing of *T. brevifolia* and a poor conversion ratio between plant and drug (2 g/4 trees) (Wani et al. 1971).

As a result, PTX became scarce and one of the most expensive drugs on the market. In order to supply an alternative production of PTX, many efforts were made to obtain the chemical synthesis of PTX with better solubility. However, the results were unsatisfying because of its complex structure. Therefore, an alternate source, 10-deacetylbaccatin III (10-DBA), derived from the needles of more abundant yew species of *T. baccata* coming from Britain (also called English yew), was used as a precursor to create new semisynthetic analogue of paclitaxel called docetaxel (DTX) or Taxotere.

Both PTX and DTX consist of a skeleton of taxane ring linked to an ester at the C-13 position (Fig. 1.4). The moieties at the C-2′ and C-3′ positions on the C-13 side chain are essential for their antimicrotubule activity. DTX differs from PTX in its chemical structure for a hydroxyl functional group on carbon 10 and a tert-butyl carbamate ester on the phenylpropionate side chain, and this small alteration makes

it more water-soluble (Gueritte-Voegelein et al. 1994). In 2010, FDA approved cabazitaxel (CTX) (Fig. 1.4), another semisynthetic derivative of PTX, for the treatment of hormone-refractory metastatic prostate cancer. CTX shows lower drug resistance than PTX and DTX, thanks to its reduced affinity to the efflux pump P-gp (Yassine et al. 2016).

1.5.1 Mechanisms of Action and Resistance of Taxanes

Taxanes are antimitotic drugs extensively used in chemotherapy. The antitumor activity is related to their ability to stimulate microtubule polymerization which results in mitotic arrest in cancer cells and induces apoptotic cell death or a senescence-like G1 state. Taxanes exhibit a different binding site on microtubules from those of podophyllotoxin and vinca alkaloids (Stanton et al. 2011). Indeed, vinca alkaloids are microtubule-destabilizing agents, while taxoids stabilize the microtubule and increase microtubule polymerization preventing Ca^{2+}- or cold-induced depolymerization and consequent disassembly. However, both classes of drugs strongly suppress microtubule dynamics resulting in similar block of mitosis. According to this common mechanism, vinca alkaloids and taxanes can be coupled in chemotherapy to improve their clinical efficacy (Fanale et al. 2015).

PTX binds to the β-tubulin, which is located on the inside surface of the microtubule, resulting in mitotic arrest and consequent apoptosis by activation of caspase and Bcl-2 proteins, responsible for mitochondrial membrane break. DTX shows higher affinity for the β-tubulin site than PTX and induces tubulin polymerization at 2.1-fold lower critical tubulin concentration. Indeed, DTX was more efficient than PTX to produce cytotoxicity in vitro and in an extensive range of murine neoplasms (Yadav and Gupta 2015). Despite the success of taxanes as antitumor agents, drug resistance represents a major obstacle to the clinical efficacy of these drugs in the treatment of cancer. Drug resistance to taxanes can emerge at various stages of treatment. There are several mechanisms involved in chemoresistance such as an increase of multidrug resistance proteins, tubulin mutation, and overexpression of class III β-tubulin that increases the rate of microtubule detachment. The multidrug resistance proteins represent the main mechanism involved in loss of drug efficacy. PTX and DTX are actively transported by the multidrug resistance-associated protein 2 and 7 (MRP-2 and MRP-7, respectively) that export drugs, accumulated into the cells, through the cellular membrane passing up their toxicity (Brooks et al. 2003). Unlike PTX and DTX, CTX shows poor affinity for MRP-2 and MRP-7, thanks to its extra methyl groups that make it also able to cross the blood-brain barrier (Yassine et al. 2016). Another mechanism of drug resistance involves tubulin mutation that leads to alteration of the expression of tubulin regulatory proteins such as stathmin and microtubule-associated proteins (MAPs) including tau protein. These proteins regulate microtubule dynamics by interacting with microtubules or tubulin dimers, thus modulating the sensitivity of cancer cells toward taxanes. MAPs and

tau proteins stabilize microtubules and allow their polymerization and rescue, while stathmin destabilizes MTs by sequestrating tubulin heterodimers (Orr et al. 2003).

Alteration in the expression level of MAPs has been reported in drug-resistant to taxanes and other antimitotic agents. For example, in breast cancer, high tau (a protein) expression reduces the capacity of PTX to reach its binding site on tubulin. As a result, evaluation of tau expression could be used as a marker to select patients for PTX therapy (Spicakova et al. 2010). Taxane resistance has also been related to expression of different β-tubulin isoforms like overexpression of βIII-tubulin that results to be significantly upregulated in the taxane-resistant tumors and is a predictive biomarker for the clinical response to PTX and DTX. The active improvement of new agents, not sensitive to MRP, MAPs, and class III β-tubulin, may provide new prospect for the treatment of refractory cancer (Rebucci and Michiels 2013).

1.5.2 Paclitaxel

1.5.2.1 Clinical Use

PTX has significant activity in a wide range of solid tumors. It was initially approved for the treatment of early-stage and late-stage ovarian cancer in combination with platinum compounds (Kumar et al. 2010). In 1994 PTX was approved for the treatment of metastatic breast cancer in combination with other anticancer agents, including lapatinib, ixabepilone, and trastuzumab (Satoh et al. 2014). PTX has also demonstrated effectiveness in the treatment of many other cancers including head and neck, prostate, esophageal and bladder cancers, SCLC and NSCLC, and AIDS-related Kaposi's sarcoma (Gill et al. 1999).

1.5.2.2 Schedule of Administration

PTX is usually administered at a dose of 175 mg/m^2 over 3 h or 135–175 mg/m^2 over 24 h every 3 weeks in patients with ovary and breast cancer. However, even after almost two decades of its clinical use, sufficient studies of dose response have not been completed in many tumors. Prolonged exposure over 96 h might have extensive efficacy, but it was disclaimed by a large prospective study (Markman et al. 1998). Because of the hepatic metabolism and biliary excretion, PTX doses should be decreased in patients with hepatic disorders, while dose modification is not required in patients with renal disorder, based on the minimum participation of renal clearance (Eklund et al. 2005).

1.5.2.3 Clinical Pharmacokinetics

PTX shows a nonlinear pharmacokinetic behavior due to distribution, metabolism, and elimination mostly with high dose levels that increase the plasma concentration and reduces the elimination pathways (Rowinsky 1995). PTX is insoluble in water and has an extensive binding to plasma proteins, large volume of distribution, minimal renal clearance, and biliary and fecal elimination. PTX requires parental administration, due to poor bioavailability and first-pass metabolism in the liver. PTX is formulated with Cremophor EL, a polyethoxylated castor oil derivative used to dissolve PTX and other drugs. Cremophor EL is associated with hypersensitivity reactions to PTX. A slower infusion rate and premedication with dexamethasone, diphenhydramine and histamine H2 antagonist such as ranitidine, cimetidine, or famotidine 30 min before infusion of PTX is require to prevent PTX-induced allergic reactions (Szebeni et al. 1998). In order to eliminate these limitations and decrease toxicity, Abraxane, a novel albumin-bound PTX formulation, has been approved in 2008 for metastatic breast cancer. Abraxane delivers PTX as a colloidal suspension of albumin nanoparticles reconstituted with 0.9% sodium chloride solution before intravenous infusion. This novel formulation does not require the need for Cremophor EL and is not associated with hypersensitivity reactions; thus premedication is not necessary (Yamamoto et al. 2011).

1.5.2.4 Toxicity

Myelosuppression, manifested as neutropenia, represents the main toxicity of PTX, significantly accentuated by higher doses and prolonged infusion, which results in increased risk of infections and immunocompromise. Fortunately, the duration of neutropenia is usually brief and reversible, whereas thrombocytopenia and anemia are uncommon (Rowinsky 1995).

Severe hypersensitivity reactions characterized by dyspnea, hypotension, urticaria, and bronchospasm have occurred in 2–4% of patients receiving PTX within few minutes after drug administration. These symptoms can be induced by the drug itself or by Cremophor EL, and their incidence is reduced in patients pretreated with corticosteroids and histamine H1 and H2 antagonists.

Peripheral neuropathy represents the most important non-hematological toxicity associated with PTX administration. This effect is both time- and dose-dependent and is characterized by numbness and paresthesia in a glove-and-stocking distribution (Mielke et al. 2005). Peripheral neuropathy is also attributable to Cremophor EL but, unlike hypersensitivity reactions, it persists even with Cremophor EL-free PTX formulations (Ibrahim et al. 2002). Other toxicities observed with PTX treatment include bradycardia, alopecia, nausea, and diarrhea (Arbuck et al. 1993). Cardiotoxicity may manifest when PTX is associated with doxorubicin, because PTX mediates the conversion of doxorubicin to doxorubicinol, a metabolic intermediate that is responsible for irreversible myocardial damage (Minotti et al. 2001).

1.5.3 Docetaxel

1.5.3.1 Clinical Use

DTX shows similar clinical activity to PTX. It is approved for use in ovarian cancer, SCLC and NSCLC, and breast cancer, and it also has major activity in head and neck cancer, gastric cancer, and bladder cancer.

1.5.3.2 Schedule of Administration

DTX has been exclusively evaluated at a dose of 100 mg/m^2 given intravenously every 3 weeks or in combination with mitomycin 12 mg/m^2 intravenously every 6 weeks or vinblastine 6 mg/m^2 intravenously every 3 weeks. Dose reductions are required for patients with elevations in serum concentrations of hepatocellular enzymes and alkaline phosphatase and in case of hyperbilirubinemia (Clarke and Rivory 1999).

1.5.3.3 Clinical Pharmacokinetics

DTX shows a linear pharmacokinetic behavior because it is usually administered over 1 h showing higher tolerance than the 3-week schedule of PTX (Clarke and Rivory 1999). However, like PTX, DTX strongly binds plasma proteins: it is metabolized by hepatic cytochrome P-450 and has minimal renal clearance, whereas biliary and fecal elimination is more than 80% (Marre et al. 1996).

1.5.3.4 Toxicity

The main toxicities of DTX include hypersensitivity, myelosuppression, and fluid retention. Myelosuppression mainly consists of neutropenia but, unlike PTX, does not seem to be related to doses and infusion durations. Although DTX is not formulated with Cremophor EL, but it is solubilized in polysorbate 80 (Tween 80), another polyethoxylated surfactant, hypersensitivity is manifested in 33% of patients receiving DTX (Rowinsky et al. 1996). However, this effect is reduced with an adequate premedication with histamine antagonists and corticosteroids. Fluid retention manifests with edema, pleural effusions, and ascites, is due to increased capillary permeability, and is accelerated with higher doses, resulting in treatment postponement or termination (Cortes and Pazdur 1995). However, like hypersensitivity, premedication with corticosteroids reduces the onset of this toxicity (Rowinsky et al. 1996). Other reported side effects following DTX administration are pruritic maculopapular rash, desquamation of the hands and feet, and alopecia. Like PTX, DTX induces peripheral neurotoxicity, but it's an uncommon effect, whereas, regarding cardiac

toxicity, DTX appears to induce left ventricular diastolic dysfunction (Shimoyama et al. 2001).

1.5.4 Cabazitaxel

1.5.4.1 Clinical Use

CTX, marketed as Jevtana, is a semisynthetic taxane, approved in 2010 for treatment of hormone-refractory prostate cancer with brain metastases, previously treated with DTX. In other studies was evaluated the possibility to use CTX in NSCLC, head and neck cancer, gastroesophageal cancer, and urothelial cancer (Nightingale and Ryu 2012). However, the main indication for CTX remains the treatment of prostate cancer.

1.5.4.2 Schedule of Administration

CTX is normally administered at the dose of 25 mg/m^2 intravenously every 3 weeks for a maximum of 10 cycles with prednisone 10 mg orally daily or mitoxantrone 12 mg/m^2 intravenously every 3 weeks (Mita et al. 2009). CTX should be used with caution in patients with hepatic disorders to prevent drug accumulation and following toxicity (Nightingale and Ryu 2012).

1.5.4.3 Clinical Pharmacokinetics

CTX has an extensive binding to plasma proteins and a large volume of distribution and presents a triphasic half-life: a rapid phase of 4 min, an intermediate phase of 2 h, and a prolonged phase of 95 h (Paller and Antonarakis 2011). Moreover, CTX shows identical metabolism and elimination of PTX and DTX.

1.5.4.4 Toxicity

The principal dose-limiting toxicity is bone marrow suppression including neutropenia and leukopenia that is possible to reduce by using prophylactic granulocyte-colony stimulating factor (G-CSF). Non-hematological toxicities are diarrhea, nausea, fatigue, neurotoxicity, and vomiting (Mita et al. 2009). Moreover, CTX is formulated in Tween-80, which is capable to induce hypersensitivity reactions. Therefore, patients should be premedicated with corticosteroids and histamine H1 and H2 antagonists is necessary (Nightingale and Ryu 2012).

1.6 Conclusions and Future Prospects

Cancer is considered a humanitarian disaster. The number of deaths caused by cancer is constantly rising despite the huge investment in research and development of new strategies to prevent and cure the disease. Currently used methods such as chemotherapy, surgery, and radiation therapy have their limitations due to their toxic effects on nontargeted tissues furthering human health problems. Therefore, there is a demand for alternative treatments with naturally derived anticancer agents desirably from plant sources. There are remarkable reserves of organic compounds in many plants on Earth that only a very small amount of which as an anticancer compounds tested and used. It was reported that more than 60% of cancer drug available in market or in testing are based on natural products. More than 70% entities among 177 anticancer drugs approved are based on natural products or mimetic. About 25% prescription drug found globally are derived from plant sources, and nearly 121 such drug entity are in use. In the USA, clinical trials are going on more than 100 natural product-based drugs. It was also estimated that 11% of the total 252 drugs found in essential medicine list of WHO are exclusively of plant origin. It appears like a huge gap between current "best medical practices," and the way people are actually treated abides all over the world. Although combinatorial chemistry continues to play a major role in the drug development process, the trend toward the synthesis of complex natural product-like libraries has continued. Drugs derived from vinca alkaloids were utilized along with paclitaxel and other anticancer agents. These compounds have been developed into staple drugs for cancer treatment, and their success along with advancements and discoveries in naturally derived drugs has led to the emergence of new technologies for the application and dosage of anticancer compounds. Through the field of nanotechnology, nanoparticles have been used as a delivery system for drugs to reach target sites. In fact, nanoparticles enhance anticancer activities of plant-derived drugs by controlling release of the compound, and their development could be applied to control sustained drug release and help in creating drugs that are tissue-specific with reduced side effects of treatments. Nanoparticles uses for cancer treatment are of growing interest and show promises as a natural alternative to the currently available treatments.

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Chapter 2
Phytochemical and Biological Properties of *Lippia gracilis*

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2.1 Introduction

Lippia gracilis Schauer (Verbenaceae) is popularly known as "alecrim-da-chapada" or "candeia-de-queimar." It is a native plant in the semiarid region of Northeast Brazil (Fig. 2.1). It is well branched with groups of white flowers and small leaves with visible odoriferous nerves. The fruits are acheno type, very small, and having seeds that barely germinate. In popular medicine of Northeast Brazil, the leaves are used as an antiseptic to treat seborrhea and dermatoses, as well as acne, throat and mouth infections, dandruff, vaginal problems, burns, and wounds (Matos 1998, 1999, 2002; Pascual et al. 2001; Gomes et al. 2011). Despite its medicinal and cultural importance in Northeast Brazil, there are few scientific studies on this important plant. The aim of this chapter is to summarize the phytochemical and biological properties of *L. gracilis* to enlighten the recent research and development.

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Fig. 2.1 Photograph of *Lippia gracilis* found in Serra da Guia, Poço Redondo-SE, Brazil

2.2 Chemical Properties of *Lippia gracilis*

2.2.1 Nonvolatile Constituents

Isolation of three flavone glucosides (1–3) and two flavanone derivatives (4, 5) from an infusion of the leaves of *L. gracilis* was reported. Similarly, carvacrol and its glycosylated derivative (6, 7) were also reported (Fig. 2.2) (Moraes et al. 2017). However, the differentiation among seven genotypes of *L. gracilis* found in Sergipe and Bahia states, Brazil, was performed by comparing the fingerprint chromatograms of methanolic extracts from the leaves and stems. This allowed researchers to establish a correlation between the genotypes and their geographical origin, and this correlation was better for stems than it was for leaves (Gomes et al. 2010). Furthermore, naringenin (5) was isolated from methanol extract of the leaves of *L. gracilis* (Guimarães et al. 2012).

Teas that were prepared by the infusion method were characterized using the fingerprint chromatograms obtained by liquid chromatography with diode array detection (LC-DAD) from leaves of the *L. gracilis* genotypes originated from two locations (Sergipe and Bahia state, Brazil) and collected during both summer (growing under irrigated and non-irrigated conditions) and winter. The results suggested that the genotypes 107, 108, and 110 had greater resistance to drought conditions because it was not possible to observe differences between the chromatograms

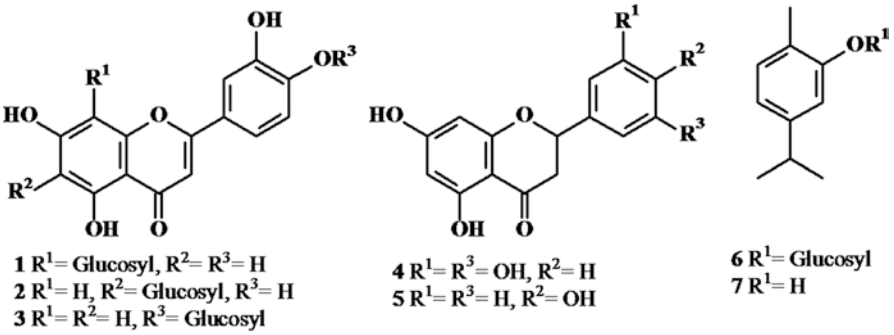


Fig. 2.2 Compounds isolated from the infusion of the leaves of *Lippia gracilis*

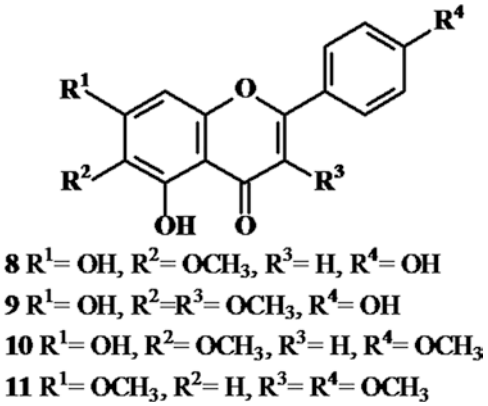


Fig. 2.3 Flavonoids isolated from the ethanolic extract of the leaves of *Lippia gracilis*

obtained from samples grown in summer under irrigated conditions or without irrigation. In addition, genotype 108 showed little chemical differentiation between samples collected in winter and summer (submitted under both forms of cultivation), which suggests that this genotype is the most adapted to the seasonal environments and types of cultivation. This information will be important in determining the most suitable harvesting season for this species, so it can be used in traditional medicine and finding the most promising genotype to ensure the maintenance and conservation of its genetic variability in a Germplasm Bank. Furthermore, it is possible to provide important materials for genetic improvement programs, thus enabling new pharmacologically potent chemotypes to be obtained (Prado et al. 2012). Trevisan et al. (2016) observed the ethanolic extract of the leaves from *L. gracilis* and isolated the flavonoids using the high-performance liquid chromatography (HPLC)/electrospray ionization tandem mass spectrometry (ESI-MS/MS) technique, and these flavonoids were identified by comparison with the literature as 6-methoxy apigenin (hispidulin) (8), 5,7,4′-trihydroxy-3,6,-dimethoxy-flavone (9), 5,7-dihydroxy-6,4′-dimethoxy flavone (pectolinaringenin) (10), and 5-hydroxy-3,7,4′-trimethoxy flavone (11) (Fig. 2.3). They concluded that flavonoids 11 and 10 were the most abundant in the leaf extract with concentrations of 51.4 and 46.6 mg/kg of

dry weight, respectively, while the flavonoids 8 and 9 were found in the same concentration of 34.4 mg/kg of dry weight.

The phytochemical investigation of roots and leaves of *L. affinis gracilis* allowed the isolation and identification of 16 secondary metabolites belonging to several classes of natural products by using successive chromatographic separations. Thus, from the hexanic extract of the roots, it was possible to isolate lapachenol (12), friedelan-3-one (13), 3-acetoxyfriedelane (14), a mixture of β-sitosterol and stigmasterol (15), oleanolic acid acetate (16), and lantadene-A (17). The fractionation of the ethanolic extract of the roots led to the isolation of tetracosanoic acid (18), tecomaquinone (19), microphyllaquinone (20), a mixture of stigmasterol 3-*O*-beta-*D*-glucoside and sitosterol 3-*O*-beta-*D*-glucoside (21), and 5-hydroxynaphto[2,3b] furan-4,9-quinone (22). In the same study, flavonoids such as quercetin (23), hispidulin (8), and 3-methylquercetin (24) could also be isolated from the ethanolic extract of the leaves (Lima 2006) (Fig. 2.4).

2.2.2 Volatile Constituents

The major volatile compounds found in the highest frequency in the essential oil of *L. gracilis* are the structurally related monoterpenes γ-terpinene (25), *p*-cymene (26), carvacrol (27), thymol (28), and thymol methyl ether (29) and sesquiterpenes such as (*E*)-caryophyllene (30) (Fig. 2.5). The quantitative variation of major compounds was due to the geographical origin, genetics, harvest time and/or growing conditions, and abiotic stress (Table 2.1). Based on the main monoterpenes found in the essential oil of *L. gracilis* samples described in Table 2.1, it is suggested that there are at least three different chemotypes, the thymol type (**T**), carvacrol type (**C**), and intermediate type (CT), which contains both carvacrol and thymol in high percentages (with more carvacrol than thymol or more thymol than carvacrol). A comparison of the predominant monoterpenes observed in the chemical composition of the essential oil of *L. gracilis* samples (Fig. 2.5) showed that the **C** and **T** chemotypes were most frequent. Only one sample collected in the Chapada das Mesas National Park, Maranhão, Brazil, showed the non-typical *L. gracilis* major compounds 1,8-cineole and γ-terpineol in high concentrations.

Few studies considering the influence of genetics, developmental stage and age, abiotic stress (water or salt stress), seasonality, and/or circadian rhythm (Gobbo-Neto and Lopes 2007) on diversity of chemical composition of the essential oil of *L. gracilis* have been published. According to Melo et al. (2013), the differences observed in the chemical composition of the essential oil of *L. gracilis* samples came from genetic differences because some genotypes were grown under the same environmental conditions and collected in the same time period of the same year. Santos et al. (2016b) used molecular markers to genetically characterize seven accessions of *L. gracilis*, which are maintained in an active Germplasm Bank of the Federal University of Sergipe (BAG-UFS), Brazil. Using molecular DNA markers, it was possible to quantify the genetic variability and infer that this germ-

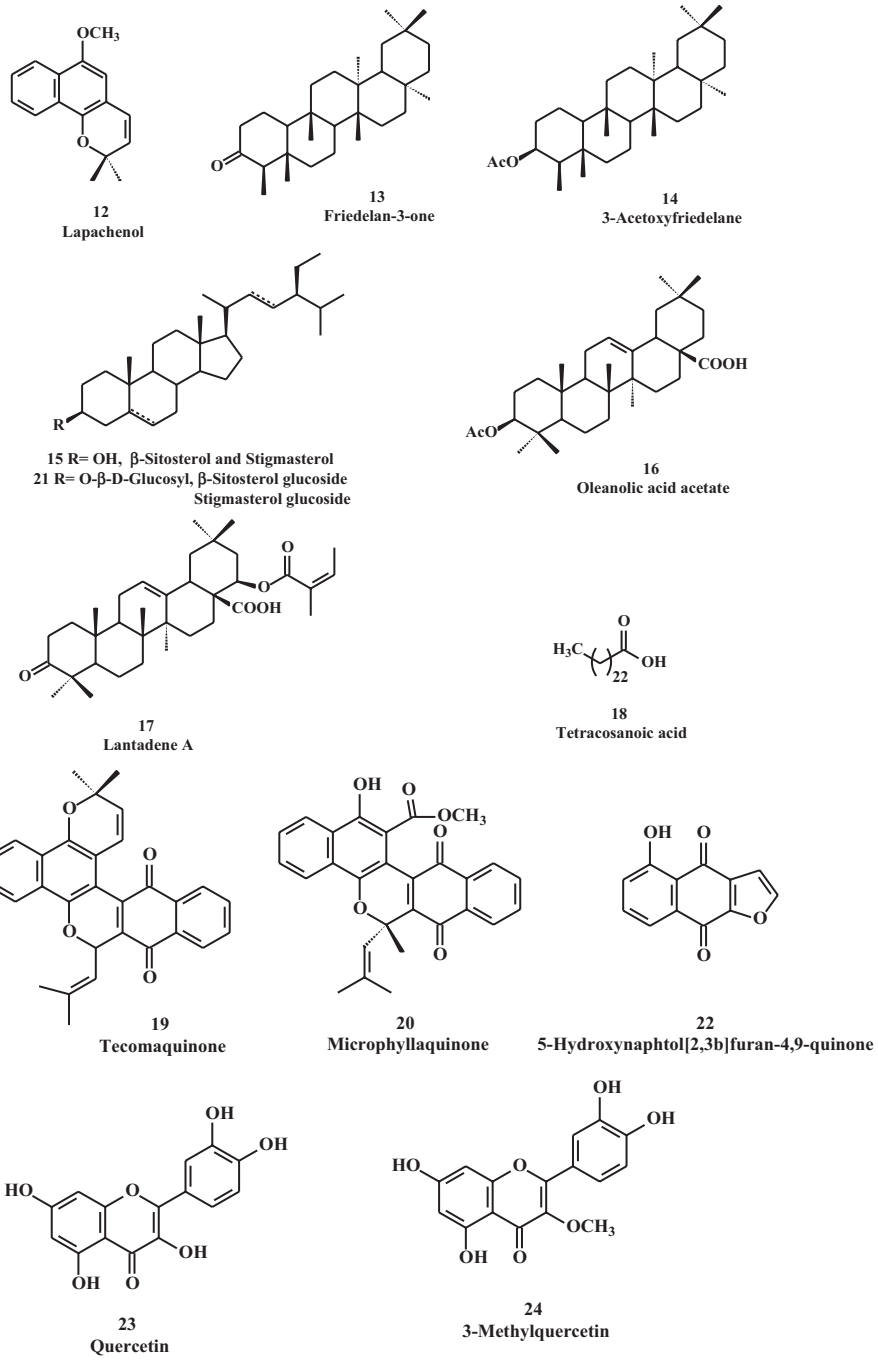
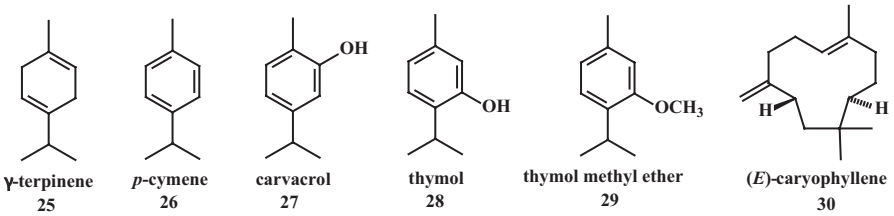


Fig. 2.4 Compounds isolated from the roots and leaves of *Lippia affinis gracilis*



γ-terpinene 25 | *p*-cymene 26 | carvacrol 27 | thymol 28 | thymol methyl ether 29 | (*E*)-caryophyllene 30

Fig. 2.5 Main compounds found in the highest frequency in the essential oils of *Lippia gracilis*

Table 2.1 Major volatile compounds found in the essential oil of *L. gracilis* from Brazil

Geographical origin	Extraction conditions	Yield (%)	Major compounds (GC peak area ≥5.0%)	References
Manaus, Amazonas	Dried leaves and inflorescences (4 h)	2.6	Carvacrol (40.4%) and *p*-cymene (11.4%) (C)	Chagas et al. (2016)
São Felix de Balsas, Maranhão	Dried leaves (100 g/3 h)	3.7	Thymol (73.5%), *p*-cymene (9.2%), and thymol methyl ether (5.4%) (T)	Franco et al. (2014)
	Dried stems (100 g/3 h)	0.4	Thymol (70.1%) (T)	
Chapada das Mesas National Park, Maranhão	Dried leaves (100 g/3 h)	8.0	1,8-cineole (56.2%), α-terpineol (12.1%), and β-pinene (6.2%)	Dias et al. (2015)
Piracuruca, Piauí	Fresh leaves (1 kg/1 h)	2.8	Carvacrol (47.7%), *p*-cymene (19.2%), and thymol methyl ether (6.2%) (C)	Matos et al. (1999)
Fortaleza, Ceará	Fresh leaves (2 h)	1.9	Thymol (30.6%), carvacrol (11.8%), and *p*-cymene (10.7%) (T)	Lemos et al. (1992) and Santiago et al. (2006)
	Fresh leaves (2 h)	0.5	Carvacrol (54.4%), *p*-cymene (10.7%), γ-terpinene[a] (8.0%), (*E*)-caryophyllene (6.1%), and bicyclogermacrene (5.1%) (C)	Pessoa et al. (2005), Santiago et al. (2006), and Lima et al. (2008)
	Fresh leaves (2 h)	–	Carvacrol (50.1%), *p*-cymene (10.7%), γ-terpinene (8.0%), and (*E*)-caryophyllene (6.0%) (C)	Neto et al. (2010)
	Fresh leaves (1 kg/1 h)	1.7	Carvacrol (31.5%) (C)	Trevisan et al. (2016)

(continued)

Table 2.1 (continued)

Geographical origin	Extraction conditions	Yield (%)	Major compounds (GC peak area ≥5.0%)	References
Crato, Ceará	Fresh leaves 7:00 a.m. (77 g/2 h)	0.6	Thymol (44.4%), carvacrol (22.2%), *p*-cymene (6.2%), β-pinene (5.6%), and (*E*)-caryophyllene (5.6%) (TC)	Bitu et al. (2012 and 2015)
	Fresh leaves at 10 a.m.		Carvacrol (35.3%), methyl cinnamate (16.7%), *p*-cymene (8.0%), (*cis*)-ocimene (7.2%), and (*E*)-caryophyllene (6.0%) (C)	
	Fresh leaves at 1:00 p.m.		Methyl cinnamate (21.0%), thymol (17.2%), carvacrol (17.1%), *p*-cymene (7.8%), and thymol methyl ether (6.3%) (CT)	
	Fresh leaves at 4:00 p.m.		Methyl cinnamate (38.2%), carvacrol (19.8%), linalool (8.4%), and β-pinene (5.7%) (C)	
	Dried leaves (35.6 g/2 h)	2.6	Thymol (21.3%), carvacrol (20.9%), α-pinene (19.4%), and *p*-cymene (8.6%) (CT)	
Felipe Guerra, Rio Grande do Norte	Fresh leaves (under salt stress)	–	Carvacrol (42.1%), thymol (32.4%), and *p*-cymene (17.1%) (CT)	Ragagnin et al. (2014)
Buíque, Pernambuco	Fresh leaves (100 g/ 2 h)	1.2–2.8	Carvacrol (36.4–45.3%), *p*-cymene (18.1–26.2%), thymol (7.6–10.3%), and thymol methyl ether (4.9–6.0%) (C)	Neves et al. (2008)
Ouricuri, Pernambuco	Fresh leaves (100 g/2 h)	0.4–0.9	Thymol (35.6–39.2%), γ-terpinene (14.9–20.5%), and 4-methoxyaceto-phenone (10.1–12.4%); α-copaene (3.8–6.3%) (T)	
Petrolândia, Pernambuco	Leaves	–	Carvacrol (41.8%), *p*-cymene (17.9%), thymol (10.1%), and γ-terpinene (6.3%) (C)	Albuquerque et al. (2006, 2012)
Pernambuco	Fresh leaves (350 g/4 h)	–	Carvacrol (45.6%), *o*-cymene (9.5%), and γ-terpinene (8.9%) (C)	Almeida et al. (2015)
Grown in the BAG-UFS, São Cristóvão, Sergipe	–	–	Carvacrol (23.5%), *p*-cymene (15.8%), γ-terpinene (14.2%), menthol (11.0%), thymol (7.3%), (*E*)-caryophyllene (7.0%), and thymol methyl ether (5.4%) **(C)**	Marreto et al. (2008)

(continued)

Table 2.1 (continued)

Geographical origin	Extraction conditions	Yield (%)	Major compounds (GC peak area ≥5.0%)	References
Grown in the BAG-UFS, São Cristóvão, Sergipe	Dried leaves (100 g/4 h)	2.8	Carvacrol (44.4%), *o*-cymene (9.4%), γ-terpinene (9.2%), (*E*)-caryophyllene (8.8%), and thymol methyl ether (5.9%) (C)	Silva et al. (2008) and Guilhon et al. (2011)
Grown in the BAG-UFS, São Cristóvão, Sergipe	Dried leaves (water stress) (100 g/4 h)	2.1	Thymol (32.7%), *p*-cymene (17.8%), thymol methyl ether (10.8%), carvacrol (7.5%), γ-terpinene (7.1%), and (*E*)-caryophyllene (6.5%) (T)	Mendes et al. (2010)
	Dried leaves (nonstress) (100 g/4 h)	1.1	Thymol (24.1%), *p*-cymene (15.9%), thymol methyl ether (11.2%), γ-terpinene (10.9%), (*E*)-caryophyllene (8.2%), and carvacrol (5.3%) (T)	
Tomar do Geru, Sergipe	Dried leaves (1 kg/3 h)	2.8	Thymol (24.0%), *p*-cymene (15.9%), thymol methyl ether (11.7%), γ-terpinene (10.9%), and (*E*)-caryophyllene (7.8%) (T)	Teles et al. (2010)
Grown in the BAG-UFS, São Cristóvão, Sergipe	Dried leaves (100 g/160 min)	4.8[b]	Carvacrol (27.6%), thymol (18.0%), *p*-cymene (16.2%), γ-terpinene (12.1%), thymol methyl ether (6.1%), and (*E*)-caryophyllene (5.1%) (CT)	Carvalho et al. (2013)
Poço Redondo, Sergipe	Fresh leaves (50 g/2 h)	4.0	Thymol (55.5%), *p*-cymene (10.8%), thymol methyl ether (10.5%), and γ-terpinene (5.5%) (T)	Ferraz et al. (2013)
Tomar do Geru, Sergipe	Dried leaves (140 min)	1.6	Genotype 106: Thymol (61.8%), thymol methyl ether (9.1%), (*E*)-caryophyllene (8.3%), and *p*-cymene (6.6%) (T)	Melo et al. (2013)
		2.1	Genotype 109: Carvacrol (54.6%), *p*-cymene (11.0%), γ-terpinene (9.4%), and (*E*)-caryophyllene (5.1%) (C)	
		2.4	Genotype 110: Carvacrol (48.9%), *p*-cymene (12.9%), and γ-terpinene (11.8%) (C)	

(continued)

Table 2.1 (continued)

Geographical origin	Extraction conditions	Yield (%)	Major compounds (GC peak area ≥5.0%)	References
Tomar do Geru, Sergipe (genotype 106)	Dried leaves (75 g/140 min)	1.3	Rainy season, 1-year-old: Thymol (59.3%), (*E*)-caryophyllene (8.6%), thymol methyl ether (8.3%), and *p*-cymene (6.7%) (T)	Cruz et al. (2014)
	Dried leaves (75 g/140 min)	1.4	Dry season: Thymol (56.8%), thymol methyl ether (10.8%), *p*-cymene (8.1%), (*E*)-caryophyllene (7.3%), and 1,8-cineole (5.0%) (T)	
	Dried leaves (75 g/140 min)	1.4	With irrigation: Thymol (56.8%), thymol methyl ether (10.8%), *p*-cymene (8.1%), (*E*)-caryophyllene (7.3%), and 1,8-cineole (5.0%) (T)	
	Dried leaves (75 g/140 min)	1.6	Without irrigation: Thymol (53.6%), (*E*)-caryophyllene (12.0%), thymol methyl ether (10.1%), and *p*-cymene (7.3%) (T)	
	Dried leaves (75 g/140 min)	3.1	4-year-old: Thymol (55.2%), (*E*)-caryophyllene (9.9%), thymol methyl ether (7.8%), and *p*-cymene (5.8%) (T)	Santos et al. (2016a)
Tomar do Geru, Sergipe (genotype 107)		1.7	Rainy season, 1-year-old: Carvacrol (43.2%), γ-terpinene (13.5%), *p*-cymene (11.5%), and (*E*)-caryophyllene (6.2%) (C)	Cruz et al. (2014)
		2.0	Dry season: Carvacrol (43.7%), γ-terpinene (13.7%), *p*-cymene (12.9%), and thymol methyl ether (5.4%) (C)	
		2.0	With irrigation: Carvacrol (43.8%), γ-terpinene (13.7%), *p*-cymene (12.9%), and thymol methyl ether (5.4%) (C)	
		2.3	Without irrigation: Carvacrol (46.0%), γ-terpinene (13.1%), *p*-cymene (8.9%), (*E*)-caryophyllene (6.3%), and thymol (5.0%) (C)	
		2.5	4-year-old: Carvacrol (38.0%), γ-terpinene (16.1%), *p*-cymene (11.9%), and (*E*)-caryophyllene (6.1%) (C)	Santos et al. (2016a)

(continued)

Table 2.1 (continued)

Geographical origin	Extraction conditions	Yield (%)	Major compounds (GC peak area ≥5.0%)	References
Tomar do Geru, Sergipe (genotype 108)		1.6	Rainy season, 1-year-old: Carvacrol (47.1%), *p*-cymene (11.7%), γ-terpinene (8.8%), and thymol methyl ether (5.9%) (C)	Cruz et al. (2014)
		2.2	Dry season: Carvacrol (44.0%), γ-terpinene (9.9%), *p*-cymene (12.5%), and thymol methyl ether (7.0%) (C)	
		2.2	With irrigation: Carvacrol (43.9%), *p*-cymene (12.1%), γ-terpinene (11.5%), and thymol methyl ether (6.2%) (C)	
		2.3	Without irrigation: Carvacrol (45.0%), *p*-cymene (11.2%), γ-terpinene (9.6%), and thymol methyl ether (6.2%) (C)	
		3.2	4-year-old: Carvacrol (39.7%), *p*-cymene (12.7%), γ-terpinene (11.0%), and thymol methyl ether (6.0%) (C)	Santos et al. (2016a)
Tomar do Geru, Sergipe (genotype 109)		1.3	Rainy season, 1-year-old: Carvacrol (49.0%), *p*-cymene (13.0%), γ-terpinene (8.5%), and (*E*)-caryophyllene (7.8%) (C)	Cruz et al. (2014)
		1.9	Dry season: Carvacrol (48.3%), γ-terpinene (10.5%), *p*-cymene (13.3%), and thymol methyl ether (5.9%) and (*E*)-caryophyllene (5.3%) (C)	
		1.9	With irrigation: Carvacrol (48.3%), *p*-cymene (13.3%), γ-terpinene (10.5%), and thymol methyl ether (5.7%) (C)	
		2.0	Without irrigation: Carvacrol (51.3%), *p*-cymene (11.0%), γ-terpinene (8.5%), (*E*)-caryophyllene (6.8%), and thymol methyl ether (5.0%) (C)	
		2.3	4-year-old: Carvacrol (40.4%), *p*-cymene (14.8%), γ-terpinene (10.4%), (*E*)-caryophyllene (7.9%), and thymol methyl ether (5.0%) (C)	Santos et al. (2016a)

(continued)

Table 2.1 (continued)

Geographical origin	Extraction conditions	Yield (%)	Major compounds (GC peak area ≥5.0%)	References
Tomar do Geru, Sergipe (genotype 110)		1.5	Rainy season, 1-year-old: Carvacrol (48.9%), *p*-cymene (12.9%), and γ-terpinene (11.8%) (C)	Cruz et al. (2014)
		2.1	Dry season: Carvacrol (45.4%), γ-terpinene (11.3%), *p*-cymene (14.0%), and thymol methyl ether (6.0%) (C)	
		2.1	With irrigation: Carvacrol (46.9%), *p*-cymene (14.3%), γ-terpinene (11.5%), and thymol methyl ether (5.9%) (C)	
		1.9	Without irrigation: Carvacrol (52.2%), γ-terpinene (11.3%), and *p*-cymene (10.8%) (C)	
		2.6	4-year-old: Carvacrol (43.3%), γ-terpinene (13.1%), and *p*-cymene (12.7%) (C)	Santos et al. (2016a)
Rio Real, Bahia (genotype 201)		1.9	Rainy season, 1-year-old: Carvacrol (35.3%), γ-terpinene (21.1%), *p*-cymene (13.7%), thymol (5.8%), and (*E*)-caryophyllene (6.3%) (C)	Cruz et al. (2014)
		2.4	Dry season: Carvacrol (36.6%), γ-terpinene (19.6%), *p*-cymene (13.2%), thymol (7.0%), and (*E*)-caryophyllene (5.3%) (C)	
		2.9	With irrigation: Carvacrol (36.6%), γ-terpinene (19.5%), *p*-cymene (13.2%), thymol (6.9%), and (*E*)-caryophyllene (5.3%) (C)	
		3.0	Without irrigation: Carvacrol (35.2%), γ-terpinene (20.2%), *p*-cymene (11.8%), thymol (6.2%), and (*E*)-caryophyllene (8.2%) (C)	
		3.3	4-year-old: Carvacrol (28.2%), γ-terpinene (21.8%), *p*-cymene (12.9%), and (*E*)-caryophyllene (7.4%) (C)	Santos et al. (2016a)

(continued)

Table 2.1 (continued)

Geographical origin	Extraction conditions	Yield (%)	Major compounds (GC peak area ≥5.0%)	References
Rio Real, Bahia (genotype 202)		1.5	Rainy season, 1-year-old: Carvacrol (47.3%), *p*-cymene (13.3%), γ-terpinene (12.0%), and thymol methyl ether (5.1%) (C)	Cruz et al. (2014)
		2.7	Dry season: Carvacrol (44.4%), *p*-cymene (12.8%), γ-terpinene (12.5%), and thymol methyl ether (6.0%) (C)	
		2.8	With irrigation: Carvacrol (44.4%), *p*-cymene (12.8%), γ-terpinene (12.5%), and thymol methyl ether (6.0%) (C)	
		2.5	Without irrigation: Carvacrol (46.1%), *p*-cymene (11.9%), γ-terpinene (11.4%), thymol methyl ether (5.1%), and (*E*)-caryophyllene (5.0%) (C)	
		4.7	4-year-old: Carvacrol (34.3%), *p*-cymene (15.7%), γ-terpinene (12.3%), and (*E*)-caryophyllene (5.1%) (C)	Santos et al. (2016a)
Olindina, Bahia	Fresh leaves (1 kg/1 h)	1.2	Carvacrol (38.4%), *p*-cymene (14.5%), γ-terpinene (10.6%), thymol (8.0%), thymol methyl ether (6.0%), and (*E*)-caryophyllene (5.0%) (C)	Matos et al. (2000)

BAG-UFS: Active Germplasm Bank, Federal University of Sergipe, maintained at the Experimental Farm Rural Campus, located in São Cristóvão municipality, Sergipe State, Brazil; (C), carvacrol chemotype; (T), thymol chemotype; (CT), carvacrol/thymol chemotype. [a]Identified as α-terpinene in the reference cited, but this identification was a mistake based on the retention index value; therefore, it must be γ-terpinene. [b]yield (mL/plant).

plasm collection of *L. gracilis* is genetically diverse. Ragagnin et al. (2014) evaluated the effect of salt stress on chemical composition of the essential oil of *L. gracilis* and found that there was no effect on the yield or the concentration of the prevalent constituents of the essential oil (thymol, carvacrol, and *p*-cymene). Cruz et al. (2014) studied the influence that irrigation and seasonality have on chemical composition of the essential oil of *L. gracilis* genotypes. Their results showed a little variation of the chemical compounds among different climatic conditions, except for genotype 106, which was rich in thymol. In addition, it was suggested that the existence of two chemotypes, one with a high thymol content (T chemotype) and one with a high carvacrol content (C chemotype), remained constant, regardless of the harvest season and irrigation. They concluded that *L. gracilis* can be cultivated with or without irrigation and harvested in the rainy and dry seasons, with little chemical variability. Bitu et al. (2015) studied the envi-

ronmental factors, such as circadian rhythm (collection time), that affect the yield and chemical composition of the essential oil from fresh and dried leaves of *L. gracilis* growing in the municipality of Crato, in the semiarid region of Ceará state, Northeast Brazil. The results showed that different times of collection qualitatively and quantitatively affect the major components in essential oil from fresh leaves. Thymol showed the highest variation in collections throughout the day (3.5–44.4%). Interestingly, methyl cinnamate was observed in increasingly high percentages at 10:00 a.m. (16.7%), 1:00 p.m. (21.0%), and 4:00 p.m. (38.2%) and some monoterpenes, such as *cis*-ocimene (7.2% at 10:00 a.m.) and linalool (8.4% at 4:00 p.m.), which were not detected when leaves were collected in early morning (at 7:00 a.m.). Santos et al. (2016a) evaluated the effect that the harvest time and geographical origin have on the essential oil of seven *L. gracilis* accessions. The essential oil samples exhibited significant variation among accessions only in 4-year-old plants. Except for one accession, which showed thymol as the main compound, the other six accessions presented carvacrol as the major compound. In addition, harvest time reduced the content of carvacrol or thymol in essential oil from all accessions. Thus, the harvest time and geographical origin both qualitatively and quantitatively affected the chemical composition of the essential oil of all accessions. Similar to all monoterpenoids, thymol and carvacrol are derived from the IPP (isopentenyl diphosphate) and DMAPP (dimethylallyl diphosphate), generally by plastidic MEP (methyl-erythritol-4-phosphate) pathway (Ramak et al. 2013). The well-known biosynthetic pathway of thymol and carvacrol from *Thymus vulgaris* by aromatization of γ-terpinene to *p*-cymene followed by hydroxylation of *p*-cymene was stablished by Poulose and Croteau (1978) (Fig. 2.6). However, Crocoll et al. (2010) and Crocoll (2011) demonstrated that the biosynthetic pathway used cytochrome P450 monooxygenases (CYP) to convert γ-terpinene into thymol and carvacrol in oregano and thyme. Furthermore, this study suggested that γ-terpinene is directly converted to thymol and carvacrol with *p*-cymene as a side product because it was not accepted as a substrate (Fig. 2.6). The original pathway prediction suggested two separate oxidation steps (γ-terpinene to *p*-cymene and *p*-cymene to thymol or carvacrol) (Poulose and Croteau 1978). To date, there is no information available on monoterpene biosynthesis in *Lippia* species, but it is established knowledge that thymol from *T. vulgaris* is biosynthesized by the aromatization of γ-terpinene to *p*-cymene, followed by the hydroxylation of *p*-cymene (Kutzner et al. 2014). A deeper knowledge about thymol and carvacrol biosynthesis in *L. gracilis* is important and could prove useful for increasing the yield of essential oil in this plant, especially in secondary metabolism under stress conditions. In addition, other factors, such as the method of extraction, the collection time, and the environmental conditions under which the plants are cultivated, may influence the concentrations of chemical compounds in this species.

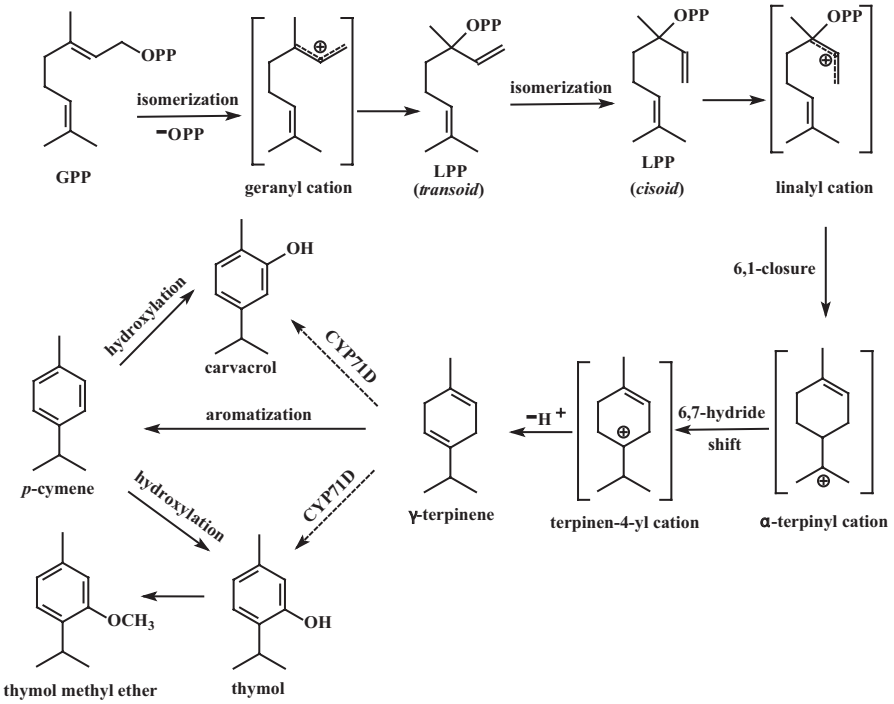


Fig. 2.6 Proposed biosynthetic pathway for the main monoterpenes found in the highest frequency in the essential oils of *Lippia gracilis*. *GPP* geranyl diphosphate, *LPP* linalyl diphosphate. (Adapted from Alonso and Croteau 1991; Nhu-Trang et al. 2006; Crocoll et al. 2010; Crocoll 2011)

2.3 Biological Properties of *Lippia gracilis*

2.3.1 Cytotoxic and Antitumor Properties

Ribeiro et al. (2012) investigated the cytotoxic potential of *L. gracilis* essential oil against tumor cell lines. The results showed that it inhibited 94.9% human melanoma (MDA-MB-435) and 95.1% colon carcinoma (HCT-8) tumor cell lines with IC_{50} values lower than 5 μg/ml for both cell lines. Similarly, the cytotoxic effect of the essential oils extracted from leaves of the three genotypes of *L. gracilis* (LGRA-106, LGRA-109, and LGRA-201) was investigated against tumor cell lines, murine melanoma (B16), human cervical carcinoma (HeLa), and human breast carcinoma (MCF-7), as well as normal cell lines, embryonic mouse fibroblasts (3T3), and human lung fibroblasts (MRC5). The results showed the cytotoxic activity in both normal and tumor cells at concentrations lower than 100 μg/ml. The major compound found in LGRA-106 essential oil was thymol (40.5%), whereas LGRA-109 and LGRA-201 contained 45.8% and 32.6% carvacrol, respectively (Melo et al. 2014). Moreover, the essential oil from the leaves of *L. gracilis* caused G_1 arrest in HepG2 cells

accompanied by the induction of inter-nucleosomal DNA fragmentation without affecting the cell membrane integrity. Cell morphology consistent with apoptosis and a remarkable activation of caspase-3 were also observed, which suggested an induction of caspase-dependent apoptotic cell death. In vivo antitumor study showed tumor growth inhibition rates of 38.5–41.9% in sarcoma 180-bearing mice (Ferraz et al. 2013).

2.3.2 Anti-inflammatory, Antinociceptive, and Antioxidant Activities

Anti-inflammatory and antinociceptive activities of the essential oil from the leaves of *L. gracilis* have been reported, with an inhibition of paw edema and carrageenan-induced cell migration observed as indicators of anti-inflammatory response, and the antinociceptive activity of the essential oil was evaluated by writhing test (Mendes et al. 2010). The essential oil inhibited the inflammatory process induced by subcutaneous carrageenan injection, thereby reducing cell migration, exudate volume, extravasated protein, and inflammatory mediators (nitric oxide, prostaglandin E2, TNF-α, and IFN). These results showed that the essential oil of *L. gracilis* can inhibit the inflammatory responses by blocking the nitric oxide pathway. It can also attenuate hypernociception by blocking the opioid and cholinergic systems (Guilhon et al. 2011). In addition, Guimarães et al. (2012) reported that the oral administration of methanol extract from the leaves of *L. gracilis* reduced both the number of writhes in the writhing test and the time of paw licks in both phases of the formalin test. An anti-inflammatory effect on peritonitis induced by carrageenan was also observed. The essential oil from the leaves of *L. gracilis* was able to scavenge the DPPH radical (Franco et al. 2014).

2.3.3 Antimicrobial Effect

The antifungal activity of the essential oil of *L. gracilis* was evaluated against *Trichophyton rubrum*, *Cladosporium sphaerospermum*, *C. cladosporioides*, *Geotrichum candidum*, *Trichoderma viride*, *Torula herbarum*, *Paecilomyces* sp., *Fusicoccum* sp., *Curvularia lunata*, *Aspergillus nidulans*, *A. flavus*, and *A. niger* by several investigators (Albuquerque et al. 2006; Melo et al. 2013; Franco et al. 2014). Similarly, antibacterial activity was evaluated against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *S. epidermidis*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella choleraceuis-diarizonae*, *Enterobacter asburiae*, *E. hormaechei*, *Bacillus thuringiensis*, *B. cereus*, and *Klebsiella pneumoniae* (Pessoa et al. 2005; Albuquerque et al. 2006; Neto et al. 2010; Dantas et al. 2010; Bitu et al. 2012). In addition, 5% solution of the essential oil of *L. gracilis* under in vivo conditions showed the antibacterial activity in diabetic rats (Neto et al. 2010). However, the amebicidal activity of the essential oil of *L. gracilis* against *Acanthamoeba polyphaga* trophozoites was also reported (Santos et al. 2016c).

2.3.4 Larvicidal, Molluscicidal, and Acaricidal Effects

The larvicidal effect of the essential oil of the leaves of *L. gracilis* was investigated against larvae of *Aedes aegypti* between the third and fourth stages. The lethal concentrations of 50% (LC_{50}) were reported after 24 h of exposure at 98 ppm (Silva et al. 2008). Similarly, the LC_{50} in larvae of *A. aegypti* in the third stage was 26.3 μg/ml (Santiago et al. 2006), 56.2 μg/ml (Lima et al. 2008), and 282 μg/ml (Dias et al. 2015). The molluscicidal activity against *Biomphalaria glabrata* and the toxicity to brine shrimps (*Artemia salina*) were also investigated for the essential oil from the leaves of *L. gracilis*. The value LC_{50} was 62.2 ppm for *B. glabrata* and 23.6 ppm for *A. salina* (Lima et al. 2008; Teles et al. 2010). Cruz et al. (2013) investigated the acaricidal effect against *Rhipicephalus* (*Boophilus*) *microplus* larvae and engorged females. They reported the LC_{50} values (1.31 and 4.66 mg/ml) for the essential oils against larvae and engorged females for genotypes LGRA-201 and LGRA-106. Similarly, Chagas et al. (2016) found a LC_{50} of 3.21 mg/ml for the essential oil isolated from the leaves of *L. gracilis* against larvae. Moreover, Costa-Júnior et al. (2016) compared the acaricidal effects of essential oils extracted from two different genotypes (106 and 201) of *L. gracilis* in organophosphate-resistant and organophosphate-susceptible strains of *R.* (*B.*) *microplus*. The essential oils of both genotypes were more effective against the organophosphate-resistant strain than the organophosphate-susceptible strain.

2.4 Conclusions and Future Prospects

The nonvolatile constituents of *L. gracilis*, including flavone and flavanones, and the major volatile compounds found in the highest frequency in essential oils are carvacrol, *p*-cymene, γ-terpinene, thymol, and thymol methyl ether. In addition, at least three different chemotypes are found, the thymol type (T), carvacrol type (C), and intermediate type (CT), which combines carvacrol and thymol in high percentages. Biological studies of *L. gracilis* showed the cytotoxic, antitumor, anti-inflammatory, antinociceptive, antioxidant, antibacterial, fungicidal, leishmanicidal, amebicidal, larvicidal, molluscicidal, and acaricidal effects. Thus, *L. gracilis* could be a potential candidate for the development of new drugs against a vast range of diseases.

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Chapter 3
Use of Indian Indigenous Fruits in Cancer Prevention and Treatment

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3.1 Introduction

Cancer is one of the leading causes of death and accounted for approximately 8 million deaths in 2008 globally (Ferlay et al. 2010). Realistic appraisals are that by the year 2020, the incidence of cancer is expected to rise threefold, and their number and deaths are likely to increase disproportionately in developing countries, where the resources to treat the disease burden are minimal (Are et al. 2010). Epidemiological and experimental studies conducted in the past have established that the interaction between biological or environmental factors and genetic predisposition directly results in cancer (Lichtenstein et al. 2000; Mucci et al. 2001; Irigaray et al. 2007; Raffelsbauer 2012) and that factors like diet, obesity, smoking, infections, radiation, stress, lack of physical activity, and environmental pollutants substantially contribute to the initiation and promotion of carcinogenesis in most organs in humans (Irigaray et al. 2007; Raffelsbauer 2012; Anand et al. 2008). It can therefore be inferred that avoiding exposure to cancer-causing physical, chemical, and biological agents, performing regular physical activities, and consuming a healthy diet are the optimum means of evading the risk of cancer (Anand et al. 2008).

The rate of occurrence of cancer among humans was largely divergent between countries and also among the individuals who had a change in their diet habits and patterns when they migrated to foreign countries (Baliga et al. 2009) and association between lifestyle, including diet, and cancer risk has been compiled and presented by the World Cancer Research Fund and the International Association of Cancer Registries in 2007 (Raffelsbauer 2012). Epidemiological and preclinical studies have indicated that an increased consumption of fruits, vegetables, and

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grains is simpler and practical means of reducing cancer incidence significantly (Anand et al. 2008; Baliga et al. 2009). The beneficial role of fruits and vegetables in preventing cancer was further substantiated by reports, where individuals consuming Mediterranean and Okinawa diet (mainly comprised of fruits and vegetables) had reduced incidence of cancer (Willcox et al. 2009; Colomer and Menéndez 2006). Furthermore, a meta-analysis of case-control studies reported that consumption of fruits reduced the risk of esophagus, lung, stomach, bladder, and colorectal cancers with a significant reduction in the risk of lung and bladder cancer (Riboli and Norat 2003). Worldwide data suggests that the diet to cancer ratio in the Western and developing countries was approximately 30% and 20%, respectively, making it the second most preventable cause of cancer after tobacco abstinence.

India is one of the world's largest biodiverse regions and is a home for a wide variety of fruiting trees. The trees indigenous to India are of great diversity which represents 344 species of fruits including *Mangifera indica* (family Anacardiaceae), *Emblica officinalis* (family Phyllanthaceae), *Eugenia jambolana* (*Syzygium cumini* or *Syzygium jambolana* or *Eugenia cuminii*) (family Myrtaceae), and *Aegle marmelos* (family Rutaceae). Although these fruits are originally indigenous to India, they can be found growing in various tropical and subtropical regions of the world and have evolved over centuries and substantially contributed to human civilizations. The fruits of wild trees are regularly consumed as part of the diet worldwide, and the excess are sold, processed into pickles, murabbas, jams, and juices. Another beneficial use of fruits is in the traditional Indian medicine system of Ayurveda and various folk systems of medicine, where these fruits are used either alone or in combination with other plants to treat various ailments. Various scientific studies have validated the ethnomedicinal use of these indigenous fruits as an anticancer agent. The present chapter emphasizes on the usefulness of these fruits in cancer prevention/treatment and also on the underlying mechanisms mediated by the phytochemicals present in these fruits.

3.2 Ethnomedicinal Use of Indian Indigenous Fruits as Anticancer Agents

3.2.1 Mangifera indica

Mangifera indica L., commonly called as mango, is often referred to as the "king of fruits" in the tropical countries (Shah et al. 2010). Mango is one of the most important indigenous fruits of India and also the choicest fruit crops of Asia and the tropical and subtropical regions of the world (Morton 1987). Although originally indigenous to Eastern parts of India, Burma, and the Andaman Islands, mango is currently found cultivated/growing in Pakistan, Sri Lanka, Malaysia, Australia, America, and Africa (Shah et al. 2010). The fruiting of the tree is annual. Mango is oval in shape with a slightly compressed fleshy drupe, approximately 8–12 (max. 30) cm long and is attached at the broadest end on a pendulous stalk. The color of

the mango skin is usually green, yellow, or tinge of red. The underlying flesh is yellow-orange in color and is found in varying quality including soft, sweet, juicy, and fiber-free. Some of the varieties are of high quality, turpentine flavored, and fibrous in wild seedlings (Shah et al. 2010). Mangoes are either eaten raw, used to prepare dessert and fruit salad, or used in dry cereal, gelatin, custards, or ice cream. Mango pulp is canned in syrup and made into jam, marmalade, jelly, or nectar; green mangoes are usually used to prepare chutneys and pickles (Morton 1987). Some of the varieties of mango which have evolved in India include Alphonso (Hapus), Bangalora (Totapuri), Banganapalli (Baneshan, Safeda), Bombai (Malda), Bombay Green, Dashehari, Fernandin, Kesar, Kishen Bhog, Langra, Mankur, Mulgoa, Neelum, Samar Bahisht Chausa, Suvernarekha, Vanraj, Aakhurasa, Sundari, Mallika, Mundappa, Himsagar, Kanchakhai, and Godabari, with hybrids such as Malika (Neelum × Dashehari), Ratna (Neelum × Alphonso), Sindhu (Ratna × Alphonso), Arka Aruna (Banganapalli × Alphonso), and Arka Neelkiran (Alphonso × Neelum). In addition to these, the exotic varieties available in India are Fazli, Suvarnarekha, Gulab Khas, Langra, and Alphonso in Pakistan; Embrapa Roxa 141 and Embrapa Alfa 142 in Brazil; Haden, Irwin, Tommy Atkins, and Keitt in USA; and Julie Peter in West Indies (Morton 1987).

Phytochemical analysis has indicated that mango contains many vitamins including ascorbic acid, thiamine, riboflavin, and niacin (Shah et al. 2010). It also contains the pigment carotenoids like provitamin A, lutein, and β-carotene; polyphenols like gallic acid, caffeic acid, quercetin, kaempferol, catechins, tannins, xanthone and mangiferin, isomangiferin, tannins, gallic acid derivatives, and lupeol; and certain high-molecular-weight gallotannins. Furthermore, the pulp of mango contains omega-3 and omega-6 polyunsaturated fatty acids and an unusual fatty acid, cis-9, cis-15-octadecadienoic acid (Shah et al. 2010). Some of the chemicals present in mango are represented in Fig. 3.1. The use of mango for the treatment of a variety of medical conditions has been in practice since time immemorial. Mango has been reported to be beneficial against a wide array of medical conditions: dentifrice, antiseptic, astringent, diaphoretic, stomachic, vermifuge, tonic, and constipation (Morton 1987; Shah et al. 2010). Mango is also a diuretic and is used to treat diarrhea, dysentery, anemia, asthma, bronchitis, cough, hypertension, insomnia, rheumatism, toothache, leucorrhoea, hemorrhage and piles, abscesses, stings, heat stroke, miscarriage, anthrax, blisters, tympanitis, colic, glossitis, indigestion, bacillosis, bloody dysentery, liver disorders, excessive urination, tetanus, and wounds in the mouth (Shah et al. 2010).

The anticarcinogenic property of polyphenols extracted from various varieties of mango (Francis, Kent, Ataulfo, Tommy Atkins, and Haden) was studied by Noratto and colleagues on cultured cancer cell lines: Molt-4 leukemia, A-549 lung, MDA-MB-231 breast, LNCap prostate, and SW-480 colon cancer cells and the noncancer colon cell line CCD-18Co (Noratto et al. 2010). The results showed that the equivalent concentrations of extract did not affect the growth of noncancer colonic myofibroblast CCD-18Co cells indicating its selective action against cancer cells. The highest effect of chemoprevention of the Ataulfo and Haden varieties of mango was mediated by the ability of the polyphenols present in these varieties to increase

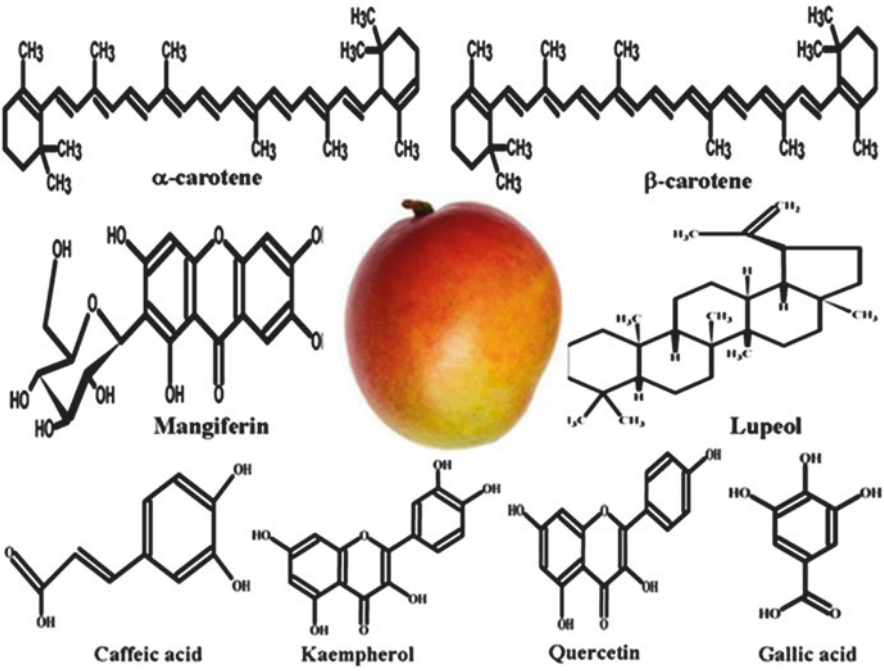

Fig. 3.1 Mango fruit and some important phytochemicals

the mRNA expression of pro-apoptotic biomarkers and cell cycle regulators, arresting cell cycle, and decreasing the generation of reactive oxygen species (Noratto et al. 2010). Mango juice possesses certain anticancer properties where it has been reported to reduce the number of transformed foci in the neoplastic transformation assay with BALB/3T3 cells and also arrest the HL-60 cells in the G(0)/G(1) phase (Percival et al. 2006). In addition, series of studies by Prasad and colleagues demonstrated that mango juice and one of its active constituents, lupeol, were able to initiate apoptosis in the prostate of Swiss albino mice and also in LNCaP cells by modulating the cell growth regulators and reducing androgen (Prasad et al. 2008a, b) and DMBA-induced oxidative stress (Prasad et al. 2007).

Mangiferin, a principal constituent of mango, is essentially a polyphenolic antioxidant and a glucosyl xanthone and possesses antioxidant, anti-lipid peroxidation, antioxidant, antiviral, immunomodulation, cardiotonic, hypotensive wound healing, antidegenerative, and antidiabetic activities (Matkowski et al. 2013). Preclinical studies on mice have shown the protective effects of mangiferin against chemical-induced bowel carcinogenesis (Yoshimi et al. 2001), lung carcinogenesis (Rajendran et al. 2008a, b, c, d), ultraviolet light-induced skin damage (Petrova et al. 2011), and deleterious effects of ionizing radiation (Jagetia and Baliga 2005). In addition, mangiferin demonstrated the attenuation of radiation-induced DNA damage on cultured human peripheral blood lymphocytes and normal human intestinal epithelial cells (Jagetia and Venkatesha 2005; Lei et al. 2012). Experiments on cultured HeLa,

HT29, and MCF7 cells have shown that mangiferin when combined with oxaliplatin increased cell death and apoptosis which mediate protection of DNA against radiation damage by downregulating NFκB (du Plessis-Stoman et al. 2011). Studies assessing the mechanism of action of mangiferin have reported that treating HL-60 cells with mangiferin resulted in a concentration and time-dependent increase in the cell cytotoxicity and arrested G2/M phase by enhancing the expression levels of CDC2 mRNA and cyclin B1 mRNA (Yao et al. 2010). Some of the other phytochemicals present in mango which have been shown to possess anticancer properties include carotenoids (Carranco et al. 2011; Tanaka et al. 2012; Sharoni et al. 2012), lupeol (Chaturvedi et al. 2008; Saleem 2009; Siddique and Saleem 2011), gallic acid (Li et al. 2003; Ow and Stupans 2003; Heber 2008), caffeic acid (Touaibia et al. 2011), quercetin (Gibellini et al. 2011; Mendoza and Burd 2011; Dajas 2012), and kaempferol (Chen and Chen 2013).

3.2.2 Emblica officinalis *Gaertn. or* Phyllanthus emblica *Linn*

Commonly known as Indian gooseberry or amla, *Emblica officinalis* Gaertn or *Phyllanthus emblica* Linn is an important plant of medicinal value and is extensively used in Ayurveda and other traditional and folk medicine systems (Baliga and Dsouza 2011). It has been used as a vegetable to prepare various cuisines and is also used to prepare pickle, chutneys, murabba, and fresh juice and is marketed as juice concentrates (Baliga and Dsouza 2011). Amla belongs to the family Euphorbiaceae which is native to India and is also found in Pakistan, Uzbekistan, Sri Lanka, Southeast Asia, China, and Malaysia (Baliga and Dsouza 2011). The fruits of amla are fleshy and smooth striated, yellowish green in color and globular in shape, and contain obovate-obtusely triangular six-celled nut. The four main varieties of amla found mostly in the forest regions include Banarasi, Francis (Hathijhool), Chakaiya, and wild Himalayan. Recent advancements in the field of horticulture have resulted in the development of certain hybrids, namely, Kanchan (NA4), Krishna (NA5), NA6, NA7, NA 10, and BSR-1, 2, and 3. These newer varieties of amla are high yielding and fruit yearly; as a result of which they are cultivated and propagated regularly (Baliga and Dsouza 2011).

Phytochemical analysis has indicated amla to be a rich source for vitamin A and also contains gallic acid, ellagic acid, chebulinic acid, chebulagic acid, emblicanin-A, emblicanin-B, punigluconin, pedunculagin, citric acid, ellagitannin, trigallayl glucose, pectin, corilagin, isostrictiniin, quercetin, kaempferol 3-O-alpha L (6″ methyl) rhamnopyranoside, and kaempferol 3-O-alpha L (6″ ethyl) rhamnopyranoside (Baliga and Dsouza 2011). Some of the chemicals present in amla are represented in Fig. 3.2.

Preclinical studies on amla have established various pharmacological properties, which include antibacterial, antifungal, antiviral, antidiabetic, hypolipidemic, antiulcerogenic, free radical scavenging, antioxidant, antimutagenic, anti-inflammatory, immunomodulatory, antipyretic, analgesic, antitussive, antiatherogenic, adaptogenic,

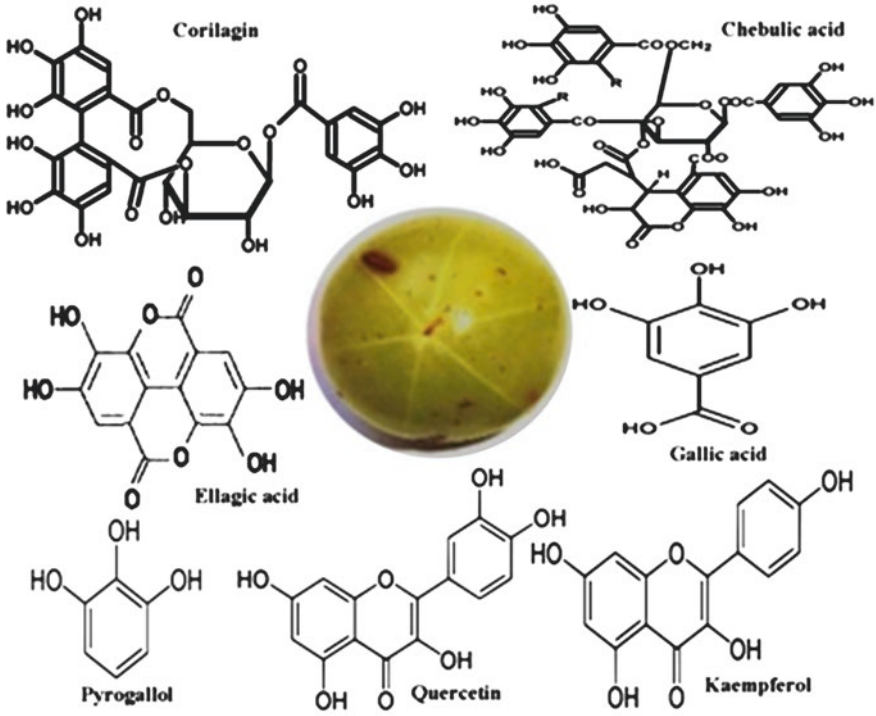

Fig. 3.2 Amla fruit and some important phytochemicals

snake venom neutralizing, gastroprotective, antianemia, anti-hypercholesterolemia, wound healing, antidiarrheal, antiatherosclerotic, nephroprotective, and neuroprotective properties (Baliga and Dsouza 2011). Additionally the phytochemicals kaempferol (Chen and Chen 2013), quercetin (Dajas 2012; Mendoza and Burd 2011; Gibellini et al. 2011), ellagic acid (Bell and Hawthorne 2008; Nepka et al. 1999), and gallic acid (Heber 2008; Li et al. 2003; Ow and Stupans 2003; Baliga and Dsouza 2011) have been shown to possess beneficial effects.

Cytotoxic properties of amla have been demonstrated on various cell lines such as L 929 (Jose et al. 2001); B-lymphoid Raji, T-lymphoid Jurkat, erythroleukemic HEL, and erythromyeloid K562 (Khan et al. 2002); MCF7 and MDA-MB-231 (Lambertini et al. 2004); hepatocellular carcinoma (HepG2); and lung carcinoma (A549) cells (Pinmai et al. 2008). In addition, amla has been reported to induce apoptosis in Dalton's lymphoma ascites and CeHa cell lines (Rajeshkumar et al. 2003). Additionally studies have also shown that amla did not exhibit cytotoxicity on Chinese hamster ovary cell lines suggesting it possess selective cytotoxic properties against the neoplastic cells (Sumantran et al. 2007). To substantiate the cell culture studies, experiments with laboratory mice with Dalton's lymphoma have shown that administering amla was effective in reducing the growth of solid tumor and in increasing the survival time (Jose et al. 2001). In addition to the whole extract,

experiments have also shown that the phytochemicals in amla such as norsesquiterpenoids, phenolic compounds, and proanthocyanidin polymers have shown in vitro antiproliferative effects in B16F10, HeLa, and MK-1 cells (Zhang et al. 2004). Furthermore, antiproliferative properties of pyrogallol, a catechin of amla, were observed in human lung cancer cell lines H441 (lung adenocarcinoma) and H520 (lung squamous cell carcinoma) which were mediated by apoptosis that inhibited cell growth (Yang et al. 2009).

Studies conducted in the past have reported amla to possess chemopreventive properties, where amla inhibited chemical-induced skin carcinogenesis (Sancheti et al. 2005), hepatocarcinogenesis (Rajeshkumar et al. 2003; Sultana et al. 2004, 2008; Chen et al. 2011) and oral carcinogenesis (Krishnaveni and Mirunalini. 2012). In addition, in vivo studies have demonstrated that amla prevents DNA damage induced by several genotoxic agents including lead, aluminum (Dhir et al. 1990), nickel (Dhir et al. 1991), cesium chloride (Ghosh et al. 1992), arsenic (Biswas et al. 1998), cadmium (Khandelwal et al. 2002), chromium (Sai et al. 2003), 3,4-benzo(a) pyrene (Nandi et al. 1997), benzo(a) pyrene (Sharma et al. 2000), and 7,12-dimethylbenz(a) anthracene (Banu et al. 2004). Cumulatively, these observations clearly indicate the usefulness of amla as an antimutagenic and anticarcinogenic agent and validate their regular use for health benefits.

Effective protection of the cardiac myoblasts H9c2 cells when cytotoxicity was induced by doxorubicin was observed after treatment with amla extract in laboratory animals (Wattanapitayakul et al. 2005). Similarly, protection of human hepatocellular carcinoma (HepG2) and lung carcinoma (A549) cells against doxorubicin- and cisplatin-induced cytotoxicity was observed in laboratory animals after they were given amla extract (Pinmai et al. 2008). Furthermore, results from in vivo analyses showed amla to be effective against radiation-induced ill effects (Hari et al. 2004; Singh et al. 2005; Jindal et al. 2009) and cyclophosphamide-induced suppression of humoral immunity (Haque et al. 2001) and DNA damage (Sharma et al. 2000). Based on these findings, amla can be regarded as a fruit with selective antineoplastic, chemopreventive, and radioprotective properties.

3.2.3 Eugenia jambolana *Lam*

Colloquially known as Malabar plum, black plum or jamun, *Eugenia jambolana* Lam. (Syn. *Syzygium cumini* Skeels or *Syzygium jambolana* DC or *Eugenia cuminii* Druce) belongs to the family Myrtaceae (Baliga et al. 2011a; Baliga 2011). This evergreen tree is famous for its fruits and is native to India and is today also found growing in Asia, Eastern Africa, South America, and parts of the Unites States including Florida and Hawaii (Baliga et al. 2011a; Baliga 2011). Morphological and organoleptic features have resulted in two main morphotypes which are found in the Indian subcontinent, namely, *kaatha jamun* and *ras jaman* (Baliga et al. 2011a; Baliga 2011). The former variety is small and acidic to taste, while the latter is

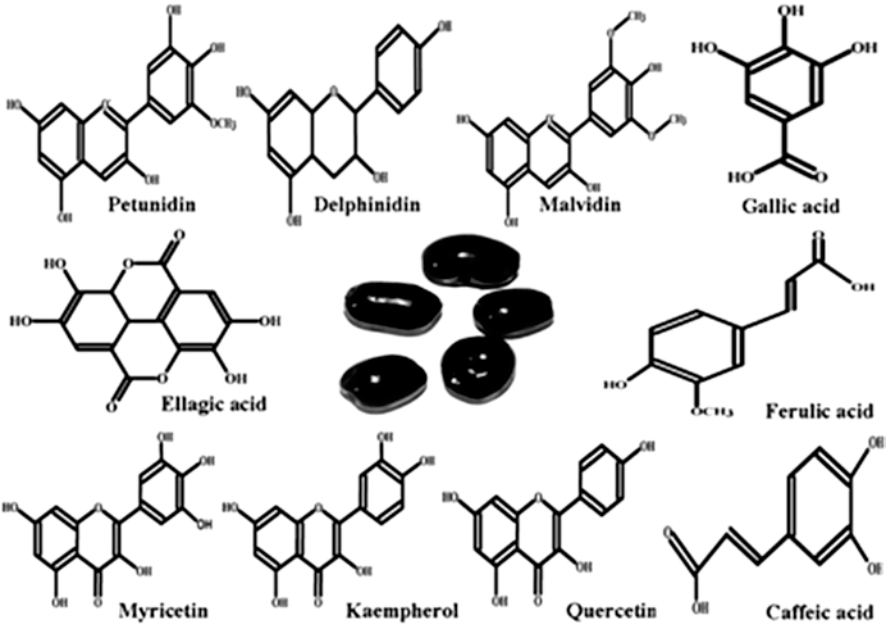

Fig. 3.3 Jamun fruit and some important phytochemicals

oblong, dark-purple or bluish, with pink, sweet fleshy pulp and small seeds (Baliga et al. 2011a; Baliga 2011).

The ripe jamun fruits are used in preparing squashes, jellies, health drinks, and wine and also for making preservatives (Baliga et al. 2011a; Baliga 2011). It is the only the berry which is reported to contain five anthocyanins – malvidin, petunidin, delphinidin, cyanidin, and peonidin – and considerable amounts of ellagic acid/ellagitannins (Aqil et al. 2012). Gallic acid, ellagic acid, corilagin, quercetin, and β-sitosterol are some of the other phytochemicals found in jamun (Baliga et al. 2011a; Baliga 2011). Some of the phytochemicals present in jamun are represented in Fig. 3.3. From the perspective of medicinal use, all parts of the jamun tree have been reported to be useful with the seed especially being one of the most useful agents in the treatment of diabetes mellitus (Baliga et al. 2011a; Baliga 2011). In addition, various pharmacological properties such as antibacterial, antifungal, antiviral, antiulcerogenic, cardioprotective, antiallergic, hepatoprotective, and antidiarrheal effects have been demonstrated in experimental animals following administration of jamun extracts (Baliga et al. 2011a; Baliga 2011). With respect to the use of jamun in cancer treatment, experiments with human cervical cancer cell lines-HeLa (HPV-18) and SiHa (HPV-16) have shown that the crude and methanolic extracts of jamun pulp induced a time-dependent increase in apoptosis (Barh and Viswanathan 2008). Another study by Li and colleagues showed that equivalent concentrations of jamun fruit extracts induced apoptosis and inhibited the

proliferation of both estrogen-independent (MDA-MB-231) and estrogen-dependent/aromatase positive (MCF-7aro) breast cell lines effectively (Li et al. 2009). However, the fruit extract of jamun did not affect the non-mutagenic and normal (MCF-10A) breast cell lines (Li et al. 2009). The most recent work on cell lines was done by Aqil and colleagues, where the antiproliferative property of jamun was tested on human lung cancer A549 cells (Aqil et al. 2012). In their study, Aqil and colleagues showed that the hydrolyzed pulp and seed extracts of jamun possess potent antiproliferative activity, which was mediated by the anthocyanidins and ellagic acid/ellagitannins present particularly in the hydrolyzed extracts (Aqil et al. 2012). Furthermore, jamun has also been reported to possess radioprotective (Jagetia and Baliga 2002; Jagetia and Baliga 2003, 2005a) and cancer prevention effects in experimentally induced skin (Parmar et al. 2010, 2011) and gastric carcinogenesis (Goyal et al. 2010) in mice.

The phytochemicals present in jamun play a crucial role in the anticancer properties of jamun. Ellagitannin and its colonic metabolite, urolithin A, have been reported to inhibit Wnt signaling, an integral process in colon carcinogenesis (Sharma et al. 2010) in addition to inhibition of the proliferation of colon cancer cells mediated by cell cycle arrest and modulation of MAPK signaling (Larrosa et al. 2006; González-Sarrias et al. 2010). A combination of suboptimal concentrations of equimolar anthocyanidins, namely, cyanidin, malvidin, peonidin, petunidin, and delphinidin, has shown to inhibit the growth of two aggressive non-small-cell lung cancer cell lines (NSCLC and H1299) synergistically causing negligible effects on the viability of non-tumorigenic cells (Kausar et al. 2012). The anticarcinogenic effect of a combination of anthocyanins was more effective than individual components, whereby greater cell cycle arrest and apoptosis and substantial suppression of the cell invasion and migration of NSCLC (Kausar et al. 2012). Mechanistic studies have indicated that the protective abilities of jamun were mediated by altering the oncogenic Notch and Wnt pathways and their downstream targets including β-catenin, c-myc, cyclin D1, cyclin B1, pERK, MMP9, and VEGF proteins, enhancing cleavage of the apoptotic mediators Bcl2 and PARP and also enhancing the inhibition of TNFα-induced NF-κB activation (Kausar et al. 2012). Inhibition of the growth of H1299 xenografts in nude mice following the administration of delphinidin was also reported by Kausar and colleagues, indicating the benefits of jamun (Kausar et al. 2012).

Several in vitro studies using cell lines and animal models have reported the antineoplastic effects of various phytochemicals present in jamun, namely, oleanolic acid, ellagic acids, isoquercetin, quercetin, kaempferol, myricetin, gallic acid, ellagic acid, betulinic acid, β-sitosterol, ellagitannin, delphinidin, petunidin, and malvidin (Heber 2008; Li et al. 2003; Ow and Stupans 2003; Touaibia et al. 2011; Dajas 2012; Mendoza and Burd 2011; Gibellini et al. 2011; Chen and Chen 2013; Bell and Hawthorne 2008; Nepka et al. 1999; Baliga et al. 2013a; Barone et al. 2009; Srinivasan et al. 2007; Kocic et al. 2011; Wang and Stoner 2008; Fimognari et al. 2008; Hou 2003). The presence of these compounds in the fruits might have contributed to the observed beneficial effects of jamun.

3.2.4 Garcinia indica *Choisy*

Garcinia indica Choisy Syn *Brindonia indica* is commonly called as kokum and belongs to the Guttiferae family. The plants are native to certain regions of India, and the trees fruit annually in summer during March to May. Kokum fruits are generally round, oblong, or oval with pointed tips and are crowned by the four parted stalkless stigma. The green raw fruits turn red to dark purple when they ripe fully and grow up to the size of a golf ball (Baliga et al. 2011b). Kokum is used to prepare chutneys, pickles, consumed as a cooling drink, and also used as an acidulant in curries. It is extensively used in culinary by the people of Maharashtra, Goa, and the coastal regions of Karnataka. The use of kokum in cooking is mainly to remove the unpleasant smell of mackerel and sardines or as an after meal digestive drink (Baliga et al. 2011b).

Kokum rinds have been reported to contain the highest concentration of anthocyanins including the cyanidin-3-glucoside and cyanidin-3-sambubioside (Baliga et al. 2011b). In addition, the rinds contain two polyisoprenylated phenolics garcinol and isogarcinol, (−) hydroxycitric acid, lactone, and citric acid (Baliga et al. 2011b). Some of the phytochemicals present in kokum are represented in Fig. 3.4. Various preclinical studies have shown that these phytochemicals possess

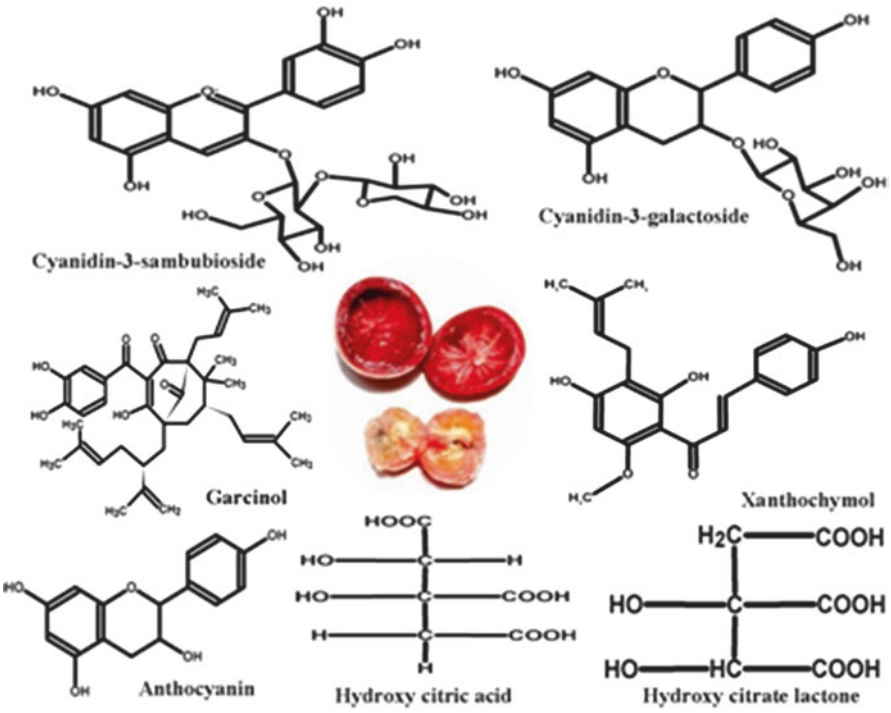

Fig. 3.4 Kokum fruit and some important phytochemicals

antibacterial, antifungal, antiulcerogenic, cardioprotective, antidiabetic, free radical scavenging, antioxidant, and anti-obesity effects (Baliga et al. 2011b).

The phytochemicals such as garcinol, isogarcinol, and xanthochymol have shown to exhibit anticancer properties which are mediated by affecting the growth and proliferation of neoplastic cells (Matsumoto et al. 2003). Of these, garcinol has shown to be an effective neoplastic agent against various human cell lines including human leukemia HL-60 cells (Pan et al. 2001); HeLa cells (Balasubramanyam et al. 2004); human breast cancer cells MCF-7, MDA-MB-231, and BT-549 (Ahmad et al. 2010, 2012a, b); hepatocellular carcinoma Hep3B cells (Cheng et al. 2010); human colorectal cancer cell line HT-29 (Liao et al. 2005); human prostate cells LNCaP, C4-2B, and PC3 (Ahmad et al. 2011); and human pancreatic BxPC-3 and PANC-1 cells (Ahmad et al. 2011; Parasramka and Gupta 2011). Furthermore, garcinol exhibited selective cytotoxicity against neoplastic cells only and had no effect against the proliferation of the non-tumorigenic MCF-10A cells when equivalent concentration of garcinol was assessed (Ahmad et al. 2010).The mechanism of action of apoptosis mediated by garcinol has been reported in several studies. Garcinol is known to trigger the release of cytochrome C into the cytosol, decrease the antiapoptotic gene Bcl-2, increase pro-apoptotic genes Bad and Bax, process procaspase-9, activate caspase-3 and caspase-2 and Wnt signaling pathways, degrade PARP and regulate EMT, and inhibit the activation of cell signaling pathways including Src, MAPK/ERK, STAT-3, and PI3K/Akt (Pan et al. 2001; Ahmad et al. 2011, 2012a, b; Cheng et al. 2010; Liao et al. 2005). In addition to these, garcinol is also reported to downregulate NF-κB-regulated genes and concomitantly inhibit constitutive NF-κB activity (Ahmad et al. 2010) and inhibit histone acetyltransferases p300 in both in vitro and in vivo systems (Arif et al. 2009; Mantelingu and Reddy 2007). The latter prohibits histone acetyltransferases activity-dependent chromatin transcription, without affecting the transcription from DNA template (Balasubramanyam et al. 2004). Furthermore, garcinol inhibits cell invasion and decreases the tyrosine phosphorylation of the focal adhesion kinase – the major signaling mediator of integrin-mediated cell-matrix contact-regulated cellular proliferation, migration, and apoptosis of HT-29 (Liao et al. 2005). Garcinol also reduced the levels of MMP-7 protein level and inhibited expression of MMP-7 in IL-1β-induced HT-29 cells (Liao et al. 2005) and to sensitize A549 lung and HeLa cervical carcinoma cells to the cytotoxic effects of ionizing radiation by inhibiting the DSB and NHEJ repair pathways, without affecting the cell cycle checkpoint (Oike et al. 2012).

The chemoprevention property of garcinol was demonstrated by Yoshida and colleagues where garcinol fed rats showed a reduction in 4-NQO-induced oral carcinogenesis (Yoshida et al. 2005). Upon immunohistochemical analysis, Yoshida et al. concluded that the preventive effect of garcinol was mediated by decreasing cell proliferation (BrdU-labeling index and cyclin D1-positive cell ratio) and reducing the levels of cyclooxygenase-2 (Yoshida et al. 2005). A recent study by Chen and colleagues demonstrated that garcinol inhibited DMBA-induced cheek pouch carcinogenesis by suppressing leukotriene B4 biosynthesis and inhibiting inflammation and cell proliferation in the oral epithelium in experimental hamsters

(Chen et al. 2012). Furthermore, a concentration-dependent reduction in AOM-induced colonic aberrant crypt foci was observed in rats following garcinol treatment (Tanaka et al. 2000).

The anthocyanins present in kokum have been reported to possess beneficial effects against cancer (Kocic et al. 2011; Wang and Stoner 2008; Fimognari et al. 2008; Hou 2003). Cyanidin-3-glucoside, the anthocyanin present in high concentration in kokum rind, effectively induced a dose-dependent inhibitory effect on the migration and invasion of A549 human lung carcinoma cells which are highly metastatic (Chen et al. 2006). This effect was mediated by decreasing the expressions of MMP-2 and u-PA genes, inhibition of the activation of c-Jun and NF-κB, and concomitantly increasing the expression of TIMP-2 and PAI genes (Chen et al. 2006). In addition, cyanidin-3-glucoside has been reported to possess antioxidant properties which in turn help in preventing mutagenesis and carcinogenesis (Elisia and Kitts 2008). Additionally Kitts and coworkers (Elisia and Kitts 2008) have also showed that cyanidin-3-glucoside protected the Caco-2 colon cancer cells against peroxyl radical (AAPH)-induced oxidative damage and associated cytotoxicity. Cyanidin-3-glucoside pretreatment to JB6 cells inhibited both UVB- and TPA-induced transactivation of NF-κB and AP-1 and expression of COX-2 and TNF-α, blocked TPA-induced neoplastic transformation in JB6 cells, and inhibited proliferation of a human lung carcinoma cell line A549 (Ding et al. 2006).

Studies on laboratory mice showed that cyanidin-3-glucoside reduced the number of nonmalignant and malignant skin tumors in mice which had TPA in DMBA-initiated skin carcinogenesis (Ding et al. 2006). Cyanidin-3-glucoside also reduced the size of A549 tumor xenograft growth and stopped metastasis by inhibiting the migration and invasion of A549 tumor cells (Ding et al. 2006). The chemopreventive properties of kokum were confirmed when cyaniding-3-glucosidase decreased the intestinal adenomas in Apc (Min) mice (a genetic model of human familial adenomatous polyposis) which were fed kokum for 12 weeks (Cooke et al. 2006).

3.2.5 Aegle marmelos *(L.) Corr. Serr*

Bengal quince, Indian quince, holy fruit, golden apple, and bael or Bilva are some of the common names of *Aegle marmelos*, which is one of the most important plants in ancient India (Baliga et al. 2011c, 2013). Indian inhabitants have used bael for over five millenniums for religious, medicinal, and dietary purposes (Baliga et al. 2011c, 2013). The fruits of this plant are round, pyriform, oval, or oblong; pulp is pale orange, pasty, sweet, resinous, and highly aromatic; and the seeds are flat, oblong in shape (Baliga et al. 2011c, 2013). Different varieties of bael have been found in India and are classified based on the fruit size and organoleptic characteristics (Baliga et al. 2011c, 2013). *Mitzapuri*, *Rampuri*, *Basti number 1*, *Azamati*, *Khamaria*, *Kaghzi gonda*, *gonda number 1*, *gonda number 2*, *gonda number 3*, *Kaghzi Etawah*, *Sewan large*, *Deoria large*, *Chakaiaya*, *Baghael*, *Lamba*, *Darogaji*, and *Ojha* are a few to name (Baliga et al. 2011c, 2013). The ripe fruits of bael are

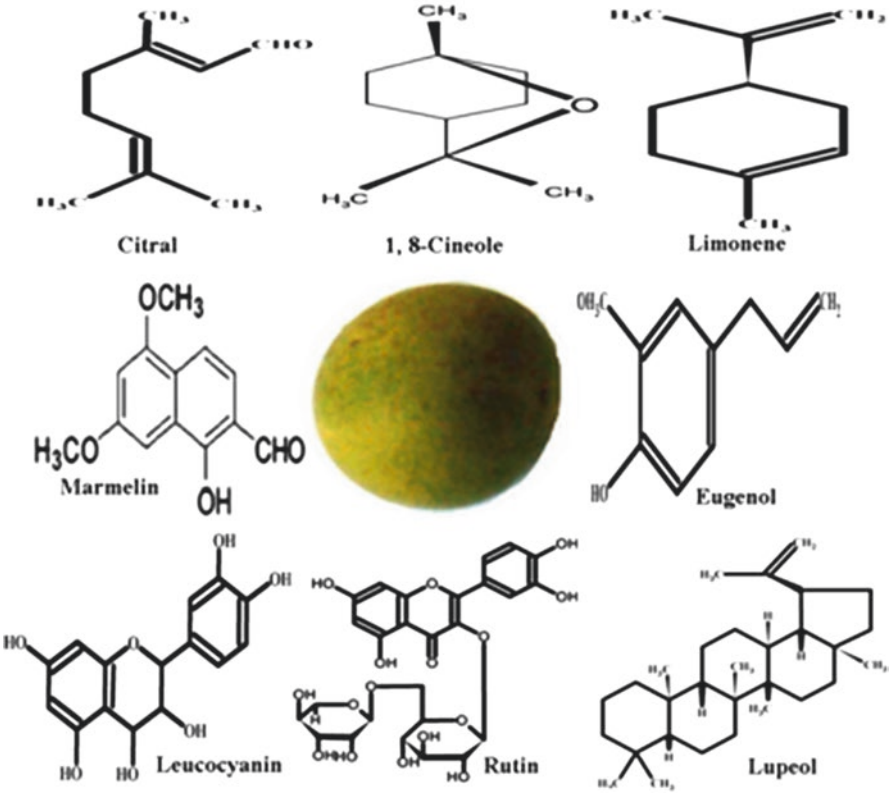

Fig. 3.5 Bael fruit and some important phytochemicals

used to prepare cooling drinks (sherbet), jam, marmalade, murabba, and syrups, used as dessert and as an ingredient for preparing cakes, or eaten with Indian bread (Baliga et al. 2011c, 2013). They are also consumed for breakfast in Indonesia and packed into tea bags and consumed in Thailand (Baliga et al. 2011c, 2013). The dried unripe fruits are astringent, digestive, and stomachic and are prescribed for diarrhea and dysentery with spells of constipation (Baliga et al. 2011c, 2013). In addition to the fruits, the tender leaves are also used to prepare a curry known as "Bael saar" by certain communities in Western part of Karnataka in India.

Bael contains various bioactive compounds such as carotenoids, phenolics, alkaloids, pectins, tannins, coumarins, flavonoids and terpenoids, and volatile compounds like limonene, β-phellandrene, p-cymene, linalool, α-cubebene, citronellal, cineole, citronella, citral, cuminaldehyde, β-cubebene, β-caryophyllene, hexadecane, pulegone, α-humulene, verbenone, and carvone (Baliga et al. 2011c, 2013). Aegeline, aegelenine, marmelin, o-methyl halfordinol, alloimperatorin, furocoumarins, psoralen, o-isopentenyl halfordinol, and marmelosin are some of the coumarins present in bael (Baliga et al. 2011c, 2013). Some of the phytochemicals present in bael are represented in Fig. 3.5.

Bael is associated with various pharmacological properties which have been established by various preclinical studies. Antibacterial, antifungal, antiviral, antiulcerogenic, cardioprotective, antiallergic, hepatoprotective, antidiarrheal, anti-inflammatory, antipyretic, analgesic, decreasing the thyroid hormone concentration, antifertility, antidiabetic, antilipidimic, and cardioprotective effects are some of the properties due to which bael has been traditionally used in Indian medicine (Baliga et al. 2011c, 2013). The methanolic extract of bael fruit possesses cytotoxic effect on human breast adenocarcinoma cells (SKBR3) in vitro (Moongkarndi et al. 2004) and prevents chemical-induced skin carcinogenesis in mice (Agrawal et al. 2011) and reduces the ill effects of ionizing radiation in mice (Jagetia et al. 2004). In addition, phytochemicals present in bael such as citral (3, 7-dimethyl-2, 6-octadien-1-al), 1, 8-cineole, d-limonene, eugenol, and marmelin have been shown to possess potent antiproliferative effects on various cultured neoplastic cells (Baliga et al. 2013; Jaganathan and Supriyanto 2012). In addition, in vitro assays using the leaf extract have shown antiproliferative properties against various human tumor cell lines including leukemic K562, T-lymphoid Jurkat, B-lymphoid Raji, erythroleukemic HEL, melanoma Colo38, and breast cancer MCF7 and MDA-MB-231 cell lines (Khan et al. 2002; Lampronti et al. 2003) and in Ehrlich ascites carcinoma-bearing Swiss albino mice (Jagetia et al. 2005a, b).

3.3 Conclusions and Future Prospects

The Indian indigenous fruits, namely, mango, amla, kokum, black plum, and bael possess various anticancer properties including antineoplastic, chemopreventive, and radioprotective in experimental animals. Mechanistic studies for assessing the varied anti-metastatic potentiality, selective antineoplastic, chemomodulatory and radiomodulatory and systemic, genetic and reproductive toxicity are warranted in the future. Of note, these fruits have been prescribed against cancer in various traditional and folk medicines in the Indian subcontinent. Upon ubiquitous usage of these fruits in daily diet, they are expected to play a substantial role in reducing the disease and societal and economic burden caused by cancer. Lastly, the commercial benefit of marketing the juice and candies of these fruits would be immense.

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Chapter 4
Potentiality of Anticancer Plant-Derived Compounds of North-East India

Mohan Lal, Nibir Ranjan Parasar, Anil Kumar Singh, and Mohd Sayeed Akhtar

4.1 Introduction

Cancer is a severe metabolic syndrome and the leading cause of mortality and morbidity worldwide with the number of cases increasing every year (Sharma et al. 2014; ACS 2016). In developed nations, this disease ranks second in death cases after cardiovascular disorders (Mbaveng et al. 2011; Siegel et al. 2016). The incidence of mortality and prevalence from major types of cancer as estimated by International Agency for Research on Cancer of 184 countries of the world revealed that there were 8.2 million cancer deaths, and 14.1 million new cancer cases, worldwide and it is projected that by 2030 there will be 26 million new cancer cases and 17 million cancer deaths per year (Thun and De Lancey 2010). Cancer is characterized by uncontrolled proliferation and dedifferentiation of normal cell. A typical cancer cell has marked attributes, viz. sends signals of proliferation and differentiation and is capable to sustain proliferation; they have the power of invasion and angiogenesis, and they overcome apoptosis (Sharma et al. 2014). Transformation from normal cell to malignant cell involves a sequence of alterations producing genetic instabilities which accumulate in a cell. Alterations such as mutation in DNA repair genes, oncogenes, apoptotic genes, tumour suppressor genes and gene involved in cell growth and differentiation are prominent (Sharma et al. 2014).

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Cancer is caused by both internal (e.g. hormones, gene mutations and immune conditions) and external (e.g. smoking, radiation and pollution) factors.

North-East India is one of the nine global biodiversity hotspots lying between 22–30°N latitude and 89–97°E longitude. This region is blessed with varying flora with diversified topography and climatic conditions marked by high humidity, moderate temperature and high rainfall. There are abundant dense forests, swamps, marshes, etc. that engulf the region with vegetation ranging from tropical to the alpine forests. Different tribes of North-East India rely mostly on the ethnic traditional herbal medicine due to lack of adequate modern medical facilities (Syiem and Kharbuli 1999; Rosangkima et al. 2010; Tushara et al. 2010). The crude herbal preparations are applied by the herbal practitioners with additives, viz. milk, curd, ghee honey, etc., as adjuvant in order to enhance the effect of the respective herbal preparations (Behere et al. 2013). The aim of the present chapter is to focus on the potentiality of major plant-derived compounds from diversified medicinal plants of North-East India for their anticancer and chemopreventive activity and their mode of action. Moreover, their large-scale production, uses of structural analogs, and molecular docking studies of some of the selected plant-derived compounds are also discussed.

4.2 Plants as an Imperative Source of Chemopreventive Phytochemicals

Since ancient times herbal formulations are used for medicinal purposes. Herbal practitioners apply various herbal formulations which are based on different philosophies and cultural origins to heal diseases. Traditional knowledge, viz. Ayurveda, Kampo, Egyptian medicine and traditional Chinese medicine, is the science of good health and well-being (Hashimoto et al. 2000; Rosangkima et al. 2010; Sharma et al. 2014). In the recent times, hunt for novel phytochemicals for drug development based on the concepts of traditional knowledge has gained wide acceptance. Natural products derived from plants are non-toxic thereby making them ideal candidates for modern drug discovery. Only 10% of the 250,000 plant species have been investigated for therapeutic applications, and more than 50% of all the modern drugs are derived from plants. Varieties of phytochemicals and their derived metabolites are present in the bark, root, leaves, stem and flower which serve an array of pharmacological activity in human health system. Phenolics, alkaloids, flavonoids, glycosides, tannins, oils and gums are responsible compounds for various therapeutic purposes. A significant antitumour activity has been shown by altered forms of these compounds. Curcumol, betulinic acid, kaempferol, ellagic acid, dillenetin, L-borneol, taxol, tangeretin, naringin and resveratrol are some of the remarkable chemopreventive phytochemicals as lead molecules for the development of anticancer drugs (Table 4.1).

Table 4.1 Chemopreventive phytochemicals derived from plants available in North-East India

Phytochemicals	Plants	Suppressed cancerous cell lines/cancer models	Uses	References
Kaempferol	*Ageratum conyzoides*	Lung cancer (A-549), gastric carcinoma (SGC-7901), colon carcinoma (HT-29), human glioma carcinoma (U-251), breast cancer (MDA-MB-231), prostate cancer (DU-145), hepatic carcinoma (BEL-7402), mouse leukaemia (P-388)	In clinical use	Adebayo et al. (2010)
Nimbolide	*Azadirachta indica*	Lung cancer (U937), leukaemia (HL-60, THP1), skin melanoma (B16), prostate cancer (PC-3)	In preclinical development	Baral and Chattopadhyay (2004), Giri and Lakshmi Narasu (2000) and Kumar et al. (2006)
Taxol	*Taxus baccata*	Breast cancer (HER2, MDA-MB- 435), ovary (SK-OV-3 w)	In clinical use	Baselga et al. (1998) and Aggarwal and Shishodia (2005)
Δ^9-Tetrahydrocannabinol	*Cannabis sativa*	Breast cancer (MCF-7, EFM-19, MDA-MB-231), skin cancer (PDV.C57, HaCa4), brain/spine tumour (U87, U373)	In preclinical development	Casanova et al. (2003), Massi et al. (2004), Cheung and Tai (2007) and Yesil-Celiktas et al. (2010)
Curcumin	*Curcuma longa*	Breast cancer (BT-20,T-47D, SK-BR3 and MCF-7), leukaemia (HL60)	In preclinical use	Cui et al. (2006) and Magesh et al. (2009)
6-Shogaol, (6)-gingerol	*Zingiber officinale*	Breast cancer (MCF-7 and MDA-MB-231), colon cancer (HCT 116, HT 29), ovarian cancer (SK-OV-3), lung cancer (A549), melanoma (SK-MEL-2), colorectal adenocarcinoma (HCT15)	In clinical use	De Petrocellis et al. (1998), Kim (2008) and Ligresti et al. (2006)

(continued)

Table 4.1 (continued)

Phytochemicals	Plants	Suppressed cancerous cell lines/cancer models	Uses	References
Dillenetin and betulinic acid	*Dillenia indica*	Lung cancer (U937), promyelocytic leukaemia (HL60, K562)	In preclinical development	Gandhi and Mehta (2013)
Alexin B, emodin	*Aloe vera*	Liver cancer (HepG2), breast cancer (MCF-7), cervical cancer	In preclinical development	Hussain et al. (2015) and Noorolahi et al. (2016)
Taxol	*T. baccata*	Breast cancer (BT-474, SK-BR-3 and MCF7)		Klauber et al. (1997)
Dillenetin and betulinic acid	*Dillenia pentagyna*	T-cell lymphoma	In preclinical development	Mehta et al. (1997) and Rosangkima and Prasad (2007)
Catechin	*Potentilla fulgens*	Breast cancer (MCF-7), human glioblastoma cancer (U-87)	In clinical use	Mittal and Tripathy (2015)
Dihydroflavonol	*Blumea balsamifera*	Breast cancer (MCF-7), epidermal carcinoma of the mouth (KB), myeloid leukaemia (K562), lung cancer (NCI-H187), hepatocellular carcinoma (McA-RH7777)	In preclinical development	Norikura et al. (2008)
Eugenol, orientin, vicenin	*Ocimum sanctum*	Lung cancer (A549), human fibrosarcoma cells (HFS)	In preclinical development	Roy et al. (2007)
Oleic acid and beta-sitosterol	*Mirabilis jalapa*	Human laryngeal carcinoma (Hep-2), breast cancer (MCF-7)	In preclinical development	Rumzhum et al. (2008) and Gogoi and Nakhuru (2016)
Vinblastine, vincristine	*Catharanthus roseus*	Lung cancer (NCI-H69/P)	In clinical use	Trevor and Theodore (1993)
Epicatechin, procyanidin B_2, B_4	*Litchi chinensis*	Breast cancer (MCF-7), leukaemia (U937, K562 and HL-60), colorectal cancer (Colo320DM and SW480)	In preclinical development	Twentyman et al. (1987), Lipinsky et al. (1997) and Hsu et al. (2012)

(continued)

Table 4.1 (continued)

Phytochemicals	Plants	Suppressed cancerous cell lines/cancer models	Uses	References
Etoposide, podophyllin, teniposide, podophyllotoxin	*Podophyllum hexandrum*	Lung cancer, testicular cancer, neuroblastoma, hepatoma	In clinical use	Uden (1989) and Abdullah and Abidin (2010)
Xanthatin, xanthinosin, 4-oxobedfordia	*Xanthium strumarium*	Cervical cancer	In preclinical development	Vaishnav et al. (2015)
Carnosic acid, rosmarinic acid	*Rosmarinus officinalis*	Breast cancer (MCF7 and MDA-MB-468), leukaemia (HL60, K-562), prostate cancer (DU-145), lung cancer (NCI-H82), liver cancer (Hep-3B), ovarian cancer (r A2780)	In preclinical development	Zhao et al. (2007), Roya et al. (2008) and Tai and Cheunga (2012)

4.3 Mechanism of Action and Molecular Targets of Chemopreventive Phytochemicals from North-East India

The precise mechanism of action of the bioactive molecules performing anticancer functions is an interesting area of current research. The usual targets of these molecules are the cytosolic and nuclear factors of a cancer cell. They either directly absorb the reactive oxygen species or stimulate the antioxidant enzymes, viz. catalase, glutathione and superoxide dismutase, in a transformed cell. The metabolic conversion of a procarcinogen is blocked by a phyto-molecule, or it suppresses malignant transformation of a preneoplastic cell. The cellular and signalling events involved in growth, invasion and metastasis are also regulated by these molecules. Curcumin (diferuloylmethane), a polyphenol present as a major phytochemical in the rhizome of *Curcuma longa*, is the most prominent chemopreventive bioactive molecule studied (Aggarwal et al. 2003; Fridlender et al. 2015). It has been used as a medicine for treatment of various diseases (Sharma et al. 2005; Fridlender et al. 2015; Lee and Kim 2016). Kaempferol, the major phytochemical in *Potentilla fulgens*, acts on proto-oncogene tyrosine protein kinase (Src), Erk1/2 and Akt pathways in pancreatic cancer cells and retards their growth and migration (Hossan et al. 2014). Ellagic acid from *P. fulgens* induces apoptosis in breast and prostate cancer cells and inhibits metastasis processes of various cancer types. Rosmarinic acid present in *Ocimum basilicum* reduces the activity of DNA methyltransferase and interferes OPG/RANKL/RANK networks (Osakabe et al. 2004; Baliga et al. 2013). Besides, it also acts in colon cancer cells by reducing COX-2 activity and Erk phosphorylation. Moreover, it targets PKA/CREB/MITF pathway and NF-kB activation in melanoma

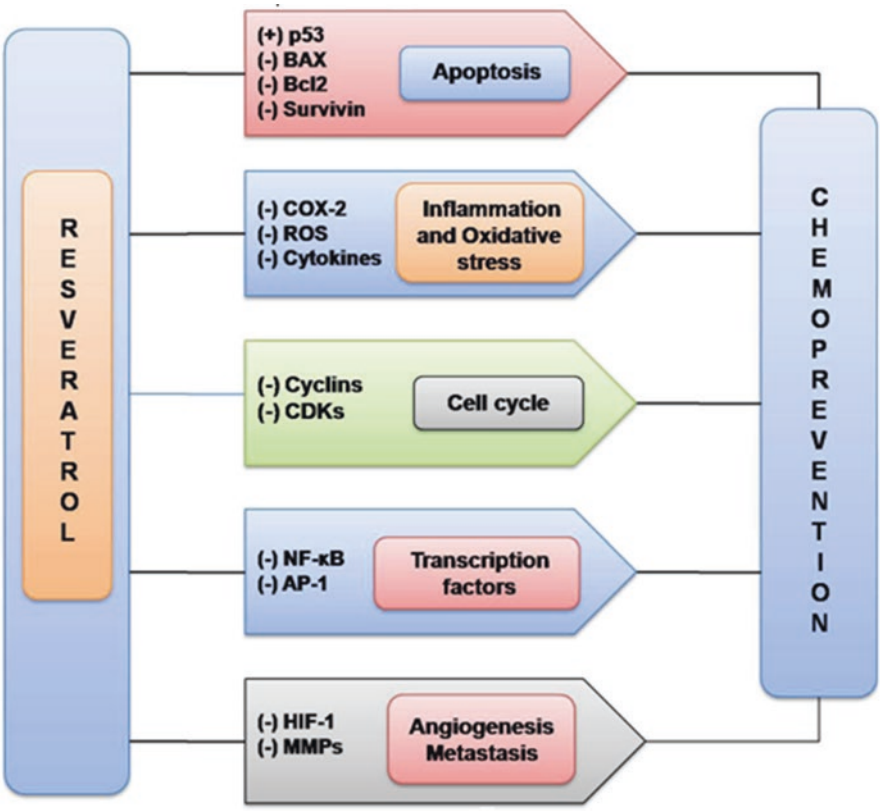

Fig. 4.1 Molecular targets of resveratrol leading to chemoprevention

and leukaemia U938 cells, respectively, thereby stimulating anti-inflammatory and antioxidant activities which consequently inhibit skin cancer (Osakabe et al. 2004; Roland et al. 2010; Baliga et al. 2013; Radhakrishnan et al. 2014). Gingerol present in *Zingiber officinale* induces caspase-dependent apoptosis in colon cancer cells by targeting the Erk1/2/JNK/AP-1 signalling (Fridlender et al. 2015). Tetrahydrocannabinol isolated from *Cannabis sativa* has been used in the past two centuries as supporting drugs for patients that receive either radiation or chemotherapies. Side effects related to these treatments such as vomiting, cachexia, nausea and loss of appetite are eased by cannabinoids (Robson 2001; Tramer et al. 2001; Ligresti et al. 2003; Massa et al. 2005; Grotenhermen and Muller-Vahl 2012). Studies imply that in the gastrointestinal system cannabinoid receptors are involved in inhibition of cell proliferation of colorectal carcinoma (Massa and Monory 2006; Varoni et al. 2016). Multiple mechanisms are performed by resveratrol in order to arrest carcinogenesis (Fig. 4.1). Paclitaxel ($C_{47}H_{51}NO_{14}$) is known as taxol, probably the most well-known anticancer drug derived from the bark of *Taxus brevifolia* Nutt. It inhibits the microtubule disassembly by binding the polymerized microtubules

(Xiao et al. 2012; Prota et al. 2013). Taxol binds to the microtubule-associated protein (MAP) microtubule complex causing further stabilization of microtubules thereby preventing mitotic spindle formation and thus inhibits mitosis as well as cell proliferation (Priyadarshini and Aparajitha 2012; Weaver 2014). Induction of multipolar divisions leads to formation of abnormal spindles bearing additional poles, and the consequence is unnatural chromosomal segregation which leads to the formation of abnormal aneuploid daughter cells that follow the apoptosis pathway (Priyadarshini and Aparajitha 2012).

4.4 Purification of Anticancer Phytochemicals

The curative efficacy of medicinal plants is determined by the quality and quantity of bioactive molecule(s) which varies with altitude, latitude, climactic conditions and seasons. Varieties of chemopreventive bioactive molecules are distributed across different parts of a plant accounting for varying levels of pharmacological activity. Development of the phytochemicals as antitumor entities becomes a daunting task owing to the synergistic effects of such bioactive phyto-constituents rather than the purified one. Purification of bioactive phytochemicals includes isolation and assay, combinatorial chemistry and bioassay-guided fractionation. Prior to fractionation of the crude plant extract, the bioactivity of the extract is confirmed by subjecting it to bioassays. Various analytical platforms are used for examination of the eluted fractions, viz. FT-IR, mass spectroscopy, HPLC and thin-layer chromatography (Fig. 4.2). Solvents should be used in an increasing polarity order of silica, Sephadex, Superdex or any other suitable matrix that can be used for fractionation. Purification is followed by in vivo examination of extracts for evaluation of anticancer activity. The killing activity of tumour and other parameters like pharmacokinetics, safety and adverse effects, dose concentration, drug interactions, etc. must be explored before the development of novel anticancer drugs. However, the major bottleneck for rapid manufacturing of medicines using natural products is the poor solubility and bioavailability of plant secondary metabolites (Guo et al. 2006). In order to meet market demands, the development and use of synthetic or semi-synthetic analogs to plant-derived substances are adopted. Morphine is a well-known example that has been modified to morphine-6-glucuronide in order to enhance its therapeutic efficacy (Parc et al. 2002). Taxol, an important plant-derived (*Taxus* sp.) anticancer drug, is present in low amounts in all *Taxus* species along with its insolubility in water. These limitations in taxol manufacturing are overcome by combining the use of 10-deacetylbaccatin III and a semi-synthetic process for production of the drug. Docetaxel is the semi-synthetic soluble analog of taxol which is widely used, and additional strategies for improvement are needed to enhance its features and also to meet future market demands of this important drug (Aggarwal et al. 2003; Sharma et al. 2005; Malik et al. 2011; Fridlender et al. 2015). Curcumin analogs have been prepared owing to its insolubility in water. Besides due to reduced absorption in the liver and in intestinal walls and systemic

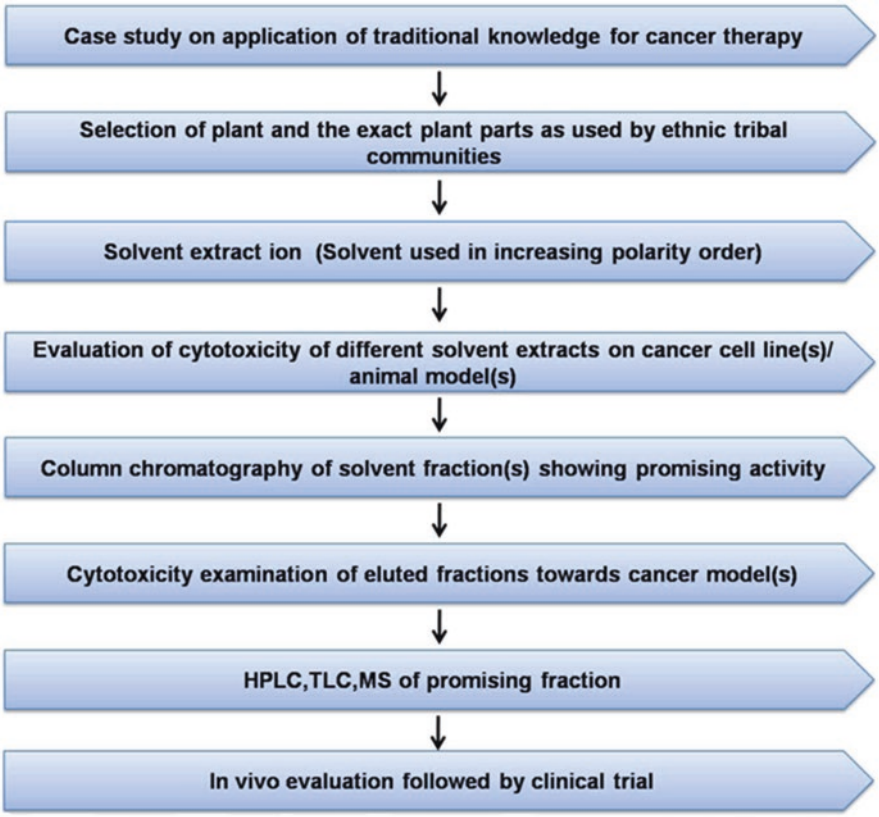

Fig. 4.2 Development of novel anticancer drugs derived from bioactive phytochemical

elimination, curcumin has poor bioavailability (Gordaliza 2007). However, the modified analog (beta-diketone monocarbonyl dienone) of curcumin has a good bioavailability and therapeutic effect as demonstrated in rodents (Twentyman et al. 1987; Mosley et al. 2007).

4.5 In Vitro Propagation of Anticancer Plants

The anticancer plants are seems to be endangered. Therefore, there is a need to raise these plants for drugs and other therapeutic applications through various standardized protocols under in vitro conditions (Kingston 2000; Malik et al. 2011). The induction of callus and proliferation of gametophytes of *T. baccata*, found abundantly in north-eastern states of Nagaland and Arunachal Pradesh, was reported by past investigators (Rohr 1973; David and Plasitra 1974). However, David and Plastira (1976) studied the mineral and phytohormone composition of culture

Table 4.2 Standardized in vitro shoot proliferation conditions of *P. fulgens*

Explants	Medium	Sucrose (%)	Auxin	Cytokinin	Root proliferation	Reference
Leaf	MS	3	IAA (1 mg/l)	BAP (1 mg/l)	Axillary	Wilken et al. (2011)
Leaf	MS	3	NAA (1 mg/l)	Kinetin (1 mg/l)	Adventitious	Klauber et al. (1997)

medium to improve callus proliferation using mature stems as explants. Moreover, callus induction studies have been also performed using different explants, viz. hypocotyls, cotyledons, young or mature stems, and the roots from young seedlings, by past investigators (Brunakova et al. 2004, 2005). These studies concluded that the young tissues are more responsive and prone to callus induction than mature plants or adult trees (Brunakova et al. 2004). However, in vitro conditions for auxiliary and adventitious shoots of *P. fulgens*, an endangered anticancer herb of higher Himalayas, using leaf as explants (Wilken et al. 2011) were tabulated in Table 4.2.

4.6 Conclusions and Future Prospects

The plant of North-East India or their derived compounds have the potential to be used as anticancer agents. The use of single drug to treat a single disease is questionable, and is a matter of debate since several decades. The recent trends in genomics, which is concerned with the genetic diversity or polymorphisms, clearly indicated that different human population need different drugs to cure the diseases. In this regard, the use of herbal medicines is gaining popularity because of its cost-effectiveness and potentials in the conventional medicinal practices. However, lacks of consistency in terms of their composition, efficacy, quality, safety, consistent manufacturing practices, regulations and approval processes lead the idea to combine the traditional and modern medicine practices. Therefore, the exploration of the plants found in North-East India is highly desired for the search of novel bioactive anticancer compounds and their mechanisms of action involved in the treatment of various types of malignant cells.

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Chapter 5
Plant-Derived Compounds in Cancer Therapy: Traditions of Past and Drugs of Future

Bee Ling Tan and Mohd Esa Norhaizan

5.1 Introduction

Cancer has become a leading cause of morbidity and mortality worldwide, and contributed to approximately 8.8 million deaths in 2015 (WHO 2017), and it is projected that without any prompt action, the number of new cases will be increased by approximately 70% in the next two upcoming decades mostly in low- and middle-income countries. In this regard, inflammation is associated with numerous diseases and severe disorders, including rheumatoid arthritis, asthma, chronic inflammatory bowel diseases, type 2 diabetes, neurodegenerative diseases, and cancer (Scrivo et al. 2011). Furst and Zundorf (2014) reported that anti-inflammatory agents primarily contain glucocorticoids, nonsteroidal anti-inflammatory, and immune-suppressant drugs. While tremendous efforts have been made over the past decades to enhance the available therapeutic options, conventional therapy seems to be not effective due to undesirable side effects. For instance, chemotherapy using synthetic drugs causes unwanted side effects, such as bleeding, hair loss, myelotoxicity, and diarrhea (Breidenbach et al. 2003). Most of the anticancer agents exhibit a narrow therapeutic effect and lack selectivity towards cancer cells. Therefore, the

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discovery of new anticancer agent from natural products has drawn a great attention among scientists in both academia and industry.

Natural products play a vital role in anticancer therapy. There are over 500 bioactive constituents from microorganisms, marine plants, and terrestrial plants which have been identified to have antioxidant, antiproliferative, or anti-angiogenic properties to suppress the tumor proliferation (Orlikova et al. 2014). Collectively, 78.6% of all approved anticancer agents are derived from natural products, with only 21.4% being synthetic ones that are not related with nature. Many evidences suggest that nature plays a crucial role in modern therapy especially in the development of drugs from natural origins (Folmer et al. 2012; Orlikova et al. 2014).

Plants exert numerous bioactive metabolites and have received great demands in the field of pharmacology due to there curative properties (Moghadamtousi et al. 2013). They act as a central player in the development of sophisticated traditional medicine particularly against cancer diseases. Prominent plant-derived compounds, such as morphine, colchicine, quinine, pilocarpine, atropine, and/or theophylline, are vitally important in the current pharmacotherapy. Due to the development of organic synthesis, many plant-derived compounds have become the first leading structures in the history of drug development (Furst and Zundorf 2014). Several examples reported by Furst and Zundorf (2014) showed that vinblastine is originally from the Madagascar periwinkle (*Catharanthus roseus*), served as a valuable anticancer drug. Paclitaxel isolated from the Pacific yew (*Taxus brevifolia*), galantamine derived from Caucasian snowdrop (*Galanthus caucasicus*), and capsaicin from chili peppers (*Capsicum* species) are promising secondary plant metabolites. Plant-derived compounds are highly used as leading structures with chemical modifications. Some of them include salicylic acid (acetylsalicylic acid), morphine (scores of derivatives), artemisinin (artemether), dicoumarol (warfarin), and camptothecin (topotecan and irinotecan). Of particular interest in this chapter, we will highlight on the emerging role of plant-derived compounds and their anticancer activities. We also provide a cohesive representation of the literature on the underlying mechanisms of action involved in the pharmacological effects of these phytochemicals.

5.2 Inflammation: A Hallmark of Cancer

Based on the hallmarks of cancer and its characteristics, inflammation and the associated cell signaling pathways have drawn an interest recently. Inflammatory environment is a cause for cancer progression and development (Balkwill and Mantovani 2001). Abnormal cell proliferation was due to deoxyribonucleic acid (DNA) damage, where their growth rates are influenced by secretion of chemokines and cytokines and the growth factors produced by modification of inflammatory cells (Balkwill and Mantovani 2001; Prasad et al. 2010). This is supported by previous findings, which indicates that natural compounds can suppress this hallmark efficiently (Folmer et al. 2012).

5.3 Currently Used Anticancer Agents

5.3.1 Anti-angiogenesis Agents

Vascular endothelial growth factor (VEGF) is one of the crucial factors responsible for inducing angiogenesis, and most of the activities are primarily modulated by vascular endothelial growth factor receptor 2 (VEGFR-2). Therefore, most of the anti-angiogenic agents target either VEGFR-2 or VEGF (Weis and Cheresh 2011). Anti-VEGF agents, including pegaptanib, ranibizumab, and bevacizumab, are usually used in the treatment of multiple solid and hematological malignancies, choroidal neovascularization (CNV), and age-related macular degeneration (AMD) (Hefner and Gerding 2014; Marinaccio et al. 2014; Solomon et al. 2014). Tyrosine kinase inhibitors, including sorafenib, regorafenib, axitinib, and sunitinib, are targeting for VEGFR-2 receptors. Interestingly, anti-VEGF agents were shown not only as anti-angiogenic agent but also as potential therapeutic for asthma, chronic obstructive pulmonary disease (COPD), and diabetic macular edema (DME), as reported by Arevalo (2014), Bandello et al. (2014), and Olivieri and Chetta (2014), suggesting a wide ranging functional potentials of anti-VEGF agents. In addition, some of the fusion proteins suppress the angiogenic molecule activities by trapping molecules effectively and inhibit the synthesis of these factors via suppression of mammalian target of rapamycin (mTOR), heat-shock protein 90 (HSP90), and cyclooxygenase (COX) pathways (Lockhart et al. 2010).

Although these anti-angiogenic agents showed a positive effect in inhibition of pathological angiogenesis, severe side effects arising from cancer patients are debilitating and result in depriving these patients in optimum and positive effects of anti-angiogenic therapy (Al-Husein et al. 2012; Elice and Rodeghiero 2012; Faruque et al. 2014). Anti-VEGF agents injected in AMD patients via intravitreal were associated with an increased risk of bleeding and cardiovascular toxicity (Elice and Rodeghiero 2012; Thulliez et al. 2014). Other undesirable adverse effects, including hepatic, cutaneous, hematological, and renal toxicities and malignant hypertension, have also been found in cancer patients who receive anti-angiogenic therapies (Ishak et al. 2014). In line with this, severe toxicities and high cost of the currently used anti-angiogenic drugs have urged an alternative approach. Scientists have concentrated on natural anti-angiogenic constituents from plants because these inexpensive molecules have a minimal or low toxicity, and were applied for centuries worldwide for the treatment in numerous diseases (Wang et al. 2015).

5.3.2 Anti-invasive and Anti-metastatic Agents

Matrix metalloproteinases (MMPs) were predominantly known for their roles in stimulating for cancer progression. MMPs exert its ability in degradation of connective tissue between the lining of blood vessels and the cells which promotes tumor

cells to metastasis (Gialeli et al. 2011). These data pave way for the development of broad-spectrum synthetic inhibitors to suppress MMP activity via interaction with Zn^{2+} ion in their active sites. Preclinical study showed that MMP inhibitors, for instance, batimastat (Davies et al. 1993), have a strong potential as anticancer agent (Gialeli et al. 2011). Batimastat, a hydroxamate derivative with poor water solubility, becomes the first MMP inhibitor evaluated in clinical trials (Macaulay et al. 1999). Batimastat has an ability to suppress several MMPs, such as MMP-1, MMP-2, MMP-7, and MMP-9, via binding to Zn^{2+} ions in the active site (Acharya et al. 2004). Compelling data has shown a promising antitumor activity of batimastat in in vivo studies on hemangioma, human ovarian cancer xenografts, mouse melanoma, and colon cancer (Watson et al. 1995; Eccles et al. 1996; Low et al. 1996). Hence, clinical studies of hydroxamate-based inhibitors were subsequently carried out (Mannello et al. 2005; Rao 2005; Vihinen et al. 2005), but the clinical data of these compounds were disappointing. From the study reviewed, marimastat was shown ineffective in a randomized phase III trial for metastatic breast cancer due to the musculoskeletal toxicity (Sparano et al. 2004).

In addition to the effects observed in hydroxamate-based inhibitors, non-hydroxamate MMP inhibitors were also used as MMP inhibitors such as rebimastat and thiol-based inhibitor SB-3CT. Rebimastat, known as BMS 275291, comprised of a thiol zinc-binding group and has been identified as a broad-spectrum MMP inhibitor. Rebimastat is a non-peptide mimetic, comprised of structural scaffold of the thiol in a deep-pocket binding, and had been shown to exert sheddase-sparing effect, thereby preventing suppression of metalloproteinases that promote the release of tumor necrosis factor (TNF), interleukin-1 (IL-1) receptor type 2, L-selectin, TNF receptor 2, and interleukin-6 (IL-6) receptor (Naglich et al. 2001). Surprisingly, a phase III trial in non-small cell lung carcinoma and a phase II trial in initial stage of breast cancer had shown an undesirable effect (Miller et al. 2004; Leighl et al. 2005).

In the past two decades, the MMP family has been tested in varieties of mammalian species, both at the protein and gene expression levels. Nearly 50 MMP inhibitors were evaluated in clinical study. Although promising preclinical data supported the MMP inhibitors as anticancer therapies, all phase III clinical trials have failed. From the studies reviewed, numerous MMP-coding genes have been knocked out in in vivo experiments, suggesting an in vivo model to study the consequences without presence of these genes. Of the study reported, MMPs are still considered as a crucial biological mediator which is implicated in many disorders. It is intriguing why, though their target ability, the development and marketing of these MMP inhibitors have been delayed so much (Vandenbroucke and Libert 2014).

While clinical studies with several MMP inhibitors are continuous, new research evidences concern about the role of MMPs which have yet to be fully defined, and the whole story turned out to be more complex than previously thought. This information suggests that all MMPs stimulate the development of cancer was a misconception, because not all MMPs have been recognized when the first clinical study was initiated. Thus, it provides evidences that not all MMPs need to be blocked at all times and in all cases (Iyer et al. 2012). Indeed, MMPs can have differential effects on tumor progression, depending on their substrates, for instance, angiogen-

esis, tumor growth and survival, invasion, and immune response mediation (Lopez-Otin et al. 2009; Decock et al. 2011; Hadler-Olsen et al. 2013). On the other hand, MMPs are also processing enzymes which selectively break several non-matrix targets, for example, clotting factors, cytokines, cell surface receptors, other proteinases, and chemokines (Vanlaere and Libert 2009) as well as tissue-remodeling enzymes.

Previous clinical study using broad-spectrum MMP inhibitors found that prolonged treatment results in an undesirable adverse effect, especially inflammation and musculoskeletal pain (Drummond et al. 1999; Skiles et al. 2004). However, this effect was reversible; thereby in the following trials, the concentration was decreased to prevent these inadvertent outcomes. Therefore, MMP inhibitor concentrations were usually shown insufficient to affect tumor biology, and thereby combination therapies were never considered. Unfortunately, two clinical trials using the MMP inhibitor tanomastat in pancreatic cancer and small-cell lung cancer were halted in the early beginning when the patients given the inhibitor exhibited significantly shorter survival compared than that of the patients given placebo (Coussens et al. 2002). These unexpected outcomes were more likely due to the broad-spectrum inhibition of MMPs and the cross-inhibition of a disintegrin and metalloproteinase (ADAM) family members and aggrecanases (ADAMs with thrombospondin motifs (ADAMTS) family members) (Edwards et al. 2008; Tan Ide et al. 2013). Nonetheless, most of the studies showed that MMP inhibitors exert a side effect; we believe that there is still hope in the suppression of MMP as a therapeutic strategy in the treatment of inflammatory-associated disorders. Therefore, natural product has played a central role in the development of significant number of drug candidate compounds.

5.4 Natural Compounds as Anticancer Agents

Natural compounds have been recognized as an excellent tool in evaluation of the molecular targets and act as therapeutic and chemopreventive compounds for biomedical applications (Kelkel et al. 2010; Schumacher et al. 2011a, b; Orlikova and Diederich 2012; Trecul et al. 2012).

Several studies have revealed that phytochemicals contained in natural products can suppress the initiation, promotion, and progression of carcinogenesis and some of their medicinal compounds hold a great promising chemopreventive and chemotherapeutic approach against cancers (Gupta et al. 2010; Lee 2010). Plants traditionally identified for the treatment of several cancer diseases (Orlikova and Diederich 2012) (Table 5.1) have seldom shown an association with the side effects compared with that of the modern chemotherapy (Jung Park and Pezzuto 2002). Realizing the potential benefits of plant-derived compounds as a source of active anticancer components, the National Cancer Institute (USA) studied nearly 35,000 plant products from 20 countries and has determined about 114,000 plant extracts for anticancer activity (Shoeb 2006). Out of the 92 anticancer drugs available prior to 1983 in the

Table 5.1 Plants with anticancer activity in vitro

Plant-derived compounds	Types of cancer cells	References
Procyanidins B1 and B2, (−)-epicatechin, (+)-catechin, phloretin, phloretin-2'-O-glycoside, quercetin, quercetin-3-O-glycoside, caffeic and chlorogenic acids	Colon cancer (Caco-2) cell line	Bellion et al. (2010)
Phloretin	Skin cancer cells	Funari et al. (2011)
Xanthone V1	Breast adenocarcinoma (MCF-7) and cervical carcinoma (HeLa and Caski)	Kuete et al. (2011)
2-Acetylfuro-1,4- naphthoquinone	Cervical carcinoma (HeLa and Caski), leukemia T-cells (PF-382), and skin melanoma (Colo38) cells	Kuete et al. (2011)
Artepillin C, quercetin, kaempferol, p-coumaric acid	Prostate cancer (LNCaP) cell line	Szliszka et al. (2011)
Cycloartane triterpenoid	Colon cancer (HT-29) cell line	Awang et al. (2012)
Daidzin, genistin, daidzein, genistein	Prostate cancer (LNCaP, C4-2B) cell line	Dong et al. (2012)
Cycloart-24-ene-26-ol-3-one	Colon cancer (HT-29) and (Caco-2) cells	Leong et al. (2016)

United States, 60% are of natural origin among the ones sold between 1983 and 1994 worldwide (Newman and Cragg 2012). About 80% of plant-derived molecules were associated with their original ethnopharmacological purposes (Tuorkey 2015; Swamy et al. 2016).

5.5 Plant-Derived Compounds Tested in Clinical Trials

Looking back the list of drugs approved in the last decades exhibits that plant-derived compounds are still vitally important in drug development. There are wealthy numbers of phytochemical studies describing new substances isolated from plants. Preclinical studies, which are in vitro, cell-based, and animal experiments on the mode of action in these substances, are available in an inconceivable quantity. However, these data are often of equivocal quality, particularly in the field of anti-inflammatory and anti-metastatic (Table 5.2) compounds. Further, literature review is overwhelming, and there are many comprehensive publications available on the respective underlying mode of action (Bellik et al. 2012; Sultana and Saify 2012; Leiherer et al. 2013; Orlikova et al. 2014). The knowledge on newly isolated components is often based on a very limited number of cell-based studies. From alterations of several key mediators of inflammatory processes, most often the transcription factor, nuclear factor-kappa B (NF-κB), the compound usually is evaluated to be an

Table 5.2 Anti-invasive and anti-metastatic properties of plant extracts or plant-derived compounds

Plant extracts or plant-derived compounds	Findings	Mechanisms	References
Glycyrrhiza uralensis	Inhibit cell migration and invasion of prostate cancer (DU145) cells	Downregulation of MMP-2 and MMP-9 and upregulation of TIMP-2	Park et al. (2010)
Chrysanthemum indicum	Suppressed proliferation and invasion of hepatocellular carcinoma (MHCC97H) cells	Downregulation of MMP-2 and MMP-9 expression and upregulation of TIMP-1 and TIMP-2	Wang et al. (2010)
Ipomoea obscura	Inhibits proliferation, invasion, migration, metastasis of melanoma (B16-F10) cells	Upregulation of TIMP, downregulation expression of inflammatory mediators via inhibition of NF-κB signaling, and inhibition of MMP-9 and MMP-2	Hamsa and Kuttan (2011)
Tripterygium wilfordii Hook F	Inhibited growth, migration, invasion, and metastasis of colon cancer (HT-29 and HCT116) cells	Downregulation of VEGF and COX-2, inhibition of cytokine receptor expression (CXCR4, TNFα, and TGF-β)	Johnson et al. (2011)
Annona muricata leaves	Effectively suppressed the migration and invasion of colon cancer (HCT-116 and HT-29) cells	Upregulation of Bax and downregulation of Bcl-2 proteins	Moghadamtousi et al. (2014)
Gypenosides	Inhibited cell proliferation and migration in colon cancer (SW620) and esophageal cancer (Eca-109) cells in dose- and time-dependent manners	Elevated intracellular ROS level and decreased the mitochondrial membrane potential	Yan et al. (2014)
Nuciferine, extracted from *Nelumbo nucifera Gaertn*	Inhibited the growth of non-small cell lung cancer (NSCLC) cells	Downregulation of β-catenin expression and its downstream targets such as c-myc, cyclin D, and VEGF-A and decreased the ratio of Bcl-2/Bax	Liu et al. (2015)
Solamargine	Inhibited migration and invasion of human hepatocellular carcinoma (HepG2) cells	Downregulation of MMP-2 and -9 expression	Sani et al. (2015)
Lupeol	Inhibited invasion of gallbladder carcinoma (GBC-SD) cells	Suppression of EGFR/MMP-9 signaling pathway	Liu et al. (2016)

(continued)

Table 5.2 (continued)

Plant extracts or plant-derived compounds	Findings	Mechanisms	References
Naringenin	Inhibited migration of lung cancer (A549) cells	Inhibition of Akt activities and reduction of MMP-2 and MMP-9 activities	Chang et al. (2017)
Enterolactone	Suppresses migration and invasion of lung cancer (A549 and H460) cell lines	Modulation of FAK-Src signaling pathway	Chikara et al. (2017)
Curcumin	Attenuates endometrial carcinoma cells migration	Slit-2 mediated downregulation of CXCR4, SDF-1, and MMP-2/MMP-9	Sirohi et al. (2017)

COX-2 cyclooxygenases-2, *CXCR-4* C-X-C chemokine receptor type 4, *EGFR* epidermal growth factor receptor, *FAK* focal adhesion kinase, *MMP* matrix metalloproteinases, *NF-κB* nuclear factor-kappa B, *ROS* reactive oxygen species, *SDF-1* stromal cell-derived factor 1, *TGF-β* transforming growth factor beta, *TIMP* TIMP metallopeptidase inhibitor, *TNFα* tumor necrosis factor alpha, *VEGF* vascular endothelial growth factor

anti-inflammatory without presenting a comprehensive in vivo study. Animal experiments are of course indispensible for the analysis of the pharmacological compound, but these models are insufficient and inconclusive, as commonly known, and do not satisfactorily reflect or show the exact condition in humans. Therefore, in the following sections, we will highlight the potential plant-derived anticancer agents that have been tested in humans, vinca alkaloids and its semisynthetic analogues, curcumin, colchicine, epigallocatechin-3-gallate (EGCG), betulinic acid, and podophyllotoxin derivatives. Chemical structures of plant-derived compounds tested in clinical trials and their sources are shown in Fig. 5.1.

5.5.1 Vinca Alkaloids and Its Semisynthetic Analogues

Compounds derived from plants play a critical role in the development of clinically useful anticancer agents. The first anticancer agents which precede into clinical trials were the vinca alkaloids, vincristine, and vinblastine from the Madagascar periwinkle, *Catharanthus roseus* (L.) (Apocynaceae), which were used as to treat testicular, lung, and breast cancers, lymphomas, leukemia, and Kaposi's sarcoma (Unnati et al. 2013). Another example is vinflunine, a dihydro-fluoro derivative of vinorelbine, which has been approved by the European Medical Agency (EMEA) in 2009 as the second-line chemotherapy in metastatic urothelial cancer (Bachner and De Santis 2008; Mamtani and Vaughn 2011). Vinflunine binds to the tubulin molecules, suppressing microtubule polymerization and the formation of tubulin paracrystals (Kruczynski et al. 1998; Bennouna et al. 2008). This binding

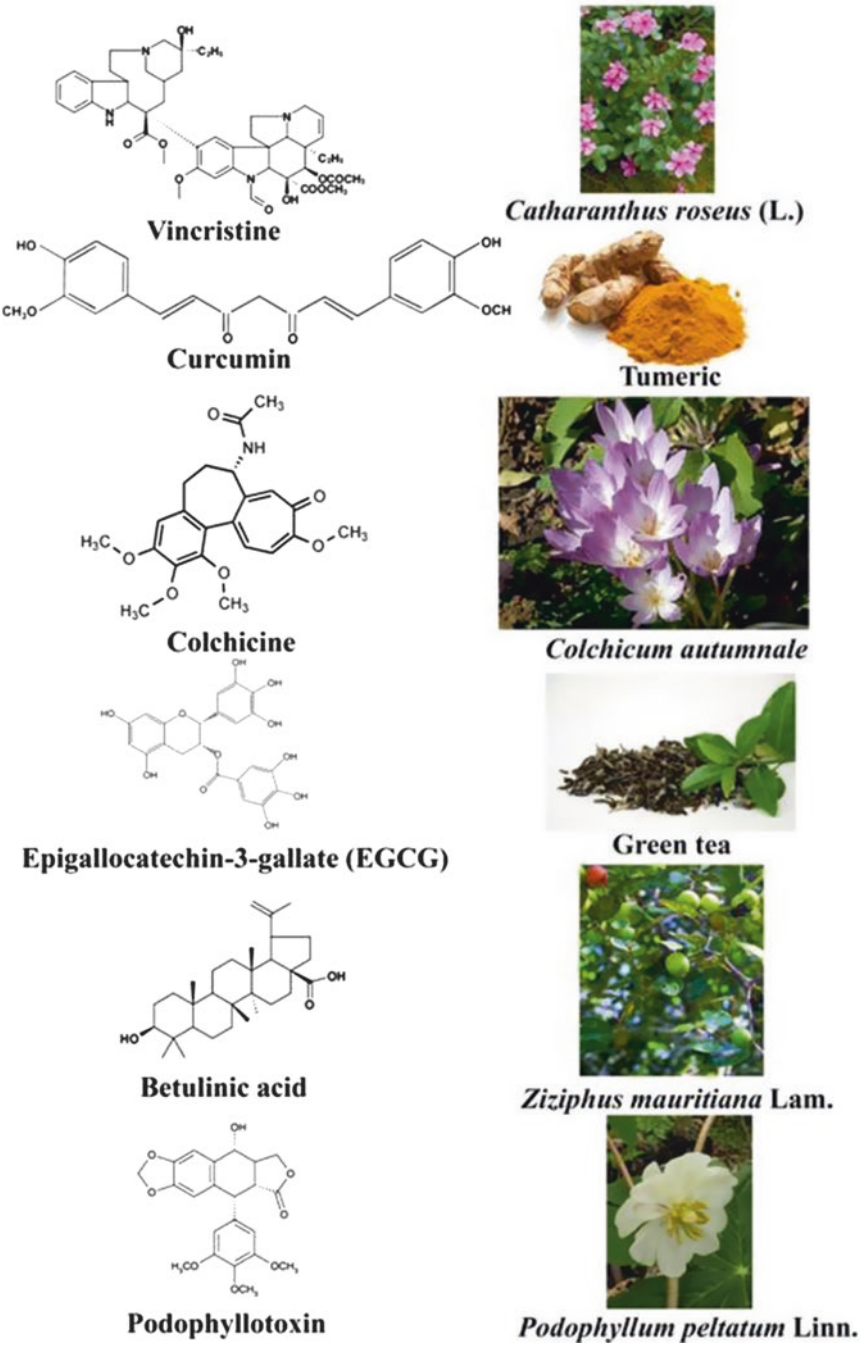

Fig. 5.1 Chemical structures of plant-derived compounds tested in clinical trials and their sources

subsequently causes the cell cycle arrest at G_2/M phase and induction of apoptosis (Kruczynski et al. 2002; Lobert and Puozzo 2008). Such results have been reported in both in vitro and in vivo studies against several types of malignant cell lines. Vinflunine is well studied in patients particularly non-small cell lung carcinoma and metastatic breast cancer in phase II/III clinical trials. Likewise, vinflunine is also being evaluated for its efficacy in advanced solid tumors in phase I/II trials (Ng 2011).

5.5.2 Curcumin

Curcumin, a polyphenol isolated from turmeric (*Curcuma longa*, Zingiberaceae), usually used as spices, exerts both anticancer and anti-inflammatory properties (Aggarwal et al. 2013; Naksuriya et al. 2014). With regard to its anti-inflammatory activity, curcumin was found to suppress predominant proinflammatory signaling cascades, including lipoxygenase (LOX), mitogen-activated protein kinase (MAPK), NF-κB, and COX pathways (Hong et al. 2004; Kim et al. 2005). Curcumin has also been reported to downregulate the secretion of prominent cytokines, for example, interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) (Shah et al. 2010). Furthermore, curcumin also blocks the expression of cell adhesion molecules, like intercellular adhesion molecule-1 (ICAM-1), which is needed for the binding of leukocytes with endothelial cells (Kumar et al. 1998). In addition, curcumin was also shown to suppress basic fibroblast growth factor (bFGF) (1 ng/mL)-induced endothelial cell proliferation in a dose-dependent manner (Arbiser et al. 1998). They concluded that 10 mg of curcumin can suppress bFGF (80 ng)-mediated corneal neovascularization in mice; however, no effect was observed in phorbol ester-stimulated *vascular endothelial growth factor* (*VEGF*) mRNA production. Treatment with 1 mM of hydrazinocurcumin-encapsulated nanoparticle in RAW264.7 macrophages causes induction of polarization of macrophages from M2 to M1 phenotype via suppression of signal transducer and activator of transcription 3 (STAT 3) (Zhang et al. 2013). In contrast, 25 μmol/L of curcumin had shown a stimulation of polarization in RAW264.7 cells into M2 phenotype via secretion of interleukin-4 (IL-4) or interleukin-13 (IL-13) and promotion of proliferator-activated receptor gamma (Chen et al. 2014; Gao et al. 2015).

Interestingly, curcumin not only present a promising anti-inflammatory profile, but also exhibit as a potential pleiotropic compound with different mechanisms of action. The clinical trial list of curcumin is explained in more detail in Gupta et al. (2013). In a study, curcumin served as an adjunct therapy or as a dietary supplement. It should be noted that bioavailability of curcumin is very low, despite continuous efforts which have been made to overcome this obstacle using chemical and technological approaches (Anand et al. 2007). Curcumin becomes an approved alternative for the prevention or treatment in one of the mentioned indications. However, it requires more studies in the future for real applicability. Taken

together, from the mounting of research evidences, it can be concluded that curcumin seems to exert a good safety with well-tolerated and nontoxicity profiles.

5.5.3 *Colchicine*

The tropolone derivative colchicine is a major alkaloid found in the plant *Colchicum autumnale* (Colchicaceae), also known as meadow saffron or autumn crocus. This plant extract has been used in gout attacks since ancient times ago. Surprisingly, the United States Food and Drug Administration (FDA) have approved colchicine for the prevention and treatment of acute gout flares as well as treatment of familial Mediterranean fever. The underlying mechanism of colchicine is well evaluated with the molecular targeting in tubulin, the binding site was characterized accurately, and the biological consequences of impairing microtubule dynamics were investigated (Bhattacharyya et al. 2008; Stanton et al. 2011). Colchicine was shown not only as a microtubule destabilizer, which exerts a strong binding capacity to tubulin (Stanton et al. 2011; Lu et al. 2012; Sivakumar 2013), but it increased cellular free tubulin to control mitochondrial metabolism in cancer cells via suppression of voltage-dependent anion channels in the mitochondrial membrane (Maldonado et al. 2010). Previous study revealed that clinically acceptable colchicine doses, in a range of 2–6 ng/mL, had a potential in the palliative treatment of cholangiocarcinoma (Wu et al. 2015) and hepatocellular carcinoma (Lin et al. 2013). These findings are further supported by another study, which observed that administration of colchicine inhibited the proliferation of human gastric cancer (AGS and NCI-N87) cell lines (Lin et al. 2016).

A wide ranging information has shown that colchicine is being approved as a drug. However, investigators are continued to conduct clinical studies to gain a better understanding in this field of application. An emerging study has been conducted using colchicine as an adjunct treatment towards inflammation-associated pathologies, including acute (Imazio et al. 2013), recurrent pericarditis (Imazio et al. 2011), and the results showed positive outcomes, including prevention of atrial fibrillation after radio-frequency ablation (Deftereos et al. 2012) and postpericardiotomy syndrome (Imazio et al. 2010). These large and well-performed investigations will definitely influence pharmacotherapy guidelines. However, due to a large number of diseases associated with inflammation, colchicine is worth for understanding further.

5.5.4 *Epigallocatechin-3-Gallate*

Epigallocatechin-3-gallate (EGCG) is a predominant bioactive constituent of green tea, *Camellia sinensis* (Theaceae). EGCG is the primary component of the green tea catechins and accounts for 50–80% of all catechins in a cup of green tea (Singh et al. 2011). EGCG has been reported to have anti-inflammatory, antioxidant,

anti-infective, anticancer, anti-angiogenetic, and chemopreventive activities (Domingo et al. 2010; Singh et al. 2011; Yang et al. 2011a; Riegsecker et al. 2013; Steinmann et al. 2013). The underlying mode of actions is also extremely large. EGCG stimulates cell cycle arrest and induces apoptosis via suppression of NF-κB and regulatory proteins in the cell cycle (Yang et al. 2011a). Moreover, it suppresses growth factor-dependent signaling, including epidermal growth factor (EGF), VEGF, and insulin-like growth factor-I (IGF-I), the MAPK pathway, COX-2 expression, and proteasome-dependent degradation (Yang et al. 2011b). Masuda et al. (2011) however reported that EGCG may modify the growth factor receptor signaling. Moreover, EGCG suppresses topoisomerase II, DNA methyltransferase 1, and telomerase, thus altering the chromatin functions (Bandele and Osheroff 2008). Unfortunately, promising preclinical data and the thorough mechanistic action, clinical research evidences conducted in the field of inflammation are insufficient. Research findings found that a beneficial effect of topical EGCG treatment against acne vulgaris in clinical trials, and is speculated due to anti-inflammatory properties of EGCG (Yoon et al. 2013), suggesting that EGCG could lessen inflammatory changes. This improvement fuelled future research to explore EGCG indications. Furthermore, a study reported by Furst and Zundorf (2014) has shown that EGCG will also be evaluated for multiple system atrophy, diabetic nephropathy, muscular dystrophy of the Duchenne type, patients with cardiac amyloid light-chain amyloidosis, fragile X syndrome, Huntington's disease, early stage of Alzheimer's disease, and Down syndrome. In addition, trials will be conducted to investigate the potential of EGCG in patients with Epstein-Barr virus and high risk for recurrent colon adenoma. Taken together, it is more likely that EGCG will expand its indication in the future, suggesting EGCG might exert enormous functional potentials.

5.5.5 Betulinic Acid

Betulinic acid is a pentacyclic triterpenoid with a lupane skeleton, isolated from *Ziziphus mauritiana* Lam. (Rhamnaceae) (Pisha et al. 1995). Triterpenoid has been demonstrated to cause cytotoxicity against brain tumor and neuroectodermal cells (Zuco et al. 2002). This finding was further confirmed by the evaluation of betulinic acid in in vivo selective proliferation inhibitory activity in athymic mice bearing human melanoma xenografts (Pisha et al. 1995). The fact that betulinic acid inducing cytotoxicity is by causing apoptosis via modulation of the intrinsic pathway as evaluated using mitochondrial membrane potential and stimulation of p38 MAPK and stress-activated protein/c-Jun N-terminal kinase (SAP/JNK) initiated by reactive oxygen species (ROS) (Laszczyk 2009). Accordingly, a betulinic acid-containing ointment was conducted in phase I/II clinical trials for the treatment of dysplastic nevi with a moderate to severe dysplasia (NIH 2010).

5.5.6 *Podophyllotoxin Derivatives*

Podophyllotoxin and deoxypodophyllotoxin have been identified as naturally occurring aryltetralin lignans (Srivastava et al. 2005). The Podophyllaceae family species including *Podophyllum peltatum* Linn. and *Podophyllum emodii* Wallich. have been recognized in the treatment of warts and skin cancer. It is also effective in the treatment of non-Hodgkin's lymphoma, lung cancer, other lymphomas, genital tumors, and Wilms' tumors (Utsugi et al. 1996; Subrahmanyam et al. 1998). The interest was also expanded to an alcohol extract of its dried roots containing podophyllin which was effective against venereal warts when applied topically. Other associated podophyllotoxin compounds including lignans were purified and introduced into clinical trials, but unfortunately it was halted due to the undesirable toxicity. Mounting research evidences conducted between the 1960s and 1970s at Sandoz Laboratories in Switzerland led to the development of teniposide and etoposide as clinical agents which are being used in the treatment of bronchial, testicular, and lymphatic cancers. Among 2069 anticancer clinical trials recorded by the National Cancer Institute (NCI) since July 2004, more than 150 are drug combinations such as etoposide toward numerous of cancers (Lee and Xiao 2005).

5.6 Mechanisms of Action of Plant-Derived Compounds as Anticancer Agent

A summary of studies on mechanisms of anticancer activity of plant-derived compounds is shown in Tables 5.2 and 5.3. Plant-derived compounds present naturally in plants may be beneficial in the amelioration of oxidative stress (Shah et al. 2010; Yoon et al. 2013). Therefore, we will focus the involving mechanisms in plant-derived compounds in the modulation of cell proliferation, inflammation, and angiogenesis.

5.6.1 *Apoptosis Induction and Inhibition of Cancer Cellular Proliferation*

Polyphenols are members of chemical constituents, present in a variety of plants and fruits, like curcumin in *Curcuma longa*, resveratrol in berries and grapes, and catechins from tea (Manach et al. 2004). These bioactive compounds show an antiproliferative effect against tumor-associated stromal cells and tumor cells, such as endothelial cells, and inhibit tumorigenesis via modulation of anti-angiogenic, antiproliferative, and antioxidant activities (Wang et al. 2015).

Table 5.3 Anti-angiogenic properties of plant extracts or plant-derived compounds

Plant extracts or plant-derived compounds	Findings	Mechanisms	References
Patrinia villosa Juss	Inhibition HUVECs proliferation, migration, and formation of tubelike structures	Induction of FAK and Akt phosphorylation	Jeon et al. (2010)
Cinnamon	Inhibition of VEGF-induced proliferation, migration, and formation of tubelike structures	Suppressed VEGFR2 kinase activity, MAPK, and STAT3 signaling	Lu et al. (2010b)
Allium ascalonicum	Inhibition sprouting and capillary tube formation in HUVECs	N.E.	Seyfi et al. (2010)
Triphala churna	100 mg/kg on matrigel assay, 40 μg/mL on CAM assay, and 40 μg/mL on HUVECs	Phosphorylation of VEGFR2	Lu et al. (2012)
Pithecellobium jiringa (Jack) Prain	Inhibition of other angiogenesis cascades including migration of endothelial cells and formation of capillary network on matrigel matrix	Arrested the growth of human endothelial cells via downregulation of VEGF expression	Muslim et al. (2012)
Catechin derivatives	1.5 mg of EGCG on HT-29 xenografts, 10 mg/kg of EGCG on 4 T1 breast cancer xenografts, 40 mg/L EGCG on MDA-MB231 cells, 30 μM EGCG on HT29 cells, and 0.75–25 μM EGCG on neutrophils	Protein kinase C, c-fos and c-Jun, STAT3, NF-κB, Erk-1/2 phosphorylation, TAM infiltration and polarization, and neutrophil migration	Jang et al. (2013)
Brucine from *Strychnos nux-vomica*	20 or 40 μM on rat aortic ring assay, 10 mg/kg on matrigel assay, and EAC tumor xenografts 5–40 μM on HUVECs	Src, FAK, Erk, Akt, and mTOR phosphorylation, VEGF, and NO production	Saraswati and Agrawal (2013)
Tylophorine from *Tylophora indica*	7.5 mg/kg on EAC tumor xenografts 2.5–20 μM on HUVECs	PI3K/Akt/mTOR signaling	Saraswati et al. (2013)
Deguelin	Treatment of deguelin showed anti-angiogenesis against cancer	Inhibition of HIF-1α-VEGF pathway	Wang et al. (2013)
Nicotiana glauca, *Tephrosia apollinea*, *Combretum hartmannianum*, and *Tamarix nilotica*	Exhibited remarkable anti-angiogenic activity	Inhibiting the sprouting of microvessels more than 60%	Hassan et al. (2014)

(continued)

Table 5.3 (continued)

Plant extracts or plant-derived compounds	Findings	Mechanisms	References
Resveratrol	5.7 μg/mL on T241 fibrosarcoma xenografts, 1.5 mg/kg of HS-1793 on FM3A breast cancer xenografts	Akt, MAPK phosphorylation, S6 protein, HIF-1α expression, secretion of IFN-γ and programming of TAM	Jeong et al. (2014)
	50 μM on A2780/CP70 and OVCAR-3 cells		
Curcumin	3000 mg/kg on HepG2 xenografts, 10 mg on mouse corneal	VEGF production, STAT3, proliferator-activated receptor gamma, IL-4 and IL-13 production, and TAM polarization	Gao et al. (2015)
	0.5–10 μM on primary endothelial cells, 1 mM of hydrazinocurcumin-encapsulated nanoparticles on RAW264.7 macrophages, and 25 μmol/L of curcumin on macrophages		
Gallic acid	Possess anti-angiogenesis against ovarian cancer	Inhibited VEGF secretion and suppressed in vitro angiogenesis in a dose-dependent manner	He et al. (2016)
Galium aparine	Inhibited the angiogenesis in MCF-7 and MDA-MB-231 cells	Decreased proangiogenic cytokines such as NRG1-β1, VEGF, and TF	Atmaca (2017)
Acorus calamus extracts	Inhibited the angiogenesis in HUVEC cells	Downregulation of Oct4 and nucleostemin	Haghighi et al. (2017)

CAM chick chorioallantoic membrane, *EAC* esophageal adenocarcinoma, *EGCG* epigallocatechin-3-gallate, *Erk* extracellular signal-regulated kinase, *FAK focal adhesion kinase*, *HepG2* human liver cancer cell line, *HIF-1α* hypoxia-inducible factor-*1* alpha, *HIF-1α-VEGF* hypoxia-inducible factor-*1* alpha-vascular endothelial growth factor, *IFN-γ* interferon gamma, *IL* interleukin, *MAPK* mitogen-activated protein kinases, *MCF-7* and *MDA-MB-231* human breast cancer, *mTOR* mammalian target of rapamycin, *NE* not elucidated, *NF-κB* nuclear factor-kappa B, *NO* nitric oxide, *NRG1* neuregulin-1, *A2780/CP70* and *OVCAR-3* ovarian cancer cell lines, *PI3K* phosphoinositide 3-kinase, *STAT3* signal transducer and activator of transcription 3, *TAM* tumor-associated macrophages, *TF* tissue factor, *VEGF* vascular endothelial growth factor, *VEGFR2* vascular endothelial growth factor receptor 2

Apoptosis is a complex process that contributes to programmed cell death involving the mitochondria (the intrinsic pathway) or the stimulation of death receptors (the extrinsic pathway). These intrinsic and extrinsic pathways cause the stimulation of caspases, including effector caspases (caspase-3, caspase-6, and caspase-7) and initiator caspases (caspase-2, caspase-8, caspase-9, and caspase-10). These two pathways then converge to activate caspase-3, which subsequently induce apoptosis (Thornberry and Lazebnik 1998). Oxidative stress and DNA damage are common signals that stimulate the mitochondrial apoptotic pathway and contribute to cyto-

chrome C release and mitochondrial membrane rupture (Thornberry 1998; Thornberry and Lazebnik 1998).

Research evidences have revealed that the anticancer ability of some dietary polyphenols, like luteolin, quercetin, apigenin, resveratrol, and genistein, may contribute to the induction of apoptosis in in vitro and in vivo studies (Gopalakrishnan and Tony Kong 2008; Surh 2008; Vauzour et al. 2010). In line with this, the apoptosis-inducing activity of EGCG has also been demonstrated to upregulate Fas expression and caspase-3, caspase-8, and caspase-9 in several cancer cell lines (Kawai et al. 2005; Nishikawa et al. 2006), as well as inhibition of BH3-interacting domain death agonist, apoptosis-suppressing proteins, B-cell lymphoma (Bcl)-2, and Bcl-extra large (Bcl-xL) (Lee et al. 2004; Nishikawa et al. 2006). Also, an earlier study found that genistein caused inhibition of breast cancer cells in a concentration and time-dependent manners with no harm toward normal breast epithelial (MCF10A) cells (Ullah et al. 2011). This is due to normal breast epithelial cells which exert no detectable copper (Daniel et al. 2005), which may partially explain their resistance observed in polyphenol-induced proliferation. The DNA damage induced by polyphenols in lymphocytes is modulated by the ROS generation. Accordingly, the anticancer activity present in the polyphenolic compounds has the potential to modulate prooxidant pathway, subsequently leading to cell death. A similar effect was also observed in resveratrol, which behave as prooxidative agents in human cancer cells (Santandreu et al. 2011). Likewise, results toward the same direction were presented by Shamim et al. (2012), who reported that polyphenol-induced apoptosis and DNA breakage in peripheral lymphocytes of pancreatic cancer at acidic pH. Overall, these data suggest that epithelial tumors have lower pH than that of normal tissues due to a high rate of glycolysis, lack of hypoxia, and vasculature, followed by lactate fermentation (Gerweck and Seetharaman 1996).

5.6.1.1 Pinocembrin

Pinocembrin has been found in numerous plants including the genera of *Piperaceae* family, which consists of 1950 species and 14 genera. Pinocembrin is a flavonoid compound derived from vegetables, fruits, seeds, nuts, spices, stems, flowers, herbs, red wine, and tea (Fig. 5.2) (Jiang and Morgan 2004; Miyahisa et al. 2006). Pinocembrin contained numerous pharmacological properties associated with inflammation by suppressing of vascular ailments, cancer growth, and bacterial colonization (Manthey et al. 2001; Touil et al. 2009). Pinocembrin found in the root of *Alpinia pricei* and *Alpinia galangal* has also been reported to exert anti-inflammatory (Hsu et al. 2010; Yu et al. 2009) and anticancer properties (Kumar et al. 2007). Furthermore, pinocembrin is also cytotoxic toward colon cancer (HCT-116) cells, with no harm against human umbilical cord endothelial cells (Kumar et al. 2007). From the study reviewed, pinocembrin activated caspase-3 and caspase-9 and mitochondrial membrane potential (MMP) activity in HCT-116 cell line (Kumar et al. 2007; Punvittayagul et al. 2011). In vivo and in vitro studies also found that pinocembrin can enhance the biological functions in medium-term

carcinogenicity and liver micronucleus in rats. This observed effect suggests that pinocembrin may protect against chemical-induced hepatocarcinogenesis (Punvittayagul et al. 2012).

5.6.1.2 Allicin

Allicin, also known as diallylthiosulfinate, is a sulfur-containing natural constituent in garlic (*Allium sativum* L.) (Fig. 5.2). Sulfur-containing components in onion and garlic are predominantly from the precursors of S-alk(en)yl-L-cysteine sulfoxides (ASCOs) and γ-glutamyl-S-alk(en)yl-L-cysteines (Kubec et al. 1999). Allicin exerts strong antimicrobial properties and thus potentially acts as a potent antibiotic in vitro (Borlinghaus et al. 2014). Furthermore, mounting evidence also indicates that allicin induces apoptosis and resulted in a redox shift in human leukemic cell lines (Miron et al. 2008). This activity subsequently resulted in the execution of cell death, both in caspase-independent (Park et al. 2005) and caspase-dependent (Oommen et al. 2004) pathways. Chu et al. (2013) had demonstrated that allicin enhanced p53-mediated autophagic cell death in hepatocellular carcinoma. The antiproliferative effect was not only shown in human leukemic and hepatocellular carcinoma cell lines, it also further induced apoptosis via mitogen-activated protein kinases/extracellular signal-regulated kinase (MAPK/ERK) and bcl-2/bax mitochondrial pathways in glioblastoma (Cha et al. 2012). Likewise, allicin also suppressed proliferation and induced apoptosis via p38 MAPK/caspase-3 signaling pathways in gastric cancer (Zhang et al. 2015). Moreover, allicin improved hypodiploid DNA content and enhanced releasing of cytochrome C from mitochondria

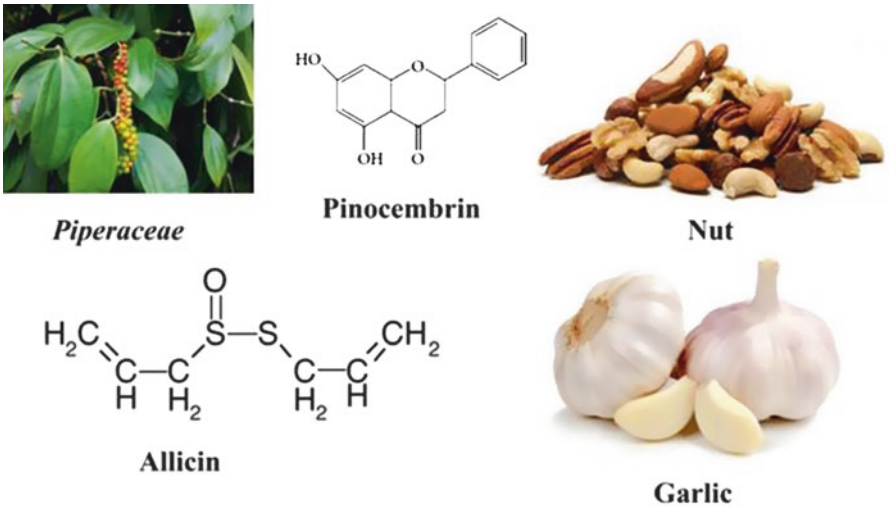

Fig. 5.2 Chemical structures of plant-derived compounds and their sources induced apoptosis and inhibited cancer cell proliferation

to cytosol capability and subsequently contributed to apoptotic colon cancer cell death (Bat-Chen et al. 2010). Additionally, the inhibition of cancer cells induced by allicin is also modulated by apoptosis-inducing factor (AIF). Of the study reported, nuclear factor E2-related factor 2 (Nrf2) is often described as an anti-apoptotic factor in the regulation of Bcl-2 and Bcl-xL expression (Niture and Jaiswal 2012; Niture and Jaiswal 2013).

5.6.2 Interfering with Inflammatory Signaling

Despite the type of inflammatory responses which may differ between diseases, inflammation and disease conditions are associated via production of inflammatory mediators by neutrophils and macrophages. COX-1 and COX-2 overexpressions produce inflammatory mediators like prostaglandin E 2 (PGE 2). Anti-inflammatory drugs with combination of nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit the inflammatory response through inhibition of infiltration and stimulation of inflammatory cells and release of mediators or inflammatory mediators (Urban 2000).

Numerous compounds mediate the COX-2 expression by modulation of MAPK signaling pathways. Indeed, p38 and ERK phosphorylation can be suppressed by curcumin (Yu and Shah 2007), resveratrol (Kundu et al. 2004), and epigallocatechin-3-gallate (EGCG) (Peng et al. 2006). Furthermore, c-Jun N-terminal kinase (JNK) stimulates the transcription factor of activator protein-1 (AP-1), which is suppressed by diallyl polysulfides from onion and garlic (Shrotriya et al. 2010). The suppression of the MAPK signaling prevents stimulations and nuclear translocation of transcription factors that interact with the specific sites of the promoter of COX-2 and ultimately suppressed the COX-2 expression. In addition to the effects observed in curcumin, resveratrol, EGCG, and diallyl polysulfides, apigenin, a flavone isolated from chamomile, has also been shown to have a similar effect against NF-κB activity. It was demonstrated to suppress IκB kinase (IKK), which resulted in inhibitory subunit of nuclear factor-kappa B alpha (IκBα) phosphorylation. IκBα sequesters NF-κB and prevents its translocation into the nucleus and the binding with its subunits (p65 and p50) and the promoter. AP-1 comprised of c-Fos and c-jun subunits has been demonstrated to suppress by curcumin in endometrium carcinoma. Similarly, this factor is also suppressed by diallyl trisulfides (Shrotriya et al. 2010), quercetin (Crespo et al. 2008), and resveratrol in non-carcinogenic mammary epithelial cells (Kundu et al. 2006).

5.6.2.1 Capsaicin

Capsaicin, a hydrophobic alkaloid derived from chili peppers (*Capsicum* species, Solanaceae), (Fig. 5.3) is recognized for its typical spiciness in the genus *Capsicum*. Capsaicin has been traditionally used as a counterirritant and topical rubefacient to

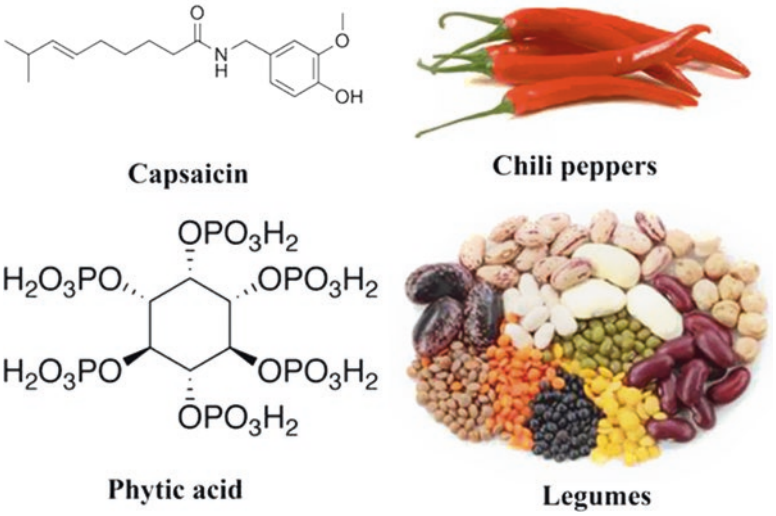

Fig. 5.3 Chemical structures of plant-derived compounds and their sources modulate inflammation

ameliorate joint and muscle pains. Capsaicin has been reported to suppress ethanol-induced gastric mucosa inflammation in rats (Park et al. 2000) and paw inflammation in arthritic rats (Joe et al. 1997). Furthermore, capsaicin has also been demonstrated to suppress inducible nitric oxide synthase (iNOS), NF-κB, and COX-2 expression in macrophages in a transient receptor potential channel vanilloid subfamily member 1 (TRPV1)-independent way (Kim et al. 2003). TRPV1 is a nonselective cation channel which has a high preference of Ca^{2+} and is primarily found in nociceptive neurons. It is often stimulated by physical and chemical stimuli, like low pH, inflammatory mediators, and heat (O'Neill et al. 2012).

5.6.2.2 Phytic Acid

Phytic acid is found predominantly in legumes, oilseed, and cereals (Fig. 5.3) (Schlemmer et al. 2009). Previous studies had demonstrated that phytic acid exerts numerous chemopreventive properties, such as anticancer and antioxidant properties (Norhaizan et al. 2011). Accordingly, phytic acid has been identified as a potential protective agent against cancer (Matejuk and Shamsuddin 2010). It inhibited different cancer cell proliferations via NF-κB activity (Agarwal et al. 2003; Kolappaswamy et al. 2009) and COX-2 pathway (Shafie et al. 2013). NF-κB is a crucial factor found in epithelial-mesenchymal transition (EMT) and survival pathways. Thus, targeting NF-κB is deemed as a promising strategy in the treatment of cancer. Research evidence shows that phytic acid inhibited the proliferation of prostate carcinoma (Kapral et al. 2008) and prevents nuclear translocation in HeLa cells and luciferase transcription activity (Ferry et al. 2002). Additionally, phytic acid

also inhibited colon cancer (Caco-2) cells through modulation of NF-κB by blocking of the p65 subunit of NF-κB and its inhibitor IκBα (Schröterová et al. 2010).

5.6.3 Modulation of Angiogenesis Signaling Pathway

Angiogenesis is the sprouting of new blood vessels from pre-existing vessels and resulted in several pathological diseases including rheumatoid arthritis, proliferative retinopathies, solid tumorigenesis, and obesity (Folkman 1990). Tumor angiogenesis is implicated by an angiogenic imbalance, where proangiogenic factors predominate over anti-angiogenic factors. Additionally, angiogenesis also causes metastasis and growth of malignant tumors. Vascular endothelial growth factor-A (VEGF-A) has been identified as a critical angiogenic mitogen (Folkman 2002). Accordingly, tumor angiogenesis is considered as a vital pharmacological target in cancer prevention and treatment (Scappaticci 2003; Dell'Eva et al. 2004). Therefore, this hypothesis has prompted in the development of the angiotherapy. Anti-angiogenic strategy can overcome the undesirable outcomes and chemoresistance resulted from the chemotherapies. Anti-angiogenic drugs targeting sprouting of new blood vessels that provide tumors with oxygen, nutrients, and blood ultimately may block the tumor proliferation and metastasis.

Avastin is a monoclonal antibody for VEGF and fluorouracil-based combination therapy. It has demonstrated an improvement in survival of metastatic colorectal carcinoma patients (Hurwitz et al. 2004). On the other hand, conventional anti-angiogenic compounds based on monoclonal antibody technology may have a limitation in terms of cost. Therefore, plant with anti-angiogenic compounds is of great demands because they are inexpensive and can produce in huge quantities (Al-Suede et al. 2012).

Plants which have various phytochemical compounds are potential natural antioxidants, including flavonoids, polyphenolic acids, phenolic diterpenes, and tannins, (Dawidowicz et al. 2006) which exert a variety of biological activities. As shown in Table 5.3, a number of plant-derived compounds have demonstrated to exert anti-angiogenic properties via modulation of several signaling pathways. These plant-derived compounds primarily contain phytochemicals which may have prominent physiological activity in the body (Liu 2003). These bioactive constituents play an essential role as antioxidants, mimic hormones, interfere with DNA replication, stimulate enzyme activities, or bind to cell walls. Compelling data have described the synergistic activity of plant-derived compounds as anti-angiogenic agents with other antineoplastic drugs (Wang et al. 2007; Sak 2012).

5.6.3.1 Resveratrol

Resveratrol, a polyphenol naturally found in berries, grapes, and other plant sources (Fig. 5.4), modulates tumor angiogenesis through several molecular pathways (Cao et al. 2004; Wang et al. 2015). In vitro studies found that resveratrol can significantly suppress VEGF expression in human ovarian cancer cells (A2780/CP70 and

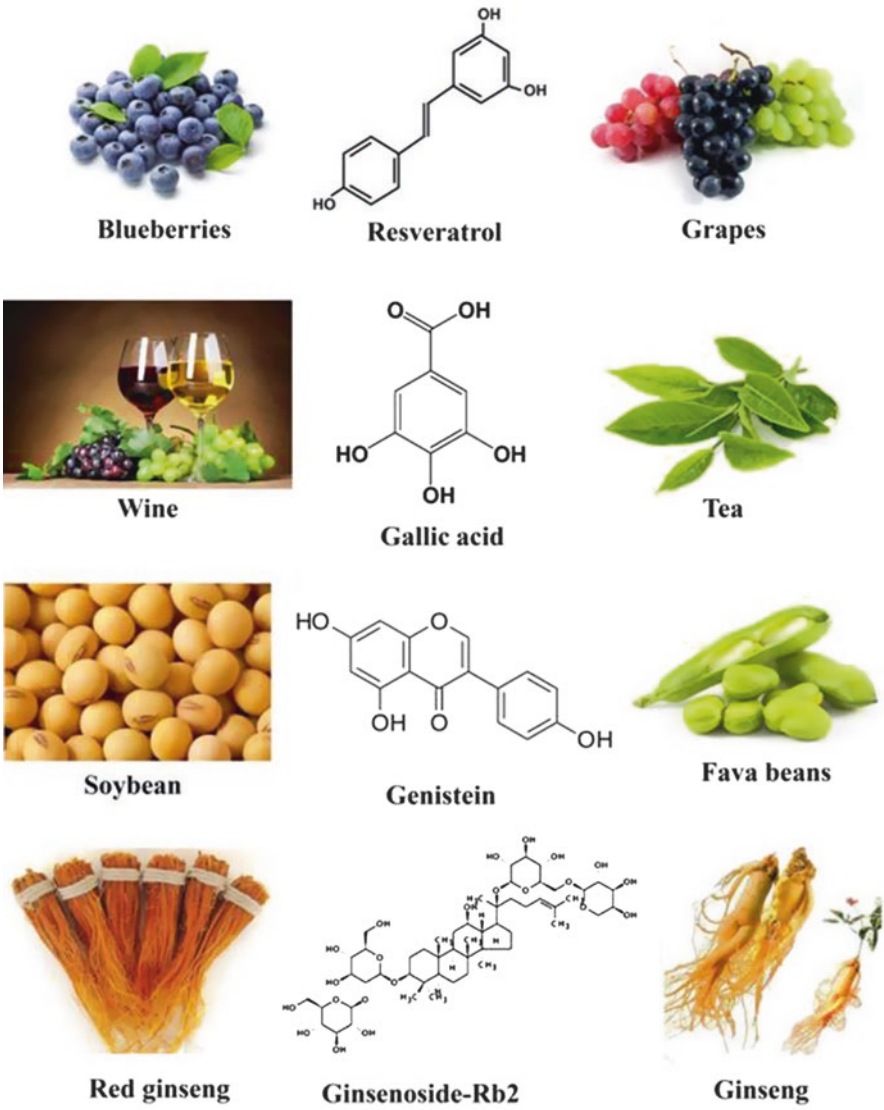

Fig. 5.4 Chemical structures of plant-derived compounds and their sources mediated angiogenesis

OVCAR-3) (Cao et al. 2004). A similar trend was also observed in in vivo study. Feeding T241 murine fibrosarcoma-bearing C57BL6 mice with 5.7 μg/mL of resveratrol has shown to suppress tumor growth via inhibition of new blood vessel formation and endothelial cell migration. In general, inhibition of cell migration was mediated through modulation of fibroblast growth factor 2 (FGF2) and vascular endothelial growth factor (VEGF) receptor-mediated stimulation of MAPK in endothelial cells (Brakenhielm et al. 2001). Moreover, resveratrol also modulates its activities via suppression of Akt- and MAPK-driven basal and insulin-like growth factor 1 (IGF-1)-mediated hypoxia-inducible factor 1 alpha (HIF-1α) expression as well as activation of proteasomal degradation of HIF-1 alpha (Cao et al. 2004).

5.6.3.2 Gallic Acid

Gallic acid is a polyphenol found primarily in berries, tea, wine, and grapes (Fig. 5.4). Gallic acid has demonstrated different biological and pharmacological properties, like antiviral, antitumor, and antibacterial activities in several human cancer cell lines including oral (Kuo et al. 2014), glioma (Lu et al. 2010a), lung (You et al. 2011), cervical (Zhao and Hu 2013), and pancreatic (Cedó et al. 2014) cancer cells. He et al. (2016) showed that gallic acid suppressed VEGF secretion and in vitro angiogenesis by HUVECs induced by the culture medium of ovarian cancer cell lines, OVCAR-3 and A2780/CP70, treated with different concentrations of gallic acid. In this study, He et al. (2016) further showed that gallic acid suppressed VEGF production via downregulation of hypoxia-inducible factor-1 alpha (HIF-1α). Hassan et al. (2014) further demonstrated that plants that are enriched with phenolic contents show a higher bio-efficacy compared than that of other plants, which adds reassuring weight to accumulating evidence showing that naturally phenolics are able to reduce the ROS in biological system. Oxidative stress generated by ROS plays a crucial role in the pathology-associated chronic disease including excessive vascularization and cancer (Kampa et al. 2007). ROS-induced cancer was shown in animal models which involved a multiple malignant transformation due to an alteration of gene expression and DNA mutations via epigenetic mechanisms and subsequently resulted an uncontrolled proliferation of cancerous cells. High expressions of ROS are usually seen in several cancerous cells (Irani et al. 1997; Yasuda et al. 1999; Yeldandi et al. 2000), and thereby suggest that ROS acts as a significant molecule in various growth-associated responses and ultimately promote tumorigenesis and angiogenesis (Ushio-Fukai and Nakamura 2008).

5.6.3.3 Flavonoids

Flavonoids, such as flavonols, flavones, flavanones, isoflavones, and anthocyanins, exhibit anti-angiogenic properties (Fotsis et al. 1997). Genistein, an isoflavonoid isolated from *Genista tinctoria* (Fig. 5.4), can suppress bFGF-mediated endothelial cell tube formation in vitro at a dosage of 150 μM by inhibition of plasminogen

activator (PA) and PA inhibitor-1 (Fotsis et al. 1993). Likewise, a low dosage of genistein (30 μM) has also been demonstrated to suppress bFGF of endothelial cells (Koroma and de Juan 1994). Other examples of plant-derived compounds are silibinin and silymarin, from the seeds and fruits of *Silybum marianum* (milk thistle), which also can suppress angiogenesis (Jiang et al. 2000; Singh et al. 2003). Feeding A/J mice with diet containing 742 mg/kg of silibinin prior to urethane administration demonstrated to inhibit growth and incidence of lung adenocarcinoma by a significant reduction in numbers of tumor-associated macrophages (Tyagi et al. 2009). Collectively, these data showed that numerous health benefits of flavonoids are attributed to their ability to act as an antioxidant.

5.6.3.4 Terpenoids and Tannins

Ginsenosides, such as ginsenoside-Rb2 and ginsenoside-Rg3, are usually isolated from the roots of red ginseng (*Panax ginseng*) (Fig. 5.4). These compounds have an ability to reduce the neovessels in murine B16 melanomas at an intravenous concentration of 10 μg or oral dosage of 100–1000 μg per mouse (Sato et al. 1994; Mochizuki et al. 1995). Nevertheless, another study also revealed that a mixture of saponins from ginseng at a dosage between 10 and 100 μg/mL may activate proliferation, endothelial cell migration, and tube formation (Morisaki et al. 1995). Besides ginsenosides, taxol is another plant-derived compound which modulates the angiogenesis. Taxol is a complex polyoxygenated diterpene derived from the bark of the Pacific yew tree (*Taxus brevifolia*). It destroys malignant tumor cells by disrupting their microtubule cytoskeleton (Foa et al. 1994) and hence demonstrates anti-angiogenic properties via inhibition of VEGF production and HIF-1α expression (Foa et al. 1994; Escuin et al. 2005).

Anti-angiogenic agent may target the endothelial cells or cancer at any necessary steps for neovascularization or carcinogenesis, including tube formation, differentiation, proliferation, or migration (Folkman 2003). Angiogenesis inhibitors may act by activating apoptosis in cells. Both in vitro experiments and in vivo models have indicated that many endogenous anti-angiogenic components may induce cytotoxicity via apoptosis (Tiwari 2012).

5.7 Effectiveness of Combined Standard Drug and Plant-Derived Compounds

Drug resistance is the primary factor that limits the chemotherapy application for cancer diseases (Waghray et al. 2015). Comparing to other monotherapy, 5-fluorouracil (5-FU) is more acceptable because sufficient concentrations usually can be administered in jaundice or hepatic dysfunction (Roderburg et al. 2011). Despite the low response rate of 5-FU monotherapy, given in combination to other

compounds, the response rates were increased until 28% (Roderburg et al. 2011). FOLFOX (5-FU, oxaliplatin, and leucovorin) regimen was reported to have a better disease control rate, higher median survival in hepatocellular carcinoma patients, and better objective response rate (Zhang et al. 2011). Therefore, administrations with combined 5-FU and other agents play a vital role in advanced hepatocellular carcinoma therapies.

Induction of apoptosis by chemotherapy drug is a complicated process, which is modulated via several signaling pathways and regulated by vast varieties of apoptosis-associated proteins (Das et al. 2010). The synergistic effect is observed in allicin and 5-FU in the induction of hepatocellular carcinoma cell death via ROS-mediated mitochondrial pathway, suggesting the therapeutic effect of allicin in hepatocellular carcinoma chemotherapy (Zou et al. 2016). From the study reviewed, allicin sensitized hepatocellular carcinoma cells to 5-FU-induced apoptosis via modulation of ROS mitochondrial pathway. In general, chemotherapy agents induce ROS and hence caused oxidative stress (Victorino et al. 2014). ROS accumulation in mitochondria may suppress the mitochondrial respiration chain and subsequently caused apoptotic cell death and mitochondrial membrane rupture (Tsuchiya et al. 2015; Gogvadze et al. 2009). A moderate elevation in ROS level was observed both in 5-FU alone and allicin groups. The ROS level has increased dramatically when the hepatocellular carcinoma cell lines are treated in combination. This finding implied the synergistic effect of ROS in combined treatment (Zou et al. 2016).

A previous study also had demonstrated that phytic acid exhibit a synergized effect along with tamoxifen and doxorubicin to suppress the proliferation of breast cancer (Tantivejkul et al. 2003). This finding indicates that phytic acid may counteract drug resistance usually observed in tumor cells and thus suggesting that it might be a useful adjunct. Interestingly, another study conducted in withanolides from *Withania somnifera* in vitro exhibited a significant reduction of human colon, breast, and lung cancer cell lines compared to that of standard drug, doxorubicin. Withaferin A, derived from the roots of *Withania somnifera*, exhibited to be more effective than doxorubicin (Jayaprakasam et al. 2003).

5.8 Conclusions and Future Prospects

Anticancer agents discovered from plant-derived compounds play a vitally important role in the treatment of cancer. Plant-derived compounds exert good immunomodulatory and antioxidant properties leading to anticancer activity. Plant-derived compounds may not serve as drugs, however they hold a great promise and indirectly provide leads in future use as a potential anticancer agents. Plant-derived compounds such as vinca alkaloids and its semisynthetic analogues and curcumin, colchicine, EGCG, betulinic acid, and podophyllotoxin derivatives have significantly affected cancer research in many aspects. They assist the researchers to gain a better understanding of the disease, providing new and efficient therapy in the development of future anticancer drugs and new mechanisms of action. Plants

represent an enormous diversity on earth; however, only a minute fraction of those have been identified. Therefore, it is expected that plants may provide potential bioactive components against numerous diseases, particularly cancer. The potential implication of the plant-derived compounds which replace conventional therapies could be significant and is warranted to be elucidated in long-term clinical trials.

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Chapter 6
Anticancer Plant Molecules for the Improvement of Immune System

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6.1 Introduction

Plants are used for human healthcare in the form of medicine as well as dietary supplements. A variety of plant molecules are being used for the treatment of pathogenic as well as non-pathogenic health issues of human. Today's most unsolved non-pathogenic health issue is cancer. Scientists and drug developers are being hilariously attracted towards molecules from natural sources for treating cancer. Out of the natural sources, plants cover the largest area of molecular source from nature. About 70% of today's natural molecule-based drugs are derived from plant sources (Chang 2002). The precised sensitivity of plant molecules for anticancer activity is being notified due to their impacts on maintaining the homeostasis of the physiological system. Plant molecules are being observed to be involved with cellular microenvironment as well as immune system to balance the homeostasis. Due to these reasons, anticancer plant molecules are also being overlooked in the ways for immune enhancement. As it is well observed in cancerous condition, immune system moves towards deflation of its activity. Therefore, inversing back the scenario by the enhancement of immune system, cancer treatment can be supported. The links between anticancer activity and immune enhancement motivate the researchers for tracing out the evidences for anticancer plant molecules which also have the ability to induce the immune system.

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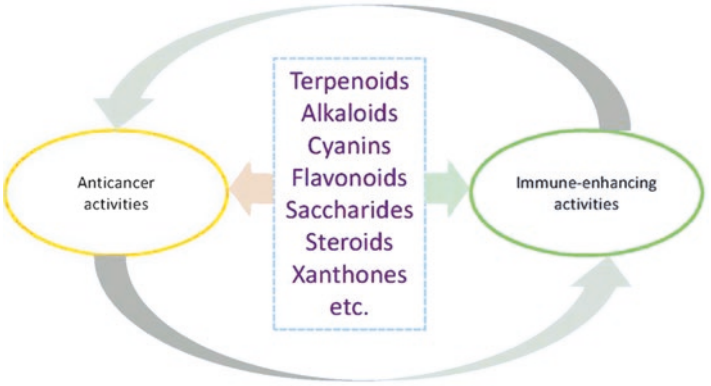

Fig. 6.1 Conceptualising the immune oncology via small bioactive molecules

Plant dietary components as well as drugs are majorly interact with the cellular microenvironment. Changes in cellular microenvironment to acidic pH by any means create a condition that motivates the cell to tend towards cancerous condition. Microenvironment is directly linked with cell-surface glycoprotein. The cellular glycoproteins transmit the signals to control nuclear transcription factors or genes via a largely distributed mesh of pathways. In this way, cellular microenvironment and cell surface becomes coordinated with immune responses. Therefore, it was assumed and concluded that cancer is linked with responses of immune system. These observations also indicate that anticancer activities are also correlated with immune responses. Therefore, it has been concluded that anticancer molecules will be of immune-enhancive nature. Considering these entire bases, the anticancer molecules are also being evaluated for their immunomodulatory activities. As we know a large number of plant-derived metabolites (terpenoids, alkaloids, cyanin, flavones, saccharides, steroids, xanthone, etc.) have potential for anticancer/antiproliferative cytotoxic activities. It is considerable that pH of these plant-derived secondary metabolites ranges near ±7.0, which creates a supportive environment for normalising the homeostasis. This information motivates the researchers for searching out the immunomodulatory specifically immune-enhancive nature of anticancer molecules from plant origin (Fig. 6.1). In this way, the most described molecules have been discussed in the following sections:

6.1.1 Terpenoids

Terpenoids as plant-derived secondary metabolites have extensive attention for chemo-preventive effects against cancer (Siveen and Kuttan 2012). They have been notified for anticancer activities through topoisomerases, cyclooxygenases, lipoxygenase and aromatase (Singh et al. 2017). It has been observed that ‘terpenoid indole alkaloids’

(e.g. vinblastine and vincristine from *Catharanthus roseus*), sesquiterpenoids (e.g. isolinderalactone from the root of *Lindera strychnifolia* and *Neolitsea daibuensis*), triterpenoid glycoside (from monk fruit), bioactive diterpenoids (as lathyranes), tetracyclic triterpenoid (cucurbitacin B), quinonemethide triterpenoid (e.g. pristimerin), pentacyclic triterpenoid (e.g. ursolic acid, celastrol), etc. are well known for anticancer activity (Mantle et al. 2000). Besides the anticancer properties, terpenoids also produce immunomodulatory activities at the system level (Baraya et al. 2017).

6.1.2 Alkaloids

Similar to terpenoids, alkaloids are also well considered for anticancer as well as immune-enhancing activities (Nowicky et al. 1991). Vinca alkaloids, taxanes, podophyllotoxin and camptothecins are being clinically used as anticancer agents (Varma et al. 2011; Yoo and Porter 1993). As plant alkaloids, sanguinarine and chelerythrine have been reported for anticancer activity against human breast adenocarcinoma cells. Alkaloids as caffeine, theophylline, gingerol, ephedrine and their derivatives have been also used for enhancement of athlete immune function (Gostner et al. 2015).

6.1.3 **Allium**

Allium spp. are known for various anticancer as well as immunomodulatory activities. Out of these *Allium sativum* holds strong potential for treatment of breast cancer (Arreola et al. 2015). Similarly flower extract of *Allium atroviolaceum* triggered apoptosis by activating caspase-3 and downregulation of Bcl-2 gene in HeLa cancer cell line. Besides anticancer activities by *Allium* spp., *Allium wallichii* and *Allium ascalonicum* have been proved as an immune-enhancive candidate by antioxidant and anti-inflammatory activities, respectively.

6.1.4 Cyanins

Anthocyanins are found from different plant sources including blueberries (Mace et al. 2014; Pribis and Shukitt-Hale 2014). These are water-soluble plant pigments of the flavonoid family (Ravindranath et al. 2004). These are recognised as natural plant pigment containing anticancer property. Their anticancer property belongs through HER-2 signalling pathway. Anthocyanin (Limtrakul et al. 2015) is known for supporting the inhibition of inflammation and cell proliferation in colorectal cancer with cilostazol and enzymatically modified isoquercitrin. Similar to flavonoids, anthocyanin from root extracts of sweet potato also shows anticancer activity against breast cancer cell lines. It is also known that *Aronia melanocarpa*

anthocyanins exhibit immunomodulatory activity in human lymphocyte and suppress the growth of human HT-29 colon cancer cells. Besides all these caspase activities were also reported by cyanin derivatives.

6.1.5 Flavonoids

Out of the all known plant secondary metabolites, flavonoids (Burkard et al. 2017) are the most observed immune-enhancing molecules along with anticancer properties. Majority of flavonoids produce clinical usable immune enhancement via NK cell activity, especially in the prevention of cancer. Quercetin (Miles et al. 2014) is a natural plant flavonoid that has the wide range health effects as anticancer and anti-inflammatory activities. Similarly, *Psoralea corylifolia*-derived neobavaisoflavone and psoralidin have been proved the TRAIL-mediated apoptosis in prostate cancer cells (Bronikowska et al. 2012; Szliszka et al. 2011). Besides the natural components, their synthetic analogues (phenoxodiol (PXD) a synthetic analogue of the plant isoflavone genistein) have also come in existence with enhanced activity (Georgaki et al. 2009). Anticancer as well as antioxidant activity of isoflavones is targeted through NF-kappa B, which is a transcription factor facilitating the angiogenesis, inflammation, invasion and metastasis (Dijsselbloem et al. 2004). Besides these, plant-originated dietary flavones were also found to regulate epigenetic modifications by altering the DNMT and HMT activities, methylation of DNA and histone proteins. Some natural flavonoid (e.g. silibinin) induces autophagy via mitochondrial dysfunction in human cancer cells (Meeran et al. 2006).

6.1.6 Saccharides

Polysaccharides (Shen et al. 2017; Ayeka et al. 2016) are ideal adjuvant in modern cancer therapy. They are nontoxic along with bioactivities such as antimetastasis, antiangiogenesis and haematopoiesis stimulator. Various polysaccharides and their protein complexes have been identified as therapeutic agents for cancer from plant sources, for example, polysaccharides from lotus seeds bear immunomodulatory and antitumour activities. Similarly, polysaccharide fraction from *Solanum nigrum* shows tumour suppression effect on breast cancer by enhancement of the host immune response (Razali et al. 2016). For enhancement of immune response, *Dioscorea* (Panthong et al. 2014) polysaccharide fraction has been notified as useful adjuvants for anticancer vaccines. Similarly, immune-enhancing activities of purslane (Li et al. 2014; You Guo et al. 2009) polysaccharides have been also observed in gastric cancer. Furthermore, tumour suppression effect via immunomodulation by polysaccharide fraction from *Solanum nigrum* has been observed for breast cancer.

6.1.7 Steroids

Steroids (Multhoff and Radons 2012) and their conjugates from plant sources are also reported for their anticancer as well as immune-enhancing properties. In this reference, *Withania somnifera* (commonly known as Ashwagandha) is a medicinal plant which produces steroidal compounds withanolides (Shareef et al. 2016). Its steroidal lactone (Grover et al. 2010) named withaferin-A arrests the tumour growth by covalent binding at cysteine-303 of β-tubulin. It is to notify that steroidal conjugates as steroidal alkaloids, steroidal saponins (Patil et al. 2014; Chhatre et al. 2014) (japonicas sp.) and glucosylated steroids (glanduliferins A and B from *Impatiens glandulifera*) are also well known for anticancer, anti-inflammatory and antibacterial activities. Steroidal saponins with a dammarane skeleton are also identified as active ingredients from ginseng plant (*Panax ginseng*), with antioxidant and anticancer activities (Baraya et al. 2017). Plant glycoside with a dammarane skeleton resembles a steroid skeleton and also has anticancer potential. Their effect on nuclear factor kappa B (NF-kB) plays a key role in immune response. It is to notify that influence on the NF-kB-inhibition activity by steroidal anti-inflammatory drugs is still unclear. But it is well described that biological activities of these steroid compounds are linked with eicosanoids and corticosteroids which are mediators of immune systems (Piotrowski et al. 2015).

6.1.8 Xanthones

Xanthone (e.g. mangiferin, a naturally occurring glucosylxanthone) is widely distributed in higher plants showing antioxidative and anticancer effects (Nunez Selles et al. 2016; Saha et al. 2016; Gold-Smith et al. 2016; Zou et al. 2014). Besides mangiferin, other xanthones have been identified from *Garcinia mangostana*, which act against human hepatocellular, breast and colorectal cancer cell lines. Furthermore furano- and pyranoxanthone natural products and caged xanthone are also of interest for cytotoxic potency and novelty in their mechanism of action to serve as clinically effective lead compounds for anticancer activity (Bronikowska et al. 2012).

6.2 Immune Enhancements by Plant Molecules

Plant molecules induce immune system in multiple ways by free radical scavenging (Saha et al. 2016), nitric oxide synthase (Shen et al. 2017), catabolism of cGMP (Petrovsky and Cooper 2015), histidine decarboxylase (Medrano et al. 2010; Galili and Anaraki 1995; Perentesis et al. 1992), neutrophil degranulation (Reina et al.

2013), thymidine uptake, xanthine oxidase, lymphocyte proliferation, antioxidant activity and synthesis of immunoglobulin. A wide variety of compounds are known which are famous for both anticancer and immunomodulatory activities: xanthonoid, polyphenols, glycosideflavonoids, proteins and peptides (Hernandez-Ledesma and Hsieh 2017), alkaloid, terpenoid, steroid, curcumin, arctigenin, glabridin and ajoene, ß-carotene, epigallocatechin-3-gallate, quinic acid and ginsan, resveratrol, osthole (7-methoxy-8-(3-methyl-2-butenyl)-2H-1-benzopyran-2-one), benzofuran derivatives (Agrawal and Pal 2013), casticin, biotoxins (snake venom, bee venom, some bacteria toxins and plant toxins), anthocyanins and procyanidins and organosulfur compounds (e.g. diallyl sulphide) (Viswanathan et al. 2014). Some of the most important plant molecules/group of compounds have been tabulated for the reference (Table 6.1).

Table 6.1 Most prominent anticancer plant molecules containing immune-modulator impacts

Molecule/compound group	Plant	Cancer/immune response	References
Anthocyanins; procyanidins	*Aronia* plants	Antioxidative, antimutagenic, anticancer, cardioprotective, hepatoprotective, gastroprotective, antidiabetic, anti-inflammatory, antibacterial, antiviral, radioprotective and immunomodulatory	Kokotkiewicz et al. (2010)
Mangiferin	–	Antioxidative, antiviral, anticancer, antidiabetic, immunomodulatory, hepatoprotective and analgesic effects	Pardo Andreu et al. (2010)
Euphorbin	*Euphorbia* spp.	Inhibition of tumour cell growth and stimulation of lymphocytes	Amirghofran et al. (2011)
Neobavaisoflavone and psoralidin	*Psoralea corylifolia*	Enhanced trail-mediated apoptosis in prostate cancer cells	Szliszka et al. (2011)
Amentoflavone and polysaccharide fraction	*Biophytum sensitivum*	Antioxidant, immunomodulatory, anticancer, anti-inflammatory, chemoprotective, antidiabetic	Bharati and Sahu (2012)
Alkaloids and polysaccharides	*Dendrobium* genus	Antioxidant, anticancer and neuroprotective activities	Ng et al. (2012)
Biotoxins	–	Novel anticancer agent	Liu et al. (2014)
Casticin	Variety of plant sp.	Anti-hyperprolactinemia, antitumour, anti-inflammatory, neuroprotective, analgesic and immunomodulatory agent	Rasul et al. (2014)

(continued)

Table 6.1 (continued)

Molecule/compound group	Plant	Cancer/immune response	References
Alkaloids (warifteine, methylwarifteine, berberine, hayatin and hayatidin)	Cissampelos pareira; *Cissampelos sympodialis*	Anti-allergic, immunosuppressive, antidepressant, anticancer, vasodilatory and muscle relaxant activities	Semwal et al. (2014)
Alkaloids, diterpenoid lactones, glycosides, steroids, phenol, aliphatic compounds and polysaccharides	*Tinospora cordifolia*	Anticancer and immunomodulatory activities	Bala et al. (2015)
Steroid sapogenin (diosgenin)	–	Anticancer, cardiovascular protective, antidiabetic, neuroprotective, immunomodulatory	Chen et al. (2015)
Polyphenols	–	Anti-inflammatory, antimicrobial, antiviral, anticancer and immunomodulatory properties	Fantini et al. (2015)
Benzofuran derivatives	Egonol, homoegonol and moracin families	Anticancer, antimicrobial, immunomodulatory, antioxidant and anti-inflammatory properties	Naik et al. (2015)
Triterpenoids, diarylheptanoids, phenylbutanoids, lignans, phenolics and flavonoids	Genus *Betula*	Immunomodulatory, anti-inflammatory, antimicrobial, antiviral, antioxidant, antidiabetic, dermatological, gastroprotective and hepatoprotective	Rastogi et al. (2015)
Taxol, diterpenoids, lignans, steroids, sterols and biflavonoids	*Taxus wallichiana* (Himalayan yew)	Antiepileptic, anti-inflammatory, anticancer, antipyretic, analgesic, immunomodulatory and antimicrobial activities	Sharma and Garg (2015)
Osthole	*Cnidium monnieri* and *Angelica pubescens*	Neuroprotective, osteogenic, immunomodulatory, anticancer, hepatoprotective, cardiovascular protective and antimicrobial activities	Zhang et al. (2015)
Curcumin, arctigenin, glabridin, ajoene, β-carotene, epigallocatechin-3-gallate, quinic acid and ginsan	*Glycyrrhiza glabra, Uncaria tomentosa, Camellia sinensis, Panax ginseng, Prunus armeniaca, Allium sativum, Arctium lappa* and *Curcuma longa*	Immunomodulatory for breast cancer treatment via anti-inflammatory and lymphocyte activation properties	Baraya et al. (2017)

(continued)

Table 6.1 (continued)

Molecule/compound group	Plant	Cancer/immune response	References
Polyphenols	–	Anti-inflammatory, antimicrobial, antiviral, anticancer and immunomodulatory agents	Costa et al. (2016)
Resveratrol	–	Enhancement of immune function and activation of NF-κb for immunomodulatory and anticancer effects	Lai et al. (2016)
Phenolic glycosides and flavonoids: eupalitin, rotenoids and alkaloid (betanin and punarnavine)	*Boerhavia*	Anti-hepatitis; against urinary disorders, gastrointestinal diseases, inflammations, skin problems, infectious diseases and asthma	Patil and Bhalsing (2016)
Xanthonoid (mangiferin, a yellow polyphenol having C-glycosyl xanthone structure)	Mango	Antioxidant, anti-inflammatory, antidiabetic, anticancer, antimicrobial, analgesic and immunomodulatory properties	Saha et al. (2016)
Organosulfur compounds (diallyl sulphide)	*Allium*	Anticancer, antimicrobial, anti-angiogenic and immunomodulatory activity	Suman and Shukla (2016)
Cycloartane triterpenoid (cimigenol)	*Cimicifuga*	Apoptotic and autophagic cell death in human colon cancer HT-29 cells	Dai et al. (2017)
Triterpenoid saponins	*Anemone*	Anticancer, immunomodulatory, anti-inflammatory, antioxidant and antimicrobial activities	Hao et al. (2017)

6.3 Conclusions and Future Prospects

The immunomodulatory studies showed that the immune system is improved by the action of anticancer molecules from the plant origin. Thus, it has been considered that anticancer plants showed anticancer activity under the flag of immune oncology of plant-based small molecules. This may be an interesting area of research for future investigators for understanding the disease as well as reliable drug development for the treatment of cancer.

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Chapter 7
Fermented Food-Derived Bioactive Compounds with Anticarcinogenic Properties: Fermented Royal Jelly As a Novel Source for Compounds with Health Benefits

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7.1 Introduction

A wide range of whole foods and their components have been shown to provide a variety of health-promoting benefits. The quest for foods that help prevent and manage certain chronic diseases has led the food industry and researchers to produce functional foods with added nutraceuticals providing antioxidant, antibacterial, anticancer, and antidiabetic properties, among other bioactivities (Ames et al. 1993;

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Gutteridge and Halliwell 1994). Nutraceuticals can be either natural or synthetic food ingredients. Natural nutraceutical components can be isolated from foods or produced through fermentation and enzymatic processing, and some of them include ω-3 fatty acids (Jacobsen and Let 2006; Hjaltason and Haraldsson 2006), probiotics (Chaila et al. 2005; Salem et al. 2006), prebiotics (Brink et al. 2005; Malcata et al. 2005), synbiotics (D'Antoni et al. 2004), vitamins (Baro et al. 2003), dietary fiber (Fernandez-Gines et al. 2004; Fernandez-Lopez et al. 2007), phytochemicals (Wolfs et al. 2006), and bioactive peptides (Korhonen and Pihlanto 2006; Thoma-Worringer et al. 2006).

Royal jelly is a milky-white secretion produced by the mandibular and hypopharyngeal glands of worker *Apis mellifera* L. bees for feeding bee larvae and the adult queen bee. The chemical composition of royal jelly on a percent weight basis includes lipids (3-6%), carbohydrates (15%), proteins (18%), water (50-60%), vitamins (less than 1%), and minerals (1.5%) (Nagai and Inoue 2004), in addition to a large number of bioactive substances including fatty acids (Vucevic et al. 2007) such as 10-hydroxy-2-decenoic acid (Caparica-Santos and Marcucci 2007), peptides (Tokunaga et al. 2004), and flavonoid phenolics (Kucuk et al. 2007). The variation of bioactive compounds such as peptides and phenolics in royal jelly depends largely on the floral sources and on postharvest conditions. Hydrolysis of food proteins from plants and animals has many industrial applications for nutraceutical, food as well as pharmaceutical uses, including the enhancement of the nutritional, functional and antioxidant properties of food products (Wang and de Mejia 2005). For example, bioactive peptides isolated from fermented foods or from the enzymatic digestion of food proteins from milk (Ryhanen et al. 2001), chickpeas, and mushrooms (Ma et al. 2006) can inhibit the angiotensin-converting enzyme (ACE), which can confer antihypertensive properties.

Although animal proteins provide the indispensable amino acids needed for growth and maintenance of health, the relatively high cholesterol and saturated fat content of animal foods and their high ecological footprint have led to the search for alternative sources of dietary proteins (Sauvaget et al. 2004). Additionally, there is increasing interest in the by-products of fermented or hydrolysed proteins, namely, protein hydolysates and their constitutive peptides, for their antidiabetic, antihyper-

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tensive, and anticarcinogenic properties. Consumption of fermented plant proteins has been associated with a multitude of health benefits including the relief of gastritis, antioxidant activity, cholesterol-lowering and antiallergenic effects as well as chemoprevention of breast, prostate, and colon cancers. There has been limited research, however, regarding the health-promoting properties of fermented royal jelly despite evidence of the presence of high levels of certain bioactive compounds in royal jelly in general. The aim of the present chapter is to discuss the various aspects related to the fermentation of foods in general and of royal jelly in particular as a source of natural nutraceutical components that can be used to develop novel functional foods.

7.2 Fermentation Process

Fermentation can be defined as a metabolic process resulting from the action of microorganisms, which can be attributed to the transformation of fermentable carbohydrates leading to the production of various organic acids, alcohol and other compounds anaerobically. Various microbial enzymes are involved in food fermentation, including proteolytic enzymes responsible for the production of peptides from protein-rich substrates. Historically, fermented foods have long been used as staples in the form of beverages and other food products in the human diet. Due to the myriad of microbial combinations that can be present in fermented foods, an extraordinary diversity of fermented food products have been consumed worldwide in nearly every culture. Despite the long historical culinary importance and popularity of fermented foods, the industrialization of food production over the past century has drastically reduced the diversity of fermented foods in the diet, particularly in Western countries (Chilton et al. 2015). Nowadays, however, there is a resurgence of consumer interest in fermented food products, motivated by mounting evidence of their health benefits (Ebner et al. 2014). The microbiota present in fermented products have recently been found to generate health-promoting effects linked to the host's immune system, energy metabolism, as well as brain health (Hovhannisyan et al. 2009). Studies have indicated a bidirectional communication between the brain and gut along the brain-gut axis, which can be adversely affected by dysbiosis of the gut microbiota and may be associated with anxiety, depression, and other illnesses (Tillisch et al. 2013). Conversely, the administration of probiotics similar to the ones found in some fermented foods has been shown to result in anxiolytic and antidepressant activities in animal models (Tillisch et al. 2013). Moreover, probiotic-rich diets have been shown to exert positive effects on stress relief and memory enhancement, potentially mediated by improvement of the gut microbiota (Messaoudi et al. 2011).

7.2.1 Health Benefits of Fermentation

Fermentation is an effective method of food preservation that reduces the risk of microbial contamination due to enrichment of antimicrobial compounds such as organic acids, bacteriocins, and sometimes ethanol as end products. In addition to

enhanced shelf life, the end products of fermentation provide new taste, flavor and texture sensations. Moreover, some foods are not edible without the microbial-mediated transformation of the food material. For example, table olives contain phenolics that are bitter in taste, which are degraded during microbial fermentation, resulting in edible olives. Another example is *Saccharomyces cerevisiae*, also known as bakers' yeast, which, either alone or with lactic acid bacteria (LAB), achieves dough leavening during bread making. Mounting evidence suggests that certain fermented foods promote human health in ways not directly attributable to the original unfermented food (Eussen et al. 2016). Many cohort studies support strong relationships between the consumption of fermented dairy foods and weight maintenance (Mozaffarian et al. 2011). Likewise, long-term prospective studies suggest reductions in the risk of diabetes, heart disease, and overall mortality by frequent consumption of yogurt (Soedamah-Muthu et al. 2013; Chen et al. 2014a). Similarly, there is evidence for antidiabetic and anti-obesity benefits of kimchi, a fermented vegetable product (An et al. 2013). Health benefits of fermented foods have also been proposed for inflammatory bowel diseases, arthritis and multiple sclerosis, although supportive clinical data are lacking (Lorea Baroja et al. 2007). In addition, the consumption of certain fermented foods has also been suggested to improve brain activity and mood (Tillisch et al. 2013; Hilimire et al. 2015). Health benefits may include immediate physiological responses, such as those provided by fermented milk consumption, which was found to reduce muscle soreness and improve glucose metabolism after acute resistance exercise (Iwasa et al. 2013).

7.2.2 Transformation of Food Constituents by Fermentation

Fermentation leads to the enzymatic breakdown of some of the raw food constituents mediated by the metabolic activity of microorganisms, which modifies the nutritive and bioactive properties of food materials with potentially beneficial effects for human health. For instance, many cheeses are well tolerated by lactose-intolerant individuals because lactose is separated into the whey during milk fermentation and is previously converted into well tolerated compounds. Yogurt is also well tolerated by lactose-intolerant individuals due to the action of beta-galactosidase enzymes produced by yogurt microbial cultures (Savaiano 2014). Microbial beta-galactosidases can survive the low pH environment of the stomach as they are protected by the buffering capacity of yogurt and physically protected within bacterial cells. On this basis, the European Food Safety Authority indicates that live microbial cultures in yogurt improve the digestion of yogurt lactose in individuals with reduced ability to digest lactose (Hill et al. 2014). One of the by-products of dairy food fermentation, conjugated linoleic acid, a fatty acid generated by the action of linoleate isomerase produced by some lactic acid bacteria (LAB), exhibits promising atheroprotective properties. Some LAB also display proteolytic activities in milk and other foods, which can increase the concentrations of bioactive peptides and polyamines (Pessione and Cirrincione 2016). Numerous studies have

isolated bioactive peptides and peptide fractions from fermented dairy products including dahi, yogurt, kefir, and sour milk. Such peptides have been shown to possess various beneficial biological activities, including antioxidant, osteogenic, antihypertensive, antithrombotic, opioid, appetite regulation (satiety), and immunomodulatory effects (Pihlanto and Korhonen 2014). There has been a particular research focus on peptides generated by fermentation which display antihypertensive activities by inhibition of the angiotensin-converting-enzyme (ACE) (Fekete et al. 2015). LAB-mediated fermentation of plant foods can also enhance the conversion of phenolics such as flavonoids into bioactive metabolites via the action of microbial phenolic acid reductase, glycosyl hydrolase, decarboxylase, and esterase enzymes (Filannino et al. 2015). The subsequent reaction of these bioactive metabolites with anthocyanidins results in the formation of pyranoanthocyanidins or 3-desoxypyranoanthocyanidins. Some of these alkyl catechols potently activate the nuclear response factor-2 (Nrf2), a key regulator of the mammalian oxidant stress response that induces expression of antioxidant and detoxifying enzymes protecting against oxidative and chemical damage (Senger et al. 2016). Fermentation can also result in the degradation or removal of undesirable or allergenic food constituents such as gliadin and phytic acid. The latter coumpound, found in cereals and grains, is a known anti-nutritional factor that chelates divalent metal ions, which can lead to micromineral deficiencies involving iron, zinc, and copper in humans and animals. The fermentation of cereal products lowers the pH, which activates the endogenous enzyme phytase responsible for removing phytic acid. Sourdough fermentation and extended fermentation times in breadmaking can also reduce the amount of fermentable compounds such as monosaccharides, disaccharides, and oligosaccharides. Reduction in the content of fermentable compounds in wheat and rye bread can improve the tolerance of these compounds in persons with irritable bowel syndrome (Laatikainen et al. 2016).

7.2.3 Biosynthesis of Bioactive and Nutritive Compounds

Fermentation leads to the synthesis of new metabolites with promising health-modulating activities, such as lactic acid (or lactate), which can attain concentrations of up to 1% concentration during LAB-mediated fermentation. Lactic acid was recently shown to reduce pro-inflammatory cytokine secretion from dendritic cells and bone-marrow-derived macrophages in a dose-dependent manner when Toll-like receptors are activated (Iraporda et al. 2015). In addition, lactate can modify the redox status by reducing the intensity of reactive oxygen species (ROS) production in enterocytes (Kahlert et al. 2016). Microbial fermentation also results in the production of B vitamins such as folate, riboflavin, and vitamin B_{12} from different non-vitamin substrates present in dairy- and plant-based foods (Sych et al. 2016). Additionally, microbial metabolites with immunomodulatory functions and neurotransmitter activity (e.g., γ-aminobutyric acid) are produced by fermentation. Food fermentation was further shown to generate proteins and

exopolysaccharides with antioxidant attributes (Li et al. 2014), immune-stimulatory activities preventing the adhesion of pathogens to the intestinal mucosa (Chen et al. 2014b), as well as hypocholesterolemic effects (Makino et al. 2016).

7.2.4 Delivery of Commensal Microbes to the Gastrointestinal Tract

Many fermented foods such as yogurt, kefir, cheese, sauerkraut, dry fermented sausage, kimchi, miso, and kombucha usually contain viable microbial cells ranging between 10^7 and 10^9 cells/ml or cells/g, which are concentrations recommended for probiotic health benefits (Derrien and van Hylckama Vlieg 2015). The consumption of fermented products can thus increase the presence of health-promoting bacteria (also known as probiotics) in the diet by approximately 10,000-fold (Marco et al. 2017). The regular consumption of these foods amounts to supplying new and beneficial (albeit transient) bacteria to the host intestinal microbiota (Chilton et al. 2015). The matrix of some fermented foods can improve the long-term survivability of these microorganisms in the food, which enhances the delivery of large numbers of beneficial bacterial strains to the gastrointestinal (GI) tract. Fermented foods are thus a promising solution for providing health-promoting probiotic strains to consumers and patients (Kort et al. 2015). It has been hypothesized that such microbial exposures are beneficial for the normal development and functioning of the neural and immune systems (Stefka et al. 2014; Campbell et al. 2016). Conversely, Western diets may be adversely affected by highly processed and over-sanitized foods which have substantially decreased the dietary exposure to health-promoting bacteria.

7.2.5 Probiotic Features of Microorganisms in Fermented Foods

Mounting evidence suggests that the regular intake of fermented products rich in live probiotic microorganisms can confer a variety of health benefits that reduce the risk of chronic diseases and other ill-health conditions. Thus, beyond their nutritional value, probiotic-rich foods have high marketing potential as they can meet consumer demand for alternatives to medicinal drugs (Chilton et al. 2015). Many of the microbial species present in fermented food products are either identical to or share physiological characteristics with species known to enhance GI tract health. Cheese produced using starter culture strains showing in vitro anti-inflammatory potential was demonstrated to protect against colitis and gut epithelial cell damage in a mouse model as opposed to control cheese which showed no protective effects (Ple et al. 2015). The concept that live microorganisms in fermented foods play beneficial roles in the host GI tract concurs with the view that the core health benefits of probiotic cultures can be ascribed to a species (LAB for instance) rather than

to select strains within a species (Hill et al. 2014). Some fermented LAB-containing foods (e.g., kimchi, sauerkraut, yogurt) are thus expected to exert beneficial effects similar to those conferred by the intake of the same species of probiotic lactobacilli provided as a supplement. For example, the ingestion of viable microorganisms from fermented foods could positively impact the intestinal epithelial immune cells and enteroendocrine cells in ways similar to the action of the isolated probiotic strains. Food-associated bacteria including *L. plantarum* and *L. rhamnosus* (Wu et al. 2015), *L. reuteri* (Gao et al. 2015), and *P. freudenreichii* (Kwon et al. 2016) have demonstrated the potential to directly modify the host response in the GI tract, which could be partly mediated by microbial metabolites such as histamine released by *L. reuteri* (Gao et al. 2015). Fermentation-related microorganisms may change the composition and functionality of the gut microbiota, although the extent of these modifications and their significance for health and chronic disease prevention remain uncertain (Kolmeder et al. 2016). These changes can occur via direct inhibition or stimulation of microbial competitors, trophic interactions involving the production of short-chain fatty acids (SCFA), as well as indirect effects by affecting the host (Kato-Kataoka et al. 2016). The above mechanisms are wide ranging and are not limited to specific strains only. As with probiotic supplements, fermentation-associated microorganisms in foods are likely influenced by gut-associated factors and by the host diet. The beneficial effects of these microorganisms on the host microbiota are expected to be relatively short-lived, but physiologically important (Zhang et al. 2016), which underscores the importance of regular dietary intakes of probiotic-rich foods. In terms of regulatory issues, only a few countries such as Canada and Italy enlist microbial species for consideration as probiotics in their regulatory guidelines, whereas foods are not allowed to use or mention "probiotics" or "contains probiotics" on food labels in most European Union member states (Kato-Kataoka et al. 2016).

7.3 Cancer As a Deadly Disease

One of the most common and deadly chronic diseases nowadays is cancer (Grandis and Sok 2004). Cancer is an umbrella term for different forms and types of this disease, which can affect any part of the body. Related terms include neoplasms and malignant tumors. Cancer arises from the transformation of normal cells into tumor cells in a multistage process that generally progresses from a precancerous lesion to a malignant tumor. Cancer can also be described as the activation or mutation of abnormal genes that control cell growth and division. It is frequently defined as the rapid production of abnormal cells that proliferate beyond their usual limits, which can subsequently damage neighboring parts of the body and spread to other organs. The latter process is known as metastasis and is a frequent cause of mortality in cancer patients. Cancer is the second leading cause of death worldwide and was responsible for 8.8 million deaths in 2015. All over the globe, nearly one in six deaths are due to cancer (WHO 2016). About 600,920 cancer deaths and 1,688,780

new cancer cases were anticipated in 2017 in the United States alone. Overall, the ratio of cancer incidence in men is 20% higher than in women, and the ratio of cancer death is 40% higher in men. Most deaths from cancer are from lung cancer (1.69 million deaths), followed by liver (788,000 deaths), colorectal (774,000 deaths), stomach (754,000 deaths), and breast (571,000 deaths) cancers (WHO 2016). Cancer evolves through several stages, starting with a single transformed cell that leads to proliferation, invasion of tissues, and, in some cases, metastasis (Shishodia et al. 2003). Cancer development is thought to result from interactions between genetic and environmental factors. The latter include dietary habits and three broad categories of external agents: physical carcinogens (e.g., ionizing radiations and UV); chemical carcinogens (e.g., tobacco smoke components, asbestos, and certain food and water contaminants such as aflatoxin and arsenic, respectively); and biological carcinogens (e.g., infections from certain bacteria, viruses, and parasites). Aging is an important risk factor for the progression of cancer as the incidence of cancer increases dramatically with age, most likely due to an accumulation of risks for specific types of cancer over time. This general tendancy is further accentuated by the reduced effectiveness of cellular reparation and protection mechanisms with aging.

7.3.1 Colorectal Cancer

Among the various types of cancers, colorectal cancer (CRC) is one of the most common causes of deaths worldwide, especially in Western countries. Over the previous quarter century, CRC has affected almost 1,000,000 individuals every year. It is estimated that 5% of Americans will be diagnosed with CRC in their lifetime (Rafter et al. 2007). Approximately 70% of CRC have been associated with environmental factors, including diet and a sedentary lifestyle. Other risk factors include age, as the incidence and death rates for CRC rise with age, and about 93% of deaths occur in the ages of 50 years and older. The median age at colon cancer diagnosis is 73 years in women and 69 years in men, which is older than the median age at rectal cancer diagnosis, which is 65 years in women and 63 years in men (Ferlay et al. 2010). Overall, colorectal cancer incidence and mortality rates are higher in men than women. The causes for this gender difference are not well understood but likely reflect complex interactions between sex-related differences in exposure to hormones and other risk factors. Insufficient physical activity is also a factor that increases CRC risk. It has been suggested that almost 30–50% of cancers can be prevented by lowering exposure to modifiable risk factors and implementing various lifestyle-related prevention strategies as well as early detection. Early detection of CRC and management of patients greatly improve CRC prognosis as there is a higher chance of recovery if diagnosed early and properly treated.

Colorectal cancer proliferates in the colon or the rectum, which are parts of the large intestine. This cancer rarely develops in the small intestine in comparison to the colorectum where it proliferates slowly and can take 10–20 years to develop.

Mostly, CRC begins as polyp, which is a noncancerous outgrowth that develops on the inner lining of the colon or rectum. Adenoma or adenomatous polyp is the most common type of polyp. Adenomas arise from glandular cells, which lubricate the colorectum by producing mucus. An estimated one-third to one-half of all individuals will eventually develop one or more adenomas (Steliarova-Foucher et al. 2005). Although all adenomas have the capacity to become cancerous, only less than 10% are estimated to result into invasive cancer. As the adenoma becomes larger, there is an increasing chance for it to develop into cancer. Adenocarcinoma is a type of cancer that develops in glandular cells and almost 96% of colorectal cancers are adenocarcinomas (Hilimire et al. 2015). Once cancer forms in the lining of the colon or rectum, it can spread into the wall of the colon or rectum, and may then reach the lymphatic or blood circulation. Typically, cancer cells first invade the nearby lymph nodes, which are bean-shaped structures that assist in fighting infections. These cancerous cells can also be transported through the blood vessels to other vital organs such as the lungs and liver. The cancer can also invade the pelvis and abdominal cavity and reach other organs and tissues such as the ovaries and peritoneum. When cancerous cells spread to distant parts of the body, it is called metastasis (Edge and Compton 2010). The extent to which cancer has spread at the time of diagnosis, also known as the cancer's stage, is crucial for prognosis and for assigning proper treatment.

A major cause of the initiation of carcinogenesis is the damage of DNA stimulated by mutagenic factors such as free radicals (Diplock et al. 1999). Carcinogenesis can be induced by mutations in the genes that control the growth and differentiation of cells and, in this manner, gene mutations may lead to initiation, promotion, and progression of the cancer. There are several mechanisms involved in the destruction and growth inhibition of cancer cells. One mechanism involves an increase of the immunological activity for identification of the cancer cells by immune factors that include tumor necrosis factor (TNF)-α produced by macrophages and TNF-β produced by lymphocytes. At the beginning of inflammation, the pro-inflammatory immune response involves fundamental pro-inflammatory cytokines, including TNF-α and interleukin (IL-1 and IL-18) (Akcay et al. 2009). As part of the inflammatory response, TNF-α, a product of immune initiation (Santander et al. 2012), induces mononuclear phagocytes to produce IL-1 and IL-6 (Hamdi et al. 2006; Powell et al. 1998). TNF-α plays a major role in affecting the tumor microenvironment and cancer progression (Balkwill 2006) via its soluble form generated by post-proteolytic cleavage or as a membrane-integrated protein (Thichanpiang and Wongprasert 2015). Inflammation and cell proliferation are regulated by TNF-α binding to the TNF main receptor (TNFR), which affects protein adaptors including the "death domain protein" (TRADD), receptor-interacting protein (RIP), and receptor associated factor (TRAF) that regulate apoptosis (MacEwan 2002). Some studies have suggested that dietary antioxidants such as β-carotene and α-tocopherol can increase the production of cytotoxic T-cells, which activates the production of cytokines and facilitates cytokine migration to cancer cells that can lead to the destruction of proliferating tumor cells. Another anticancer mechanism involves the promotion of the expression of the wild type of p53 gene that inhibits tumor cell growth. Antioxidants have been shown to promote the expression of this gene, while decreased expression of p53 mutants

promotes carcinogenesis. Vitamin A in the form of β-carotene has been suggested to inhibit carcinogenesis in this manner (MacPhee 1998; Ferguson 2001).

7.3.2 *Role of Fermentation in CRC Prevention and Amelioration*

There is strong evidence that lifestyle factors such as diet play a significant role in the development of certain cancers including CRC (Martínez et al. 2008). Increasing evidence from in vitro, animal and human studies suggests that probiotic-rich fermented milk products provide protection against CRC. Although cohort studies to date have failed to detect a significant effect of fermented milk intake against CRC, most case-control studies suggest a protective role. There are also indications of an inverse relationship between the incidence of colon cancer and probiotic consumption (Davis and Milner 2009). Additionally, interventional studies have demonstrated a change in intermediate markers of CRC risk in human subjects, from a high- to low-risk pattern, following the consumption of fermented milk or probiotic cultures (Heavey et al. 2004). Fermented products rich in live probiotic cultures may decrease the risk of exposure to carcinogens via several mechanisms including: (a) detoxification of ingested carcinogens, (b) alteration of the intestinal environment leading to decreased activation of carcinogens from pro-carcinogens, (c) increased production of SCFAs such as butyrate which enhance apoptosis, and (d) production of metabolites that hinder the growth of cancerous cells or stimulate the defense mechanisms against cancer cell invasion (Davis and Milner 2009).

Modulation of the composition of the gut microbiota has been suggested as a mechanism by which the etiology of colon cancer could be positively affected. Intestinal microflora modifications can occur via the ingestion of probiotics that directly improve the intestinal microbial balance and by ingesting prebiotics which are fermentable compounds that support the growth of probiotics, thereby facilitating specific changes in the composition and activity of the gut microbiota (Grootaert et al. 2009). Possible mechanisms by which probiotics may inhibit colon cancer involve alteration of the metabolic activities of the intestinal microflora and of the physicochemical conditions in the colon, binding of potential carcinogens, and production of antitumorigenic or antimutagenic compounds such as SCFAs. Most prebiotics are nondigestible oligosaccharides. In humans, they are not digested in the small intestine and are thus fermented in the colon where they can serve as substrates supporting the growth of probiotic microorganisms (Grootaert et al. 2009). Inulin and fructo-oligosaccharides are the most studied oligosaccharide prebiotics. Dietary fibers such as arabinoxylan, the main nondigestible polysaccharide in whole cereal grains, also possess prebiotic properties (Sengupta et al. 2006). Dietary fibers from fruits, vegetables, whole grains and legumes undergo microbial fermentation in the GI tract and some of their prebiotic properties may contribute to the pre-

vention of colon cancer (Sekhon and Jairath 2010). Fermentation mediated by dietary fiber intake can result in a selective stimulation of growth and activity of the gut microflora, especially *Lactobacilli* and *Bifidobacteria*. Moreover, microbial fermentation of dietary fibers in the GI tract leads to the production of SCFAs such as acetate, butyrate and propionate. Butyrate has been studied the most for its potential roles in colon cancer prevention and treatment as chemopreventive agent (Scharlau et al. 2009). While acting as an energy source for non-cancerous colonic cells, butyrate may also reduce the survival of tumor cells by inhibiting their proliferation and by inducing differentiation and apoptosis, which can inhibit the promotion and progression of cancer (Sengupta et al. 2006).

One human intervention trial has suggested that synbiotic products, a combination of probiotics and prebiotics, are more effective than prebiotics or probiotics alone in terms of cancer prevention (Rafter et al. 2007). Dietary fibers and prebiotics may protect against colon cancer by inhibiting the formation of carcinogens and ROS in the GI tract and by decreasing the exposure to these compounds. ROS are extremely reactive oxidants produced during many physiological processes including detoxification of foreign compounds, immune response, cell metabolism, as well as aerobic respiration (Valko et al. 2004). ROS may contribute to carcinogenesis when oxidative stress (an imbalance between the production and neutralisation of ROS) results in elevated cellular levels of ROS, which can damage DNA, lipids, and proteins, thereby contributing to the initiation, promotion, and progression of cancer (Hamer et al. 2009). Mammalian cells express different types of protective stress-response enzyme systems against oxidative stress such as catalase (CAT) and superoxide dismutase. Hydrogen peroxide (H_2O_2), a highly reactive ROS, is rapidly detoxified by CAT, and high levels of CAT expression and activity have been associated with a reduction of DNA damage and a lower risk for cancer (Skrzycki et al. 2009).

Some findings suggest that the regular ingestion of live *L. casei* might delay the reappearance of bladder tumors (Aso et al. 1995), but these findings await confirmation. The preventive effect of probiotics on carcinogenesis may be mediated via their action on the gut microbiota (Valko et al. 2004). Carcinogens are cancer-causing chemicals that can be ingested or generated by the metabolic activity of certain intestinal microorganisms (Marco et al. 2017). One hypothesis for the prevention or delay of tumor development by LAB is that these bacteria may bind to mutagenic compounds in the intestine (Isolauri et al. 2004), thereby decreasing the absorption of or exposure to these carcinogenic compounds. Mutagenic potency is usually estimated in vitro using the Ames test. However, it should be noted that mutagen-related cancer risk in humans can differ by more than a thousandfold among individuals (Kailasapathy and Chin 2000). Probiotics may also suppress the growth of bacteria that convert pro-carcinogens into carcinogens, thereby reducing the levels of carcinogens present in the gut. In that regard, the activity of the enzymes that convert pro-carcinogens into carcinogens has been used as an indicator of the capability of probiotics to limit intestinal microflora-mediated bioactivation of pro-carcinogens.

7.3.3 Mechanisms of Cancer Chemoprevention Involving Fermented Foods

Cancer chemoprevention is characterized by the use of natural, synthetic, or biologic substances to reverse, suppress, or prevent the development of cancer. The three phases of chemoprevention involve primary prevention, secondary prevention, and therapy (Scharlau et al. 2009). Primary prevention is the inhibition of initiation, the first step of carcinogenesis, by reduction of toxicity or induction of detoxification. The latter actions are called blocking activities, and occur via prevention of the formation of the ultimate carcinogens and ROS, which involves antioxidative effects (Cummings 1981). Agents that affect secondary prevention are called suppressing agents. The promotion of initiated cells to pre-neoplastic cells is inhibited by secondary prevention via the reduction of cell growth or the enhancement of differentiation and apoptosis of the initiated cells (Skrzycki et al. 2009). Blockage of the progression of pre-neoplastic cells to neoplastic cells, which occurs relatively late in carcinogenesis, is the tertiary stage of chemoprevention (Martínez et al. 2008). The putative anticarcinogenic properties of dietary fibers may be related to enhanced butyrate formation in the lumen of the GI tract (Perrin et al. 2001). Dietary fibers and prebiotics such as inulin-type fructans (β (2-1)-fructans) from chicory roots (*Cichoriumin tybus*) are fermented to SCFAs and lactic acid in the GI tract. These fermentation products may exert a variety of chemoprotective properties in the GI tract, including the modulation of glutathione-S-transferases (GSTs) and other detoxification enzymes in human colon adenoma cells and colon tumor cells (Valko et al. 2004). The mechanisms of chemoprotection by fermentation products, especially butyrate, have been suggested to include the induction of GSTs and other stress-response proteins. Low levels of GST expression in the colon may be counteracted by the production of butyrate from microbial fermentation in the GI tract (Cummings 1981), and have been shown to induce GSTP1, GSTM2, and GSTA4 in HT29 cells (Ebert et al. 2003). An upregulation of GSTs involving GSTA2 and GSTT2 has been demonstrated in primary human colon epithelial cells following incubation with butyrate (Pool-Zobel et al. 2005). Other mechanisms by which butyrate may mediate gene expression in colon cancer are by modifying the acetylation of histones at their N-terminal (lysine-rich tails) and by activating the mitogen-activated protein kinase (MAPK) signalling transduction pathway (Marks et al. 2001). The acetylation of histones depends on two classes of enzymes, called histone deacetylases (HDACs) and histone acetyltransferases (HATs). Altered HDAC or HAT activity has been detected in several cancers. Modification of the N-terminal tail of histones by acetylation and deacetylation plays a key role in modulating gene expression as it affects the interaction of transcription regulatory protein complexes with DNA. Several compounds, including SCFAs (e.g., butyrate and some analogues), were found to inhibit HDACs. Due to their clinical activity against various human malignancies, HDAC inhibitors from diverse structural groups have been studied for potential use as chemoprentative agents (Drummond et al. 2005). Butyrate inhibits cell growth and HDAC activity at

millimolar levels. In human colon cells HT29 treated with butyrate, an important accumulation of acetylated histone 4 was evidenced, which may be related to higher expression of the genes encoding GST in these cells (Drummond et al. 2005).

7.4 Fermented Royal Jelly for Novel Source of Compounds and Their Health Benefits

7.4.1 Chemical Composition of Royal Jelly

The constituents of royal jelly on a percent weight basis include water (50–65%), proteins (11–18%), carbohydrates (10–15%), lipids (4–8%), minerals (1.5%), and small amounts of vitamins and polyphenols (Karaali et al. 1988). Proteins are the major macronutrient of royal jelly, constituting about half of its dry weight. The predominant types of proteins in royal jelly include royalisin, a small protein with known antibacterial and immunoregulatory activities. In addition five main royal jelly proteins (MRJP) have been identified, namely, MRJP1, MRJP2, MRJP3, MRJP4, and MRJP5 (Albert and Klaudiny 2004; Drapeau et al. 2006; Schonleben et al. 2007). MRJP1 is a weak and acidic glycoprotein with a molecular weight of 55 kDa (Simuth 2001; Bilikova et al. 2002; Kimura et al. 2003). MRJP2, MRJP3, MRJP4, and MRJP5 are glycoproteins with molecular weights of 49, 60-70, 60, and 77-87 kDa, respectively (Schmitzova et al. 1998). Carbohydrates account for 30% of royal jelly dry weight. The main sugars in royal jelly are glucose and fructose; however, glucose constitutes 50–70% of the total sugars (Chen and Chen 1995). Sucrose is usually present as well, but in more variable concentrations (Lercker 1986). Other disaccharides and oligosaccharides are also found in royal jelly such as maltose, trehalose, raffinose, isomaltose, and melezitose (Sabatini et al. 2009). Royal jelly is known to possess some biological activities, mostly demonstrated in experimental animal models, including antimicrobial (Blum et al. 1959), antitumor (Townsend et al. 1959), and anti-inflammatory activities (Nagai et al. 2004).

7.4.2 Health Benefits of Royal Jelly

7.4.2.1 Antioxidant Activity of Royal Jelly Components

Antioxidants can be classified chemically into two major groups. The first group consists of chemical substances such as tocopherols (vitamine E) that interrupt the propagation step of the free radical cascade by donating a hydrogen atom to free radicals. The second group involves a synergistic mode of action which includes metal chelators and oxygen radical scavengers. It is comprised of compounds such as citric acid, flavonoids, and amino acids. As antioxidants reduce the oxidative cellular damage implicated in pathogenic pathways, these compounds play a key

role in the prevention of chronic diseases such as cancers and cardiovascular diseases (Surh 1999; Kris-Etherton et al. 2002; Ferrari and Torres 2003).

Royal jelly is a natural product that is a rich source of a variety of phenolic compounds, including flavonoids and cinnamic acids, which are potent antioxidants (Gomez-Caravaca et al. 2006) and have been reported to exhibit anti-inflammatory, anticarcinogenic, and immune-modulating activities (Salah et al. 1995; Serafini et al. 1996; Catapano 1997; Vinson et al. 1998). In vitro studies indicate that royal jelly shows strong antioxidant capacity based on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity (Liu et al. 2008). Food protein hydrolysates and their constitutive peptides have recently been found to exert antioxidant activities (Nagai and Inoue 2004; Kosinska et al. 2011; Samaranayaka and Li-Chan 2011; Shahidi and Zhong 2011). The protein fraction of royal jelly has demonstrated scavenging ability against free radicals such as DPPH radicals, superoxide anion radicals, and hydroxyl radicals (Nagai et al. 2004). In royal jelly protein hydrolysates, up to 29 antioxidative peptides have been identified (Guo et al. 2009), including 12 small molecular weight peptides (Ala-Leu, Arg-Tyr, Ile-Arg, Lys-Leu, Lys-Phe, Lys-Tyr, Phe-Arg, Phe-Lys, Tyr-Tyr, Tyr-Asp, Leu-Asp-Arg, and Lys-Asn-Tyr-Pro). The analysis of their antioxidative properties revealed strong hydroxyl radical scavenging activity. Three dipeptides that included Tyr residues at their C terminal end (Arg-Tyr, Lys-Tyr, and Tyr-Tyr) also had strong hydrogen peroxide scavenging activity. The antioxidant activity of these tyrosyl dipeptides was suggested to be due to the presence of Tyr residues at their C termini), as the phenolic hydroxyl group of Tyr can scavenge free radicals by donating a hydrogen atom (Guo et al. 2009). In an animal model, royal jelly supplementation to C3H/HeJ mice for 16 weeks resulted in reduced serum and kidney concentrations of 8-hydroxy-2-deoxyquanosine, a marker of oxidative stress-induced DNA damage (Inoue et al. 2003).

7.4.2.2 Antidiabetic Activity of Royal Jelly Components

As α-amylase stimulates the hydrolysis of dietary starch, inhibition of this enzyme is a mechanism by which anti-diabetic drugs can lower postprandial glucose levels in diabetic and pre-diabetic patients following the ingestion of carbohydrate-rich meals (Ankolekar et al. 2012). There seems to be no studies of anti-α-amylase activity by royal jelly and its constituents. However, royal jelly intake for 8 weeks was found to inhibit hyperinsulinemia (insulin resistance) and hypertriglyceridemia and to decrease systolic blood pressure in Wistar rats who consumed a high fructose solution (Zamami et al. 2008). Royal jelly intake did not significantly influence blood glucose levels in the latter study.

7.4.2.3 Antihypertensive Activity of Royal Jelly Components

The angiotensin-converting enzyme (ACE) is a key enzyme in the renin-angiotensin system which regulates blood pressure in the circulatory system and adjacent organs. Inhibition of ACE is a therapeutic target for pharmaceutical treatment of high blood pressure in hypertensive patients, including those with type 2 diabetes. Numerous ACE-inhibitory peptides have been discovered in a variety of food sources, sardines (Seki et al. 1999) and sour milk (Nakamura et al. 1995) for instance. ACE-inhibitory activity was also reported in hydrolysates of royal jelly proteins. In particular, three peptides isolated from royal jelly hydrolysates displayed 50% ACE-inhibitory concentration (IC_{50}) values at concentrations ranging from 0.008 to 0.018 mg/ml (Shinoda et al. 1978). An antihypertensive effect was also evidenced following the oral administration for 28 days of two peptides isolated from royal jelly given to spontaneously hypertensive rats (Maruyama et al. 2003). These findings suggest that peptides from royal jelly may be valuable antihypertensive nutraceuticals for use in the formulation of functional foods designed for hypertension management (Katsu-hiko et al. 2004). In addition to peptides, two fatty acids present in royal jelly, namely, trans-2-octenoic acid and trans-10-hydroxy-2-decenoic acid, were found to exert antihypertensive effects (Okuda et al. 1998).

7.4.2.4 Anticarcinogenic Activity of Royal Jelly Components

Several bee products contain a variety of natural compounds with known anticancer activity in vitro and in vivo (Premratanachai and Chanchao 2014), which may involve the induction of tumor cell apoptosis or cytotoxic effects on these cells (Salazar-Olivo and Paz-Gonzalez 2005). Apart from honey, bee products include beeswax, bee pollen, propolis, as well as royal jelly (Pyrzynska and Biesaga 2009). Royal jelly is broadly used in commercial health foods, medicinal products, and cosmetic products worldwide (Palmieri et al. 2003). It has long been suggested, both in folk medicine and more recently in the scientific literature, to possess therapeutic properties for common illnesses and some chronic diseases. The intake of royal jelly has been suggested to provide chemopreventive effects against leukemia and other forms of cancer, and to help alleviate cancer-related fatigue (Okuda et al. 1998). The presence of a variety of macronutrients (proteins, carbohydrates, fatty acids, and free amino acids), vitamins (B vitamins especially and trace amounts of vitamins A, C, D and E), minerals, and non-nutrient bioactive substances in royal jelly (Takenaka 1982; Echigo et al. 1986; Leigh 1999) may contribute to its anticancer actions (Pour and Lawson 1984; Klaassen and Braakhuis 2002; Giovannucci et al. 2006; Zou et al. 2006). The proteins and peptides in royal jelly have been reported to have antioxidative (Guo et al. 2005), immuno-enhancing (Terada et al. 2011), and monocyte-proliferation stimulating (Kimura et al. 2003; Okamoto et al. 2003) properties, which may also be beneficial against cancer development. The crude proteins of royal jelly have been found to inhibit the proliferation of human

breast cancer cell lines (Nakaya et al. 2007). Similarly, certain royal jelly protein fractions exhibit cytotoxicity towards human cervical carcinoma HeLa cells (Townsend et al. 1959). Immunomodulatory effects have been attributed to the major royal jelly proteins (MRJPs) (Hanes and Simuth 1992; Albert and Klaudiny 2004; Majtan et al. 2006). MRJP1, the most common protein in royal jelly, has demonstrated an immunostimulatory role via pro-inflammatory cytokine production (Majtan et al. 2006), while MRJP3 inhibits the pro-inflammatory process (Kohno et al. 2004) and regulates T helper 2 cytokine production (Okamato et al. 2003). Both MRJP 1 and 2 have been shown to stimulate macrophages to release TNF-α (Simuth et al. 2004; Majtan et al. 2006) and to exert antibacterial effects (Bilikova et al. 2009). MRJP3 stimulates the immune response via IL-6 production (Kohno et al. 2004) both in vivo and in vitro (Okamoto et al. 2003; Kohno et al. 2004). Antitumor properties of royal jellies are also thought to be mediated by the presence of apalbumin-1 and apalbumin-2 proteins, which stimulate macrophages to release the cytokines TNF, IL-1, and IL-6 (Simuth et al. 2004; Majtan et al. 2006). The major fatty acid in royal jelly, 10-hydroxy-2-decenoic acid (Townsend et al. 1961), was found to prevent the propagation of leukemia cancer cells and ascetic tumor cells (Townsend et al. 1959). It has been hypothesized that 10-hydroxy-2-decenoic acid exerts its antitumor effects partly by decreasing angiogenesis and tumor vascularization via inhibition of the vascular endothelial growth factor (VEGF) (Izuta et al. 2009a, b). Figure 7.1 summarizes the proposed mechanism by which royal jelly proteins and peptides inhibit the development of cancer tumors.

7.5 Conclusions and Future Prospects

Foods and their components can exert numerous beneficial effects in human health. To enhance these benefits, there is a need in the food and health sector to produce functional food products with specific nutraceutical and pharmaceutical properties. Phenolics and other bioactive compounds such as peptides play an important role in human health due to their ability to reduce the risk of cardiovascular diseases, diabetes, and certain types of cancer. Bioactive peptides can occur naturally in foods or may be released after hydrolysis using microbial or enzymatic treatments. Recognition of fermented royal jelly as an important functional food and source of functional ingredients is supported by evidence of various biological properties such as antitumor, antioxidant, antimicrobial, anti-inflammatory, immunomodulatory, anxiolytic, and antimicrobial effects. Developing the functional and therapeutic applications of royal jelly and its bioactive components obtained through fermentation or enzymatic bioprocesses is a hot and promising topic. Future research should aim to better understand the bioactive composition and physiological and health effects of fermented royal jelly. Further work is also needed to characterize the influence of production and processing conditions on the desired bioactive compounds and their bioactivities in vivo.

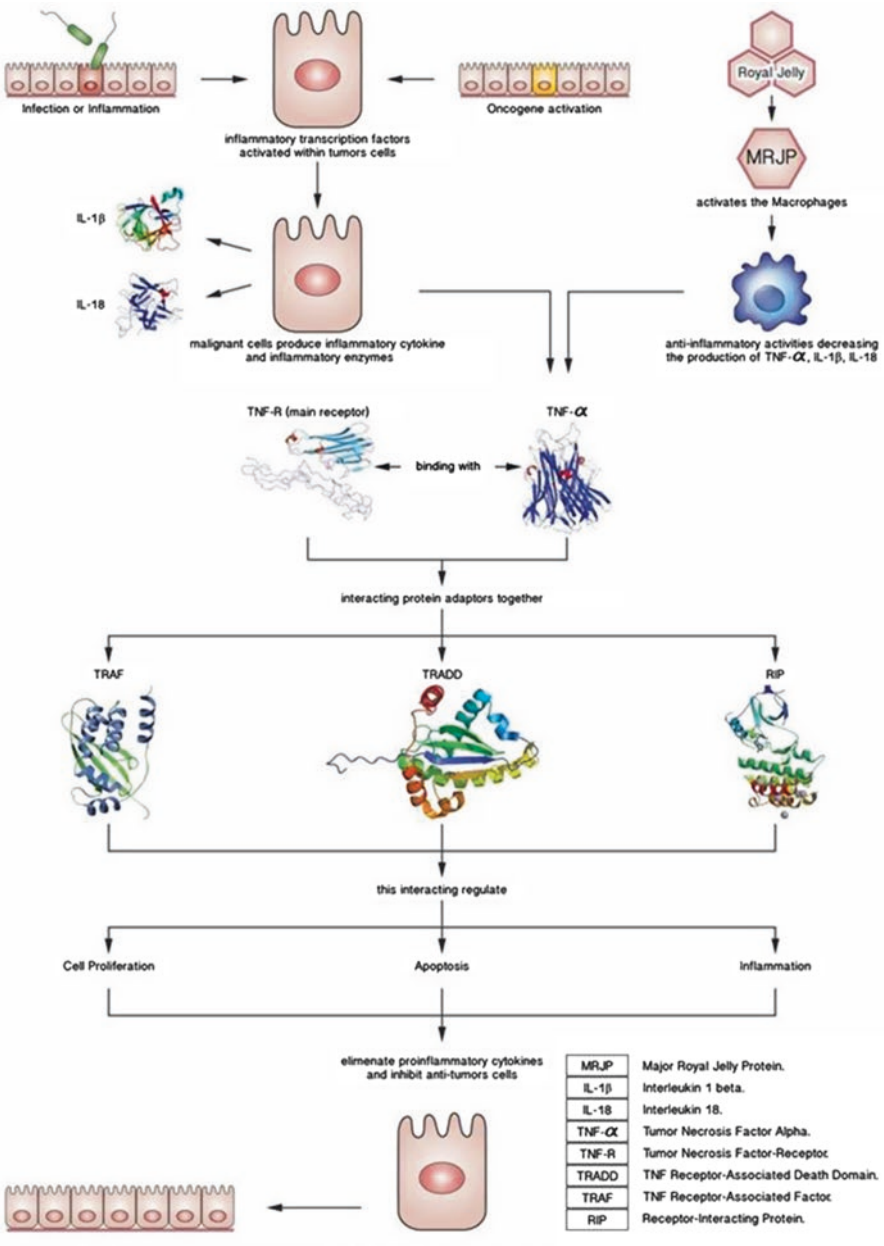

Fig. 7.1 Proposed mechanism by which royal jelly peptides and proteins exert their inhibitory activity towards tumors

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Chapter 8
Mass Spectrometry-Based Techniques for the Assessment of Pharmacological Responses of Ayurvedic Drugs

Amey Shirolkar, Manasi Malgaonkar, Amit Pawase, Sudesh Gaidhani, and Sharad Pawar

8.1 Introduction

Ayurveda is a traditional Indian knowledge for the betterment of mankind. Ayurveda believes nature has remedies for all the human health-related issues that can be treated more effectively using natural compounds. Ayurveda encompasses numerous therapies and treatment procedures that have been practised and testified since from the past thousands of years. The drug-human body's physiological interactions have been given deep thought in Ayurveda (Sarkar et al. 2013). A drug is any man-made, natural, or endogenous molecule which exerts a biochemical or physiological effect on the cell, tissue, organ, or organism, and the pharmacology is the branch of medical biology concerned with the study of drug action (Vallance and Smart 2006). Especially, it is the study of the interactions that occur between a living organism and chemicals that affect normal or abnormal biochemical function. As of now, pharmacological studies have covered almost all living entities, from human, animal, plant, and microbes, and in the course of time, mammalian organ cell lines have been also included to the list. Pharmacology deals with the fate of the drugs in a biological system; their absorption, distribution, metabolism, elimination and conversely effects executed in cellular machinery. The classical pharmacology and toxicology deal with the physiological and pathophysiological end points and reference values (Cross et al. 2015). With the evolution of mankind, the science of drugs, i.e. pharmacy, as well as science of studying drug action also has been evolved. From organ and organ systems, research focus has shifted to micromolecules, their metabolic interactions, and complex pathways comprising of numerous intrinsic

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factors (MacLellan et al. 2012). The continuous demand for a technology to handle complexity, but easy to handle it was always there. The advent of mass spectrometry (MS) responded to this demand to the utmost extent. The upgraded mass spectrometric assemblies and associated software tools contributed in the evolutionary transformation of MS from one of the tools of characterization to soul of characterization. The most fundamental changes began with size and shape of the MS; the roomful assemblies of earlier days have been replaced by bench top models (Buie 2011). The extent of automation in MS operations also has been enhanced to reduce efforts, time of users, and handling errors. Now if a worklist consisting of information about sample injection volume, acquisition method, and position of sample in sample tray is uploaded to MS, then the assembly on its own can do data acquisition for numerous samples. The era of larger preparative columns to analytical columns has been gone, and nanochips are getting generalized in the experimentation. Though the column dimensions and particle size are getting reduced upto nanometres, there is no compromise with separation abilities (Zamfir 2014). The volume of column will directly have effects on the amount of solvent consumed; therefore with size of column, the solvent consumption also has been reduced from millilitre to microlitres.

A major advance that enabled examination of protein structure by MS and MS/MS was the introduction of soft ionization techniques to volatilize biomolecules, in particular electrospray ionization (ESI) and matrix-assisted laser desorption/ionization (MALDI). The development of ionization techniques such as ESI and MALDI now allows almost any compound to be studied by MS (Glish and Vachet 2003). Matrix-assisted laser desorption/ionization (MALDI) and electrospray ionization (ESI) soft ionization methods efficiently generate intact gas-phase ions from large biomolecules with minimum fragmentation. In ESI, charged droplets are produced by passing a solubilized sample through a high voltage needle at atmospheric pressure. Desolvation occurs prior to entrance into the high vacuum of the mass spectrometer. ESI typically induces a range of charge states; because most mass spectrometers actually detect mass/charge or m/z. The resulting spectra may have many ions for each analysis. Charging can be induced by addition or loss of protons to form the MH^+, MH_2^{2+}, MH^-, $MNaH^{2+}$, etc. Generally APCI and ESI produce only molecular ions, but the multicharge ionization mechanism of electrospray can extend the mass range of the instrument to provide a mass range of greater than 80,000 Da to permit the accurate mass determination of macromolecules such as intact proteins (Thadikkaran et al. 2005; Villas-Boas et al. 2005).

The development of bioinformatics and cheminformatics tools happened parallely to mass spectrometry technology. Tools to segregate ambiguity in similar compounds like isomers and isotopes, functional derivatives have been developed. For handling whole biological system profiling, i.e. omics data statistical tools more than paired and unpaired t-tests, ANOVA and for non-parametric data Mann-Whitney U tests are required. The present day mass data analysis tools can perform post hoc and multiple testing corrections with traditional ANOVA to nullify false results (Vinaixa et al. 2012). They are also equipped with statistical models and data representation tools like box-whisker and scatter plots to traditional heat maps,

Venn diagrams, and column-bar graphs. A great progress has been achieved in creation of class-specific databases of metabolites for carbohydrates, nucleotides, amino acids, and their derivatives, and the libraries for lipids have already been established (AOCS and SimLipid lipid libraries). The database searching tools also become easier and effective; they are well efficient in screening the huge databases with spectral features of interests like mass ranges, charge states, missed cleavages, modifications, pI, host organism, and minimum detected peaks. The species-specific NCBI databases for proteins and many other metabolic libraries have been developed and maintained by scientific institutions, and as they are freely available, they are contributing tremendously in omics research especially in developing countries. Thus, the aim of this chapter is to closely look at the upgraded features of mass spectrometry techniques for chemical characterization of multicomponent Ayurvedic drugs/formulations and for the assessment of complex pharmacological responses of these interventions in various biological systems.

8.2 Pharmacology and Ayurvedic Sciences

Effective and safer alternatives to treat several human disorders can be found at Ayurveda, the traditional system of Indian medicine. Ayurvedic therapeutics is based on the laws of nature. Its holistic approach to healthcare is based on a unique understanding of the interrelationship of body, mind, and spirit. The aim of Ayurvedic medicine is to integrate and balance these elements to prevent illness and promote wellness through diet, nutrition, herbs, spices, yoga, external therapies, meditation, and daily and seasonal routines (Kizhakkeveettil et al. 2011). The current guidelines on safety of Ayurvedic drug products from agencies like WHO and Central Council for Research in Ayurvedic Sciences (Ministry of AYUSH, Govt of India, New Delhi) are in accordance with single entity/active principle, and also bioequivalence guidelines centred with the same amount of drug should be bioavailable, and similar fate of the drug is evaluated in similar ways for the combinations of the drugs. The multicomponent active principle of similar categories and multicomponents of different therapeutic categories from herbal products are combined together for symptom relief in classic allopathy practice. From the available literature on single Ayurvedic drug, it is possible to conclude that some common chemical classes also possess the marked side effects and ratio of the drug component also plays a very important role in such toxicities (Manohar 2014).

Ayurvedic compound formulations are broadly classified under the heading of Rasausadhi (predominantly metals and minerals are used for preparation and dealt in Rasashastra) and Kastausadhi (predominantly plant drugs are used for preparation and mainly dealt in Bhaisajyakalpana). It is claimed that Ayurvedic/herbal healthcare products (AHPs) are safe because of their natural origin. However, several reports exist of adulteration of AHPs with synthetic drugs. The essential ingredient in most formulations is not precisely defined. Scientific evidence from randomized clinical trials is only strong for few herbal medicines. A fundamental problem in all clinical

research of herbal medicines is whether different products, extracts, or even different lots of the same extract are comparable and equivalent. For example, *Echinacea* products can contain other plant extracts; use of different plant species (*E. purpurea*, *E. pallid*, or *E. angustifolia*) and different parts (herb, root, both) is also common and might have been produced in quite different manners (hydro- or lipophilic extraction). Even different species may be known by the same name in local language. Brahmi refers to *Centella asiatica* and *Bacopa monniera* (Bose et al. 2012). Only a small fraction of the thousands of medicinal plants used worldwide has been tested rigorously in randomized, controlled trials. Even if the animal studies or anecdotal clinical experiences are promising and use of an herb is widespread, such observations cannot predict the results of well-designed randomized, controlled trials. A recent review by Singh et al. (2014) concluded that the efficacy and safety of traditional Indian medicines are limited. The data available is mostly experimental or in animals. Most trials do not report hard efficacy endpoints and duration of observation periods is generally short. The clinical relevance of the observed effects is not always clear. For instance, most Indian trials of hepatoprotective agents are open and uncontrolled. As most acute liver conditions have a natural recovery, it is difficult to link the improvement to the herbal product (Singh et al. 2014).

Discovering new chemical entities (NCEs) from plant sources has been the first mind stuck for the plant chemists in both academics and industry. Compounds of natural origin still play a major role in development of new drugs. About 40% of the world's best-selling drugs are derived directly or indirectly from compounds of plant origin. For Ayurveda and other traditional medicines, newer guidelines of standardization, manufacture, and quality control are required. The World Health Organization also has recognized the importance of traditional medicine and has been active in creating strategies, guidelines, and standards for botanical medicines (Anonymous 2002). It is very rare to find to a single individual in global or Indian urban population who is not having an intervention or is not consuming any kind of drug. Pharmacology provides the studies to determine the safety and effectiveness of medications. The safety of medications is important for everyone; the reasons behind drug reactions are being studied and looking for ways to personalize treatment according to people's genetic make-up. Thus, the pharmacology encompasses drug composition and properties, synthesis and drug design, molecular and cellular mechanisms, organ/systems mechanisms, signal transduction/cellular communication, molecular diagnostics, interactions, toxicology, chemical biology, therapy, and medical applications and antipathogenic capabilities (Agarwal 2014). The two main areas of pharmacology are pharmacodynamics and pharmacokinetics. Pharmacology, a biomedical science, deals with the research, discovery, and characterization of chemicals which show biological effects and the elucidation of cellular and organismal function in relation to these chemicals. In broad terms, pharmacodynamics discusses the chemicals with biological receptors, and pharmacokinetics discusses the absorption, distribution, metabolism, and excretion (ADME) of chemicals from the biological systems (Vallance and Smart 2006). Pharmacology covers a vast area; it includes characterization of drug to characterization of response given by organisms for a drug. Mass spectrometry (MS) has evolved tremendously in past

few decades in terms of utility from astrophysics to forensics and from synthetic chemistry to biotechnology. Hyphenated mass spectrometric assemblies and equally developed data analysis tools have contributed awesomely in this evolutionary transformation. Here we are looking at few selected features of mass spectrometric technology to facilitate pharmacological research with special reference to Ayurvedic drugs.

8.3 Modern Techniques

NMR is a non-destructive analytical technique which is good at detecting positional isomers and has been used to analyse flux rates of metabolic pathways. However, it is difficult to analyse traditional NMR spectra of complex mixtures because of its relatively low sensitivity at the micromolar range, leaving low-abundant metabolites undetected. Comparatively, MS has higher sensitivity and detection limit can reach nano- and picomolar levels. MS can be used for selective or targeted analysis of chemical compounds that is not possible with NMR (Emwas 2015). Direct injection MS can be hassled with ion suppression caused by complex sample matrices, where the signal of many analytes with low ionization efficiencies cannot be detected. To avoid these problems, MS is often hyphenated to GC or LC to decrease sample complexity.

GC-MS is a sensitive and robust separation technique with established applications in the field of metabolomics. GC-MS has good separation resolution due to the long capillary column and readily available commercial mass spectra databases; thus annotation of unknown peaks is quicker (Fiehn et al. 2000). GC-MS typically uses electron impact ionization (EI) which is less prone to sample matrix effects, ionizes most compounds with relatively high efficiency, and generates instrument-independent mass spectra for library build-up. Mass spectra acquired from a single quadruple MS are typically different from those acquired from an ion trap MS; therefore, libraries built on different types of MS may not be generalized. GC-MS can detect metabolites such as amino acids, organic acids, carbohydrates, phosphorylated metabolites, fatty acids, and cholesterol. However, GC-MS is not suitable for analysis of large or thermolabile compounds such as nonpolar intact lipids, nucleotides, nucleotide diphosphates, cofactors, or oligosaccharides. After identification of the feature compounds, their potential secondary metabolites are screened using the predictive multiple reaction monitoring (pMRM) mode available on triple-quadruple linear ion trap mass spectrometer (QTRAP). Currently, the analysis of low-abundant metabolites remains an unresolved problem in metabolic profiling. In spite of being able to detect many metabolites, neither TOF MS nor ion trap MS performing in full scan mode is sensitive enough to detect and characterize metabolites at trace levels. Triple-quadruple (QQQ) tandem mass spectrometer (MS/MS) provides excellent sensitivity in multiple reactions monitoring (MRM) mode, but lack structural information and metabolite coverage. Typically, TOF or tandem TOF (TOF/TOF) instruments provide MS or both MS and MS/MS capabilities.

Developments in LC separation are also desirable in order to reduce sample loading and improve separation of proteins and peptides. Gel-eluted liquid fraction entrapment electrophoresis (GELFrEE) integrates gel electrophoresis separation within reverse phase LC and eliminates the need for prior electrophoresis and sample processing before injection into the LC (Goodacre et al. 2003). It has been applied to the detection of nitrotyrosine using the increased hydrophilicity of aminotyrosine (formed by reduction of nitrotyrosine with dithionite) and concomitant shift in chromatographic elution of modified peptides on reverse phase HPLC (Saeys et al. 2007). In contrast, for top-down studies, the favoured method is capillary zone electrophoresis (CZE), which allows lower sample loading and has higher separation efficiency than reverse phase HPLC (Li et al. 2014). This can facilitate detection of oxidation in complex clinical samples, where analyte concentration may be limited.

8.4 Modern Techniques in Herbal Drug Identification and Characterization

Multicomponent botanical formulations can be standardized with newer techniques such as DNA fingerprinting, high-pressure thin-layer chromatography (HPTLC), and liquid chromatography-mass spectroscopy (Patwardhan et al. 2004). The authentication methods for herbal, mineral, and marine products are microscopy, spectroscopy, spectrometry, chromatography, chemometry, immunoassays, and DNA fingerprinting. As it is well known that the efficacy of traditional herbal medicines has a characteristic of a complex mixture of chemical compounds present in the herbs, thus how to evaluate reasonably their relationship is obviously not a trivial task. Moreover, the chemical profile by itself is insufficient in determining the efficacy of drugs. This is where biochemistry, molecular biology, and cell biology are invaluable in establishing quantifiable and reproducible assays (Kamboj 2012). The advancement of analytical techniques will serve as a rapid and specific tool in the herbal research, thereby allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety, and shelf life of herbal drugs. The effective regulation and control of herbal medicines moving in international commerce also requires close liaison between national institutions that are able to keep under regular review all aspects of production and use of herbal medicines, as well as to conduct or sponsor evaluative studies of their efficacy, toxicity, safety, acceptability, cost, and relative value compared with other drugs used in modern medicine.

Chromatography is defined as a technique of isolation and identification of components or compounds or mixture of its individual components by using stationary phase and mobile phase. High-performance liquid chromatography (HPLC), also known as high-pressure liquid chromatography, is essentially a form of column chromatography in which the stationary phase consists of small particle (3–50 μm)

packing contained in a column with a small bore (2–5 mm), one end of which is attached to a source of pressurized liquid eluent (mobile phase). The three forms of high-performance liquid chromatography most often used are ion exchange, partition, and adsorption. HPLC is a popular method for the analysis of herbal medicines because it is easy to learn and use and is not limited by the volatility or stability of the sample compound. In general, HPLC can be used to analyse almost all the compounds in the herbal medicines. Thus, over the past decades, HPLC has received the most extensive application in the analysis of herbal medicines. Reversed-phase (RP) columns may be the most popular columns used in the analytical separation of herbal medicines. Although liquid and gas chromatography are powerful tools for chemical profiling of herbals, these in combination with mass spectrometric detection have become more effective and convenient because these techniques are very useful for the characterization and quantification of individual constituent in the plant extracts (Newman and Cragg 2007).

8.4.1 HPLC and Associated Techniques

With the introduction of electrospray mass spectrometry, the coupling of liquid chromatography and mass spectrometry has opened the new way to widely and routinely applied to the analysis of herbal medicines. HPLC fingerprints can be then applied for documentation of complete herbal extracts with more information, and online qualitative analysis becomes possible (Farooqui et al. 2014).

Hyphenated HPLC techniques: Moreover, combined HPLC-DAD-MS techniques take advantage of chromatography as a separation method and both DAD and MS as an identification method. DAD and MS can provide online UV and MS information for each individual peak in a chromatogram (Farooqui et al. 2014). In the analysis of the chemical components of TCMs, HPLC/MS technique is usually used for the separation and identification of a variety of similar structural compounds, and mass spectrometry is an important qualitative tool (Li et al. 2011). Alkaloids, sugar, glycosides, phenols, flavonoids, terpenes, phenylpropanoids, and steroid saponins all have been effectively analysed using HPLC/MS. Therefore for quality control purpose, for finding synthetic adulterants, and for the characterization of low-abundance targets in complex samples, with high resolution, reproducibility, and selectivity UPLC-MS, UPLC-QTOF/MS has been demonstrated to be powerful tools. Some of peaks can be characterized directly online by comparing the retention time, UV spectra, and fragmentation information with the reference. During the discovery process of novel compounds, it is important to differentiate novel from known compounds in crude extracts before starting a time-consuming process of purification. The majority of studies only focused on determining the components of herbal drugs. It is insufficient in the depth of research. Therefore, more efforts should be made to explore the relationship between the effectiveness and components of herbal drugs by using HPLC/MS techniques (Li et al. 2011).

It is widely accepted separation technique for both sample analysis and purification. In HPLC, the mobile phase is forced through the column under high pressure with isocratic or gradient elution. HPLC is the most widely used of all, and the reason for the popularity of the method is its sensitivity, its ready adaptability to accurate quantitative determinations, its suitability for separately nonvolatile species or thermally fragile ones, and, above all, its widespread applicability to substances that are of prime interest to industry, to many fields of science, and to the public.

The preparative and analytical HPLC are widely applicable in pharmaceutical industry for isolating and purification of herbal compounds. The main aim is to isolate the herbal compounds, whereas in analytical work the aim is to get the information about sample. Preparative and analytical high-performance liquid chromatography (HPLC) is widely used in pharmaceutical industry for isolating and purification of herbal compounds (Chimezie et al. 2008; Rao and Anna 2009). Vasicine, the major bioactive alkaloid of *Adhatoda vasica*, was estimated by HPLC in two polyherbal drug formulations – Shereeshadi Kashaya and Yastyadivati – and its content was found to be 18.1 mg/100 g in Shereeshadi Kashaya and 0.7 mg/100 g in Yastyadivati (Anupam et al. 1992). HPLC analysis of Senna leaves provided informations about sennoside content, kaempferol 3-*O*-D-gentiobioside, aloeemodine 8-*O*-D-glucopyranoside, rhein 8-*O*-D-glucopyranoside, torachrysone 8-*O*-D-glucopyranoside, and isorhamnetine 3-*O*-D-gentiobioside L. Standardization of the Triphala (an antioxidant-rich herbal formulation) mixture of *Emblica officinalis*, *Terminalia chebula*, and *T. belerica* in equal proportions has been reported by the HPLC method by using the RP18 column with an acidic mobile phase (Singh et al. 2007). Two HPLC methods, one combined with a photodiode array detector (LC/UV) and another with mass spectrometry (LC-MS), were reported for the analysis of aristolochic acids I and II in herbal medicines. Kankasava an Ayurvedic medicine and liquorice a Chinese medicine were analysed thoroughly using HPLC and LC-MS. Kankasava is a fermented polyherbal formulation prepared with Kanaka and other ingredients. It is used in chronic bronchitis, asthmatic cough, and breathlessness. Kankasava is analysed by RP HPLC. It is a simple, precise, accurate RP-HPLC method which was developed for the quantitative estimation of atropine in Kankasava polyherbal-branded formulations.

The advantages of GC clearly lie in its high sensitivity of detection for almost all the volatile chemical compounds. It is not convenient for its analysis of the samples of polar and nonvolatile compounds. For this, it is necessary to use tedious sample workup which may include derivatization. Therefore, the liquid chromatography becomes another necessary tool for us to apply the comprehensive analysis of the herbal medicines. On the other hand, the advantages of HPLC lie in its versatility for the analysis of the chemical compounds in herbal medicines; however, the commonly used detector in HPLC, say single-wavelength UV detector, seems to be unable to fulfil the task, since lots of chemical compounds in herbal medicines are non-chromophoric compounds. Consequently, a marked increase in the use of HPLC analysis coupled with evaporative light scattering detection (ELSD) in a recent decade demonstrated that ELSD is an excellent detection method for the analysis of non-chromophoric compounds. This new detector provides a possibility

for the direct HPLC analysis of many pharmacologically active components in herbal medicines, since the response of ELSD depends only on the size, shape, and number of eluate particles rather than the analysis structure and/or chromophore of analytes as UV detector do. Especially, this technique is quite suitable for the construction of the fingerprints of the herbal medicines.

8.4.2 Liquid Chromatography-Mass Spectrometry (LC-MS)

Liquid chromatography-mass spectrometry (LC-MS) is an analytical chemistry technique that combines the physical separation capabilities of liquid chromatography with the mass analysis capabilities of mass spectrometry. LC-MS is a powerful technique used for many applications which has very high sensitivity and selectivity. Generally its application is oriented towards the specific detection and potential identification of chemicals in a complex mixture. LC-MS is a powerful technique used for many applications which has very high sensitivity and specificity. Generally its application is oriented towards the specific detection and potential identification of chemicals in the presence of other chemicals. Raw materials like honey and animal fats can be authenticated by this technique, and the spectral fingerprints of them can be generated. A qualitative analysis of carotenoid composition was performed by HPLC/UV on samples of *Corallium rubrum* to generate a chromatogram profile (Cvejic 2007; Revathy et al. 2012). A generalized strategy was developed using LC-MS/TOF for the detection and verification of steroidal and anti-inflammatory drugs in 58 AHPs collected from various parts of India. The strategy involved recording of mass spectral information for standard drugs – including ionization mode (ESI/APCI − ve or + ve), mass spectrum, accurate mass, identification of qualifier fragments (two), extracted ion chromatograms (EICs), isotopic pattern, and determination of UV max (nm) through UV-PDA studies. Adulteration was then detected in AHPs primarily through comparison of EICs at accurate m/z for molecular ion peaks and retention time (RT) matching with the standard. It was confirmed by spiking with the standards and matching mass spectrum, accurate mass, RT of qualifier fragments, isotopic pattern, and UV spectrum of the standards with the adulterant peaks in AHPs. Dexamethasone and diclofenac were detected as adulterants in ten AHPs, whereas one AHP tested positive for piroxicam and another for dexamethasone. The study showed that LC-MS/TOF-based screening could be used as a rapid approach to monitor adulteration of steroids and anti-inflammatory drugs in AHPs (Savaliya et al. 2009). The combination of HPLC and LC-MS is currently the most powerful technique for the quality control of Chinese herbal medicine Gan-Cao (licorice) (Zhang and Ye 2009). Liquid chromatography-mass spectroscopy (LC-MS) has become a method of choice in many stages of drug development (Lee and Kerns 1999). Chemical standardization of an aqueous extract of the mixture of the 20 Chinese herbs was done using LC-MS (Ip et al. 2010). Further, LC-MS analysis of amino glycosides in natural products showed that these drugs are highly soluble in water, exhibited low plasma protein binding, and were more than 90%

excreted through the kidney (Shen et al. 2010). LC-MS, LC/NMR, and GC-MS methods are very helpful in multicomponent analysis (MCA) in the herbal extracts. These techniques are sensitive, selective, and fast where simultaneous separations as well as identification of separated components in a mixture are possible. LC and GC perform the function of separation, whereas MS performs the function of identification of components in a mixture on the basis of molecular mass and fragmentation pattern. It gives us two-dimensional information: the first-dimensional information comes in the form of retention time, whereas second-dimensional information comes from mass detector in the form of molecular mass and fragmentation pattern. These techniques are very selective and specific and allow the detection of compounds even in picogram amounts (Lee and Kerns 1999). LC-MS has been playing a more significant role in natural product research because the technique is capable of characterizing active components in plant extract, and recent publications show that application of LC-MS has been rapidly expanding into the areas of structure elucidation. Three new sesquiterpenes were isolated from *Penicillium roqueforti* and their structures were established (Dan et al. 2007). From the fruits of *Gardenia jasminoides*, several carotenoids were isolated and identified. New carotenoid, crocetin, was characterized by LC-MS (Manuel et al. 2006). LC-MS methods are very useful in determining the active components and metabolites in preclinical studies. Studies on the metabolites of piperine, an alkaloid constituent of *Piper nigrum*, led to the characterization of two metabolites in rat urine. These metabolites were characterized on the basis of LC/MS/MS and LC/NMR/MS/MS data (Bajad et al. 2003). An Ayurvedic polyherbal formulation (PHF) eye drop (Itone™) was studied through information-dependent acquisition (IDA) method using a hybrid system of ultra-high-performance liquid chromatography (UHPLC) coupled with mass spectrometry (LC-MS/MS). This study was also extended to evaluate the intraocular penetration in the rabbit eyes upon topical application using liquid chromatography-coupled tandem mass spectrometry (Velpandian et al. 2013).

8.5 Pharmacology Posttreatment Part

There is no limitation for the studies carried out under category of pharmacological activities; it may include any of these: study of anti-complementary, analgesic, anti-hyperlipidaemic, anti-artherosclerotic, anti-inflammatory, antiarthritic, antimicrobial, antioxidant, antitumour, and anti-carcinogenic (Alam et al. 2012). With the aim of identifying proteins associated with picroside biosynthesis in *Picrorhiza kurroa*, differential protein expression was studied. Mass spectrometry-based protein identification revealed altered proteins belonging to several functional categories like stress response, signalling pathways, metabolic pathways, transcription and translation factors, and energy metabolism. Proteins involved in diverse functions were identified among which the most important proteins were glyceraldehyde-3-phosphate dehydrogenase, 1-aminocyclopropane-1-carboxylate oxidase, photosystem I reaction centre subunit V, 2-oxoglutarate ferrous-dependent oxygenase, and

putative cytochrome P_{450} superfamily protein because of their role in picroside biosynthesis. These identified proteins provide an insight and a basic platform for thorough understanding of biosynthesis of secondary metabolites and various other physiological processes of *P. kurroa* (Sud et al. 2014).

8.6 Moving Through Mass Spectrometry

Till here we looked at the reported evidences of applicability of mass spectrometry in pharmacological studies involving Ayurvedic or herbal drugs, in terms of chemical characterization of drug and molecular characterization of generated physiological response. Here onwards we are looking at a case study designed to introduce readers about the potential of mass spectrometric platform. We are referring to publications of pharmacology and biochemistry departments of National Research Institute of Basic Ayurvedic Sciences (NRIBAS) in the past 3 years that cover various aspects of pharmacology. The concerned instrument is 1290UPLC-ESI-6538QTOFMS belonging to NRIBAS (CCRAS, Ministry of AYUSH, Govt of India), Pune, India. Here, ESI refers to electrospray ionization, the source of ionization of molecules that were segregated through ultrahigh-performance liquid chromatography. Quadruple time of flight is a kind of mass analyser that performs the duty of assigning the mass/charge (m/z) to molecules (Fig. 8.1).

Through LC-MS analysis, a time-specific list of compounds gets generated. In this instead of molecular weight (~molecular mass), mass by charge ratio (m/z) is denoted. Based on the sample quality and type, an average sample run through

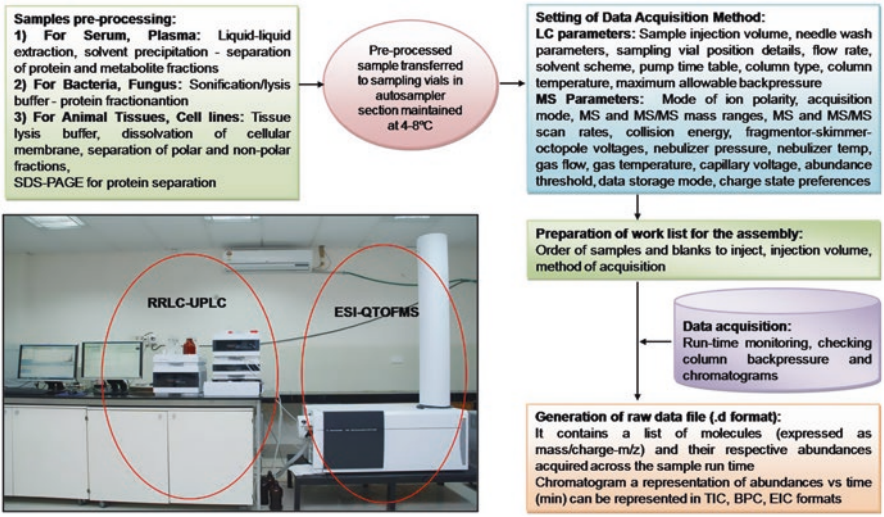

Fig. 8.1 A flowchart of working on LC-MS assembly

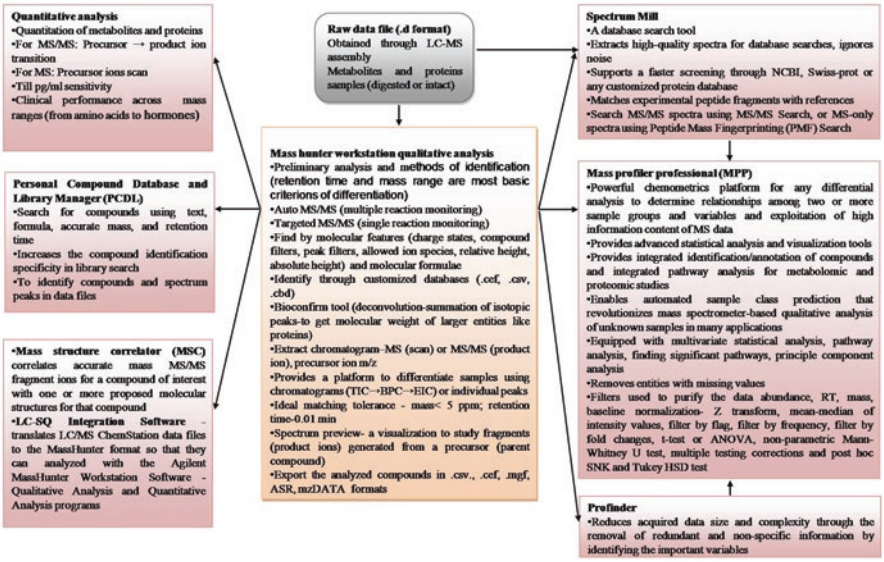

Fig. 8.2 Available software tools to facilitate pharmacological research in Agilent's LC-MS assembly, from acquiring data to confirmation and validation of results

LC-MS may yield numerous, hundreds to thousands, molecular features. These molecular features need to undergo a thorough processing with respect to their abundances and their level of expressions in study groups, and then only a conclusive data will be obtained. To do this many LC-MS manufactures supply a range of assistive software tools. With few differences the data processing workflow remains very much similar across different instrumentation.

As in this case study we are considering an Agilent instrumentation, we will consider analysis tools developed by Agilent technologies only (Fig. 8.2). The central role in data analysis is undoubtedly played by "MassHunter Workstation Qualitative Analysis software". In this software preliminary data analysis and quality testing can be carried out irrespective of objectives of the experimentation. For example, as per molecular features (charge state, mass range, abundance, peak height, peak spacing tolerance), by molecular formula compounds can be filtered out. Also using any standard compound library, the compounds can be screened through it. Allowable library formats are .cdb, .mtl, and .csv. Based on search criteria, the software will screen the metabolites of interest in libraries within the set retention time.

Over the time after utilizing features of qualitative analysis, it was observed that while processing a large omics data through MH qualitative and MPP, the complexity of the analysis can be reduced to great extent if molecular feature extraction becomes more accurate. Hence, Agilent came up with MH workstation Profinder software that reduces acquired data size and complexity through the removal of redundant and non-specific information by identifying the important variables (Fig. 8.3).

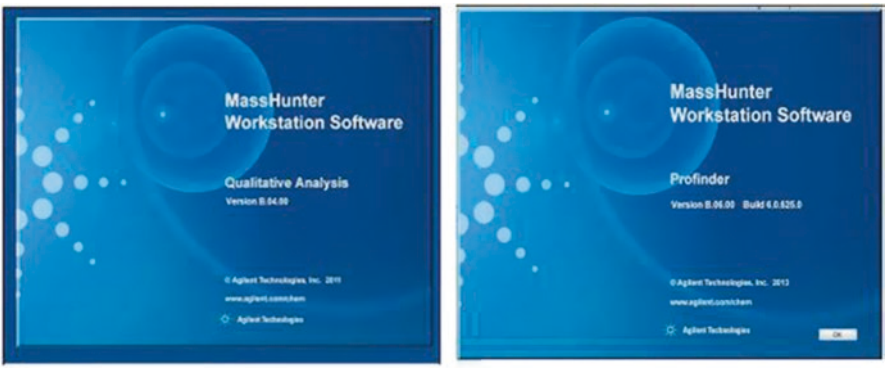

Fig. 8.3 Logos of Agilent technology's MH workstation qualitative analysis and Profinder software

The most basic and primary analysis begin with comparison of chromatograms. The MassHunter Workstation Qualitative Analysis software can be used to differentiate various categories of samples based on the visual differences in position of peaks. After doing chromatogram subtraction, the differentially expressed peaks and differences in their abundances can be found out in measurable terms. From peaks of interests, we can find out the compounds belonging to them, and by matching the fragmentation pattern, the identity can be confirmed. As shown in Fig. 8.4, through such first line of analysis, the upregulation of tetrahydrofolyl-(Glu)(2)-574.5432 Da and downregulation of 2′-deoxyinosine triphosphates-491.98 Da can be traced out based on the revelations made by the chromatogram subtractions. The chromatograms can also be represented in list mode and in overlaid mode. A represents a solvent blank and B, C, and D represent the prepared drug extract in respect to this particular solvent (Fig. 8.5), and by looking at the chromatograms, the necessary, marked chromatographic difference between the drugs and blank is concluded. The other basic representation is overlaid mode, which is more famous among others. In this kind of representation, samples belonging to different categories can be analysed in a single chromatographic frame, and with different colour schemes, the marked difference can be pointed out (Figs. 8.6 and 8.7).

After chromatogram integration, the abundant peaks get highlighted, and labels indicating their retention time (RT) get generated (Fig. 8.8). This is useful in finding out marked differences between chromatograms and discriminating peaks. After sorting out discriminated peaks, the respective MS spectrum can be extracted from it (Fig. 8.9). This extraction yields a list of most abundant fragments and their respective abundance (relative intensity); this information can be used further to assign an identity for the compounds represented by the peak (Fig. 8.10). Several online databases like HMDB, NIST, Metlin, CSIR-tandem mass database, ReSpect for phytochemicals, and MassBank help in this identification task. The peak list with abundances can be compared with references reported in these databases.

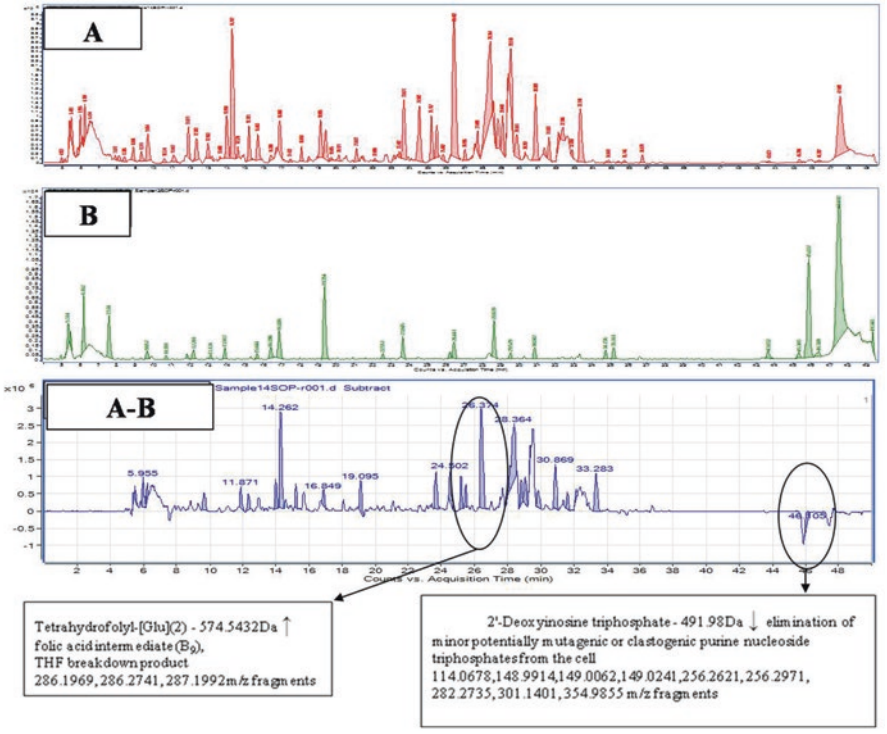

Fig. 8.4 Peak discrimination/chromatogram comparison study: The *A* and *B* chromatograms can be subtracted from each other and differentially expressed peaks can be segregated; the compounds getting represented by these peaks will be identified using fragmentation pattern

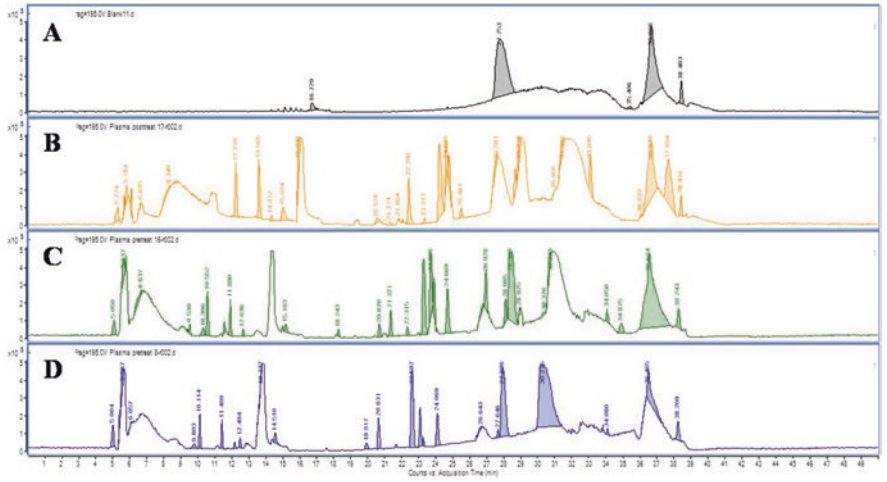

Fig. 8.5 Expression of chromatograms in list mode, where *A* blank (solvent) and *B*, *C*, and *D* chromatograms of extracted drugs

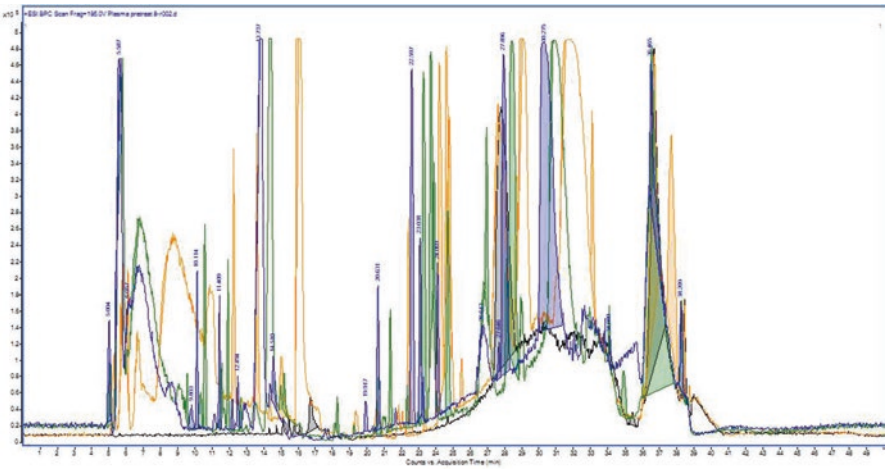

Fig. 8.6 Expression of chromatograms in overlaid mode

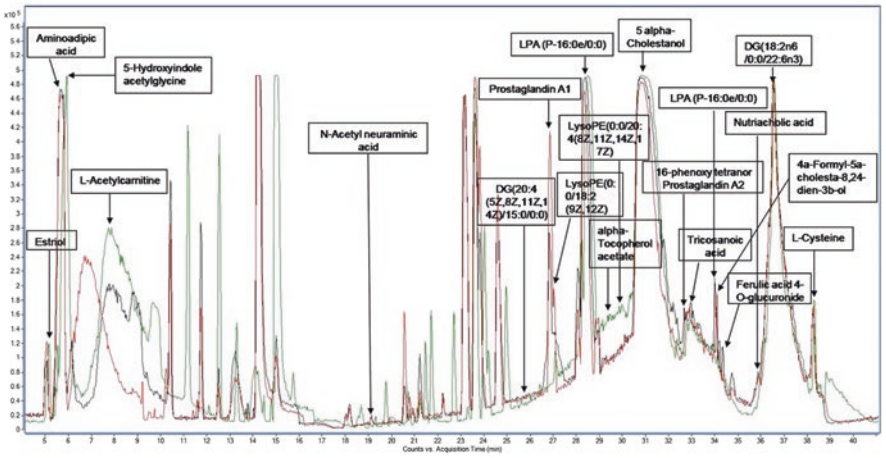

Fig. 8.7 The extracted +ESI base peak chromatogram (BPC) in overlaid mode with various categories of identified (labelled) compounds

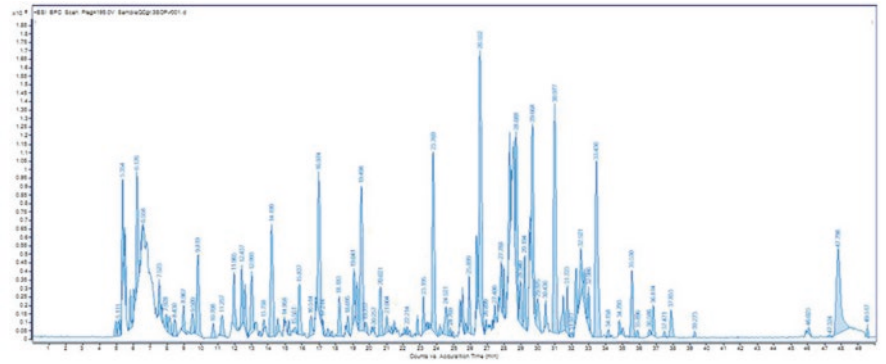

Fig. 8.8 An integrated base peak chromatogram indicating abundant peaks and retention time

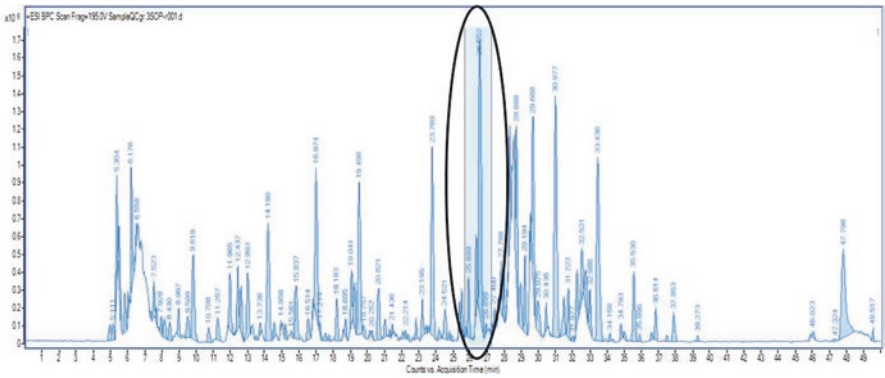

Fig. 8.9 A peak of selected MS spectrum

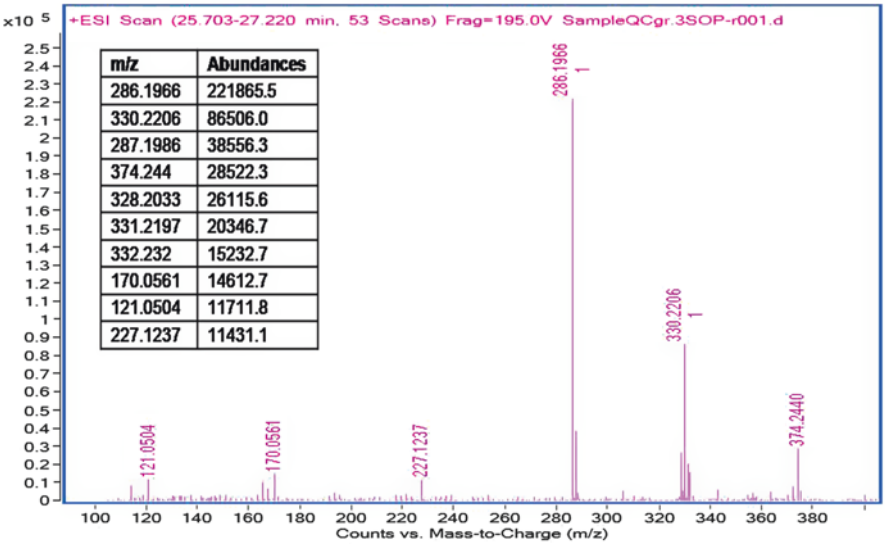

Fig. 8.10 A spectrum preview image of a peak represented at 26.552 min, showing the respective major compounds

8.6.1 Mass Spectrometry-Based Quantitative Studies

The selectivity and sensitivity of LC-MS assembly can be utilized for quantitating a compound of interest irrespective of its concentration and chemical identity. Metabolites as well as proteins can be quantitated till ρg/ml level. An added advantage is the analysis done by LC-MS is very much reliable, so there will be almost no chance of getting any false result. As every component has a unique fragmentation pattern of its own, the quantitation carried out on LC-MS is always considered as

flawless as there is no scope for getting any false-positive or false-negative values. With such advantages it is feasible to quantitate even five to ten markers simultaneously in an experimental setup. This creates a platform for pharmacokinetics-pharmacodynamics (PKPD) studies. As explained at the beginning, the former studies the effects of the drug on biological systems, and the latter the effects of biological systems on the drug. So the fate (chemical changes) of any drug molecule inside the host's body over a long period of time can be effectively tracked using the quantitative (targeted) MS/MS approach. Heavy biomolecular entities like hormones (e.g. insulin, angiotensin, prolactin, FSH, TSH) can also be quantitated on LC-MS accurately as well as more economically than the ELISA-based approaches.

Liquid chromatography-mass spectrometry (LC-MS) was carried out in +ESI mode to analyse a medicinally valuable alkaloid, camptothecin (CPT). It has anticarcinogenic activity and belongs to *Nothapodytes nimmoniana* (J. Graham) plant; its precursor was observed at 349.1743 m/z with major product ions 305.1283 and 248.0944 m/z that exactly matched with standard CPT (mol. wt. 348.352 g/mol). The method was used to plot a standard curve of CPT which was prepared from the intensities of transitions (349.1743→305.1283 m/z). High correlation value was obtained for this standard plot (R^2 = 0.9944). The quantitative analysis of CPT in various *N. nimmoniana* extracts showed parallel trend as of the ELISA-based method. A negligible retention time shift was observed in quantitative LC-MS analysis of CPT; for 21-min run time, the CPT was throughout getting eluted at 16.418 min (RT) (Fig. 8.11a–c) (Dixit et al. 2015).

HPLC/electrospray ionization mass spectrometry method is accurate and reproducible and requires less specimen, sample preparation, and analysis time over HPLC assay. The method was used to plot a standard curve for β-sitosterol was prepared from the intensities of transitions (397.50→147.0987 m/z) having regression coefficient (R^2) 0.9952. Out of eight extracts and two drugs used in the study, bark water, leaves water, and leaves hot water extracts were found to have a considerable quantity of β-sitosterol, i.e. 170, 123.5, and 19.3 ng/mL, respectively. The results showed significant differences in the distribution of β-sitosterol among different organs of *S. asoca* and drugs prepared from its bark. At the same time, Baidyanath Ashokarishta sample showed fivefold more β-sitosterol than Dabur Ashokarishta. Significant differences observed in the distribution of β-sitosterol among different organs of *S. asoca* could be helpful for the quality control of herbal medicines and provide necessary information for the rational utilization of plant resources. Considering the encouraging results obtained in this study, application of HPLC-QTOFMS in authentication of crude and processed herbal drugs over HPLC assay was proposed. This type of approaches could be helpful for the quality control of herbal medicines and provides necessary information for the rational utilization of plant resources (Gahlaut et al. 2013a).

Herbal medicines are highly complex and have unknown mechanisms in diseases treatment. Eight extracts (cold and hot water) from four different organs of *S. asoca* and two drugs were prepared, and antimicrobial activity was assessed by microbroth dilution assay. Quantitative and qualitative analysis of catechins in crude extracts was done by using targeted and auto-MS/MS. (+)-Epicatechin

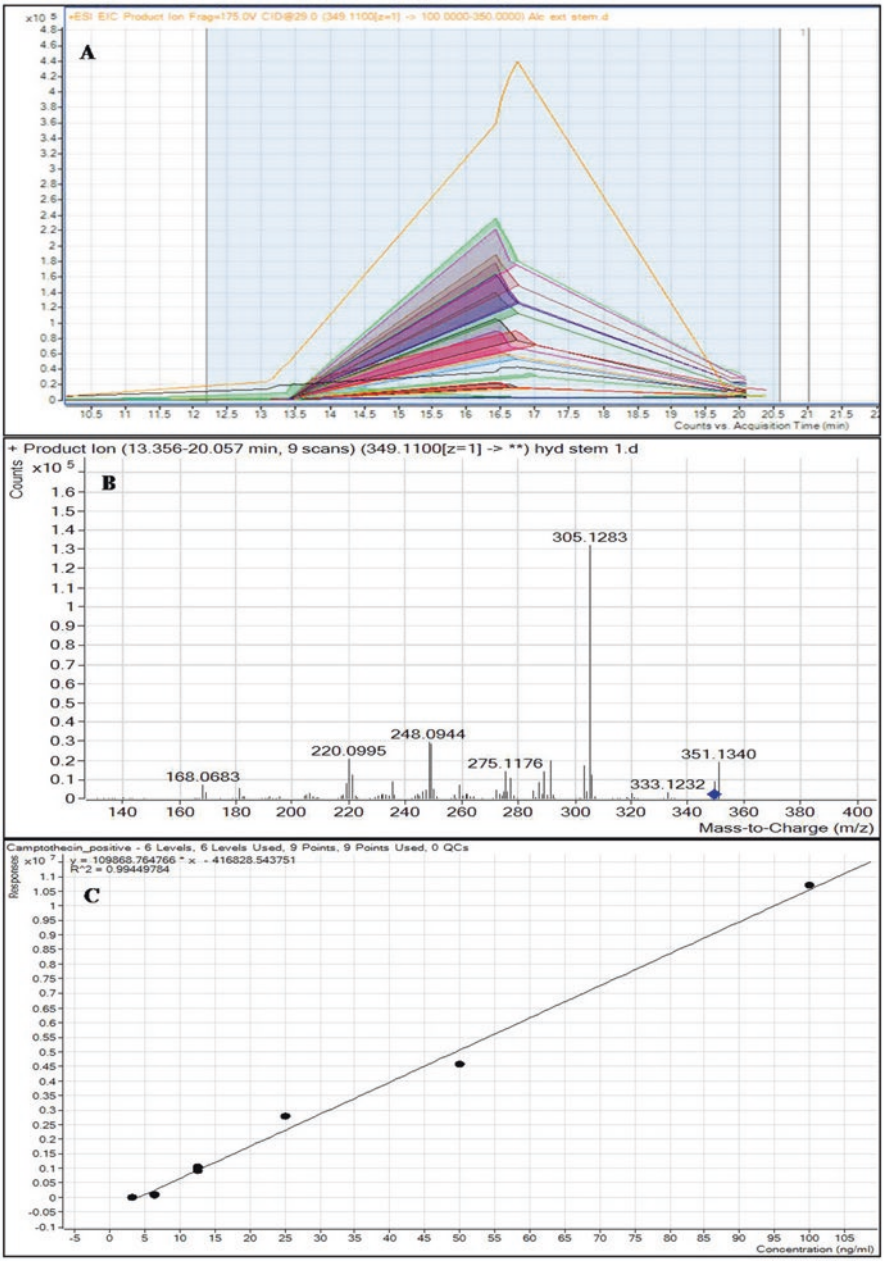

Fig. 8.11 (**a**–**c**) Least retention time variations observed in CPT analysis at 16.418 min 349.1743→305.1283 m/z transition of CPT was used to quantitate it. The blue dot (♦) represents the 349.1743 m/z parent/CPT. The standard plot of CPT had an ideal R^2 and hence was reliable

291.27→139.2374, (+)-catechin 291.27→139.2387, and (−)-epigallocatechin 307.27→163.4057 standards were used to create reference plots with $R^2 > 0.95$. (+)-Catechin and (+)-epicatechin and their biosynthesis-related compound were found to be upregulated in regenerated bark and leaves extracts. (−)-Epigallocatechin was found to be significantly higher in bark water extract as compared to others but showed low antimicrobial activity. Result showed downregulation of (−)-epigallocatechin and upregulation of (+)-catechin and (+)-epicatechin in the regenerated bark and leaves of *S. asoca*. The concentration of (+)-epicatechin in processed drugs (Ashokarishta) from Baidyanath was found to be seven times higher than that of Dabur Pvt. Ltd., but no antimicrobial activity was observed, indicating the variations among the plant-based drugs. This will be helpful in rational use of *S. asoca* parts (Shirolkar et al. 2013a).

8.6.2 Multivariate Statistical Analysis of MS Data

Initial processing of UPLCQ-TOF-MS raw data includes baseline correction, noise reduction, removal of background contaminants, and extraction of molecular features. It is being done in MassHunter Qualitative software, Version 3.1 (Agilent Technologies). The parameters used for extraction of metabolites data are usually set as mass range 50–1200 m/z, mass tolerance 5 ppm, noise elimination level 10, 2.5% of minimum intensity to the base peak intensity, minimum threshold 5000 cps, and retention time tolerance 0.01 min. The ions with identical elution profile and related m/z value are extracted as single molecular feature (MF), within the algorithm employed for full MS/MS data. Molecular features are characterized by retention time, intensity in the apex chromatographic peak, and accurate mass. Background-subtracted data are converted into compound exchange (.cef) file for further use in Mass Profiler Professional (MPP). MPP (Agilent, version B 02.02) is used for statistical evaluation of technical reproducibility and multivariate analysis. The retention time and m/z alignment across the sample sets are performed using a tolerance window of 0.2 min and 20 mDa. The MFs were reduced stepwise based on frequency of occurrence, abundance of respective molecular features in classes, and one-way analysis of variance (ANOVA). To discriminate data unsupervised methods PCA and PLS-DA are applied under the correlation and covariance methods. The metabolites specifically present in different classes get listed out. The abundant metabolites which can act as representative of their groups will be identified further using library and databases. The purpose of doing all these hard-core statistical analyses is to filter out all irrelevant, false entities and only to get true representatives. Different intensity threshold from 1000 to 10,00,000 cpu can be used for molecular feature extraction in the full retention time range. As explained in Table 8.1, LC-MS data may obtain thousands of compounds (here 79,563), it is impossible to analyse them for their trueness and fitness manually so MPP line new age analysis tools are very useful to filter out 79,308 out of these and only to retain

Table 8.1 An overview of molecular features (MFs) extracted at various intensity threshold settings (unit counts) and applied filtering steps in MPP

Intensity threshold setting (counts)	Number of MFs across the sample sets			
	Initial aligned entities[a]	Filtering by frequency[b]	One way ANOVA[c]	Filtering by fold change[d] (FC >2.0)
1000	79,563	606	262	255
5000	72,086	621	305	268
10,000	55,402	612	299	263
50,000	16,722	401	185	158
1,00,000	8323	272	133	107
10,00,000	690	40	20	17

[a]After removal of MFs extracted from blank samples
[b]Filtering criterion was the presence of a MF in at least 100% of samples at least in one group
[c]Displaying entities satisfying corrected p-value cut-off <0.05
[d]Compounds with FC cut-off of >2.0 in at least one condition pair against control condition

255 prominent MFs. Such tireless statistical analysis workflow has emerged as a great boon for researchers in field of mass spectrometry (Table 8.1).

8.6.2.1 Principal Component Analysis

Data is further processed to get molecular features which are significant and differentially expressed in the samples using one way ANOVA with Benjamini-Hochberg correction and fold change analysis. Fold change>2.0 is the most commonly used criterion. PCA is performed via transformation of measured variables into uncorrelated principal components, each being a linear combination of the original variables. Analysis of molecular features gives a clear separation in PCA space of the analysed samples (Fig. 8.12). PCA is also performed to reduce data dimensionality by performing covariance analysis. Heat map and box-whisker plots are the other famous representations used in mass spectrometry, and the changes in expression of a single or many metabolites across study categories can be visualized in heat map (Fig. 8.13), whereas box-whisker is useful in sorting out intro and intergroup variations (Fig. 8.14).

8.6.2.2 SNK Post Hoc Test

ANOVA with post hoc SNK or Tukey HSD is applied to find out the differentially and non-differentially expressed molecular features across the samples with significance ($p < 0.05$). ANOVA with Bonferroni or Benjamini-Hochberg correction and SNK or Tukey HSD post hoc is considered as highly significant and ideally represents only true characters of a data set.

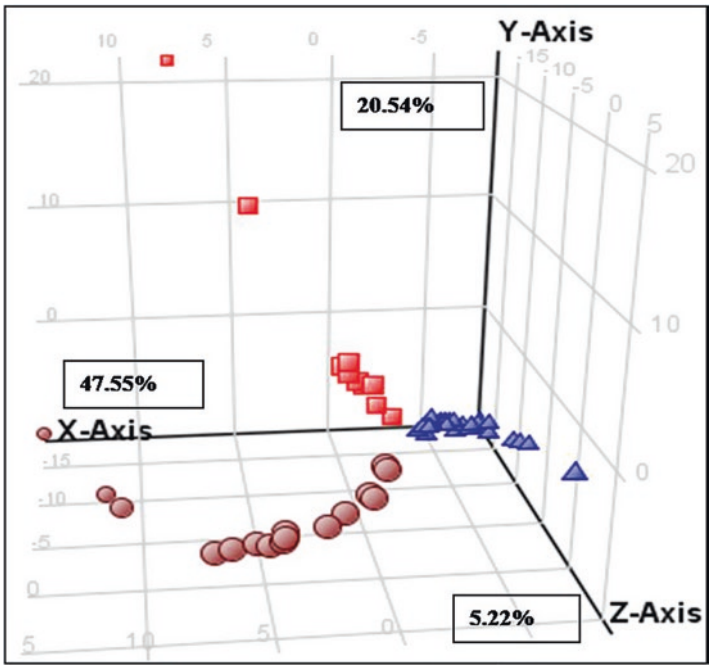

Fig. 8.12 Principal component analysis (PCA) indicates spatial relationship between three groups of metabolites. It is a self-explanatory representation of relatedness or differentiation observed in groups of metabolomes

8.6.2.3 Partial Least Square Discriminant Analysis (PLS-DA)

MFs absent in at least 75% samples of one group are normally removed to reduce the dimensionality of the data sets prior to PCA and PLS-DA. PLS-DA is a widely used supervised pattern recognition method capable of sample class prediction which is used to construct and validate a statistical model for sample classification and discrimination. The results of sample classification are presented in terms of discrimination and recognition abilities, representing the percentage of the samples correctly classified during model training and cross-validation. MPP offers five statistical models but PLS-DA is most routinely used and easy to interpret. Thus PLS-DA model provides excellent separation among the sample varieties. PLS-DA model is sufficient for sample discrimination and authentication (Table 8.2) (Gahlaut et al. 2012, 2013b, c; Shirolkar et al. 2013b; Kumari et al. 2015).

Though we repeatedly discussed here usefulness of LC-MS and statistical analysis software for metabolites (<1500 Da), this workflow is equally useful in analysing larger molecules like proteins or peptides. Without doing much changes in parameters, it can be run on either side. Peptide and protein identification is performed using Spectrum Mill software (Agilent Technologies). Each peptide mass spectrum may be searched against the NCBInr, Swiss-Prot, or any customized data-

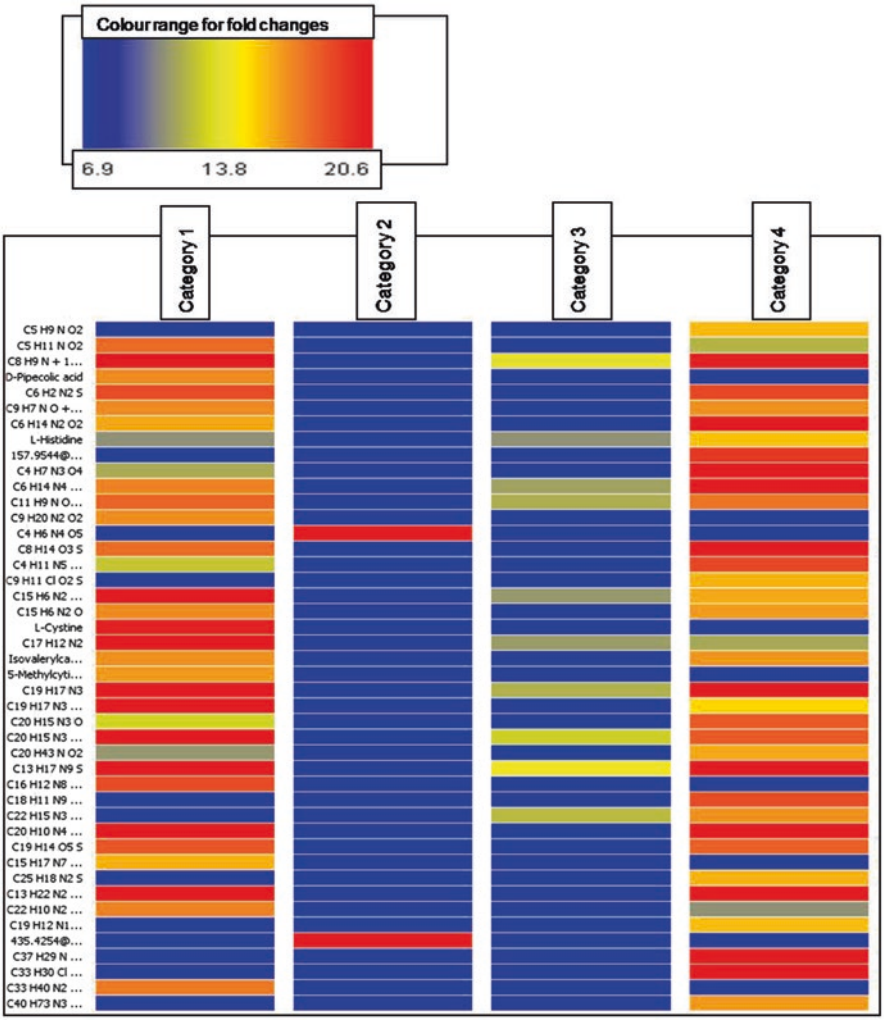

Fig. 8.13 Heat map represents changes in expression of few selected compounds across study groups. Categories 2 and 3 and 1 and 4 have similar levels of expression for these selected metabolites. The colour code for fold changes in expression has also been represented on top

base. The searches are run using the fixed modification of carbamidomethylation labelled cysteine parameter enabled and methionine oxidation as variable modification. Other parameters include MS spectral features (MH^+ 600–10,000 m/z, extraction time range 0–30 min), maximum ambiguous precursor charge 4, precursor mass tolerance ± 100 ppm, scored peak intensity (%SPI) >70, and number of distinct peptides >2 (Gahlaut and Dabur 2013). Such identified peptides can be processed for searching protein-protein interactions or prior to identification through

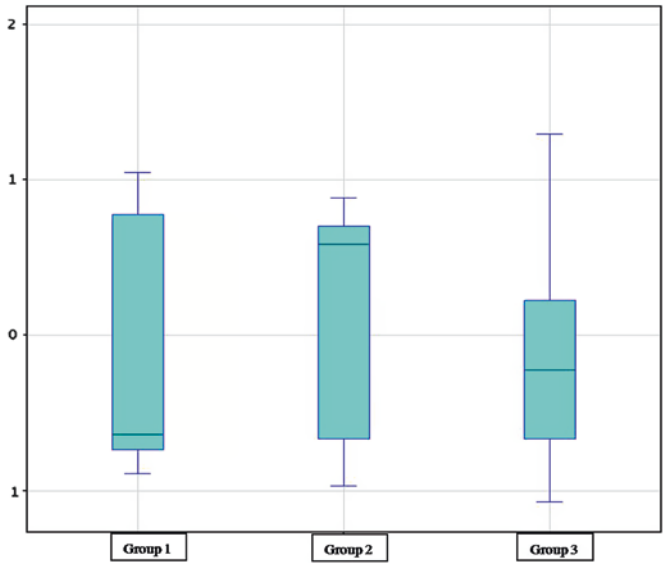

Fig. 8.14 The box-and-whisker plot is an exploratory graphic, used to show the distribution of metabolites in three groups. In this group 3 has the highest level of variability in expression on both sides (up- and downregulation) as indicated by length of whiskers

Table 8.2 Partial least square discriminant analysis (PLS-DA) yielded overall accuracy (%) for categorization of study groups

Category	Category 1 (predicted)	Category 2 (predicted)	Category 3 (predicted)	Category 4 (predicted)	Accuracy (%)
Category 1 (true)	2	0	0	0	100
Category 2 (true)	1	3	0	0	75
Category 3 (true)	3	0	1	0	25
Category 4 (true)	0	0	0	4	100
Overall accuracy	–	–	–	–	71.42

spectrum mill or after they can be imported into MPP. All the above described processes can be run with a protein (digested or undigested) data as well. In MPP we can run a multi-omics experiment involving metabolites and proteins belonging to a same experimental setup, this is actually a simulation of a living entity (i.e. host organism), and very meaningful results will be obtained through it.

8.7 Conclusion and Future Prospects

The multifaceted applicability of mass spectrometry-based platforms for profiling, pharmacokinetics, and pharmacodynamics studies in human, rat, bacterial, fungal, plant, and cell line systems has been revealed by the ascend in the related scientific publications in the past decade. Whole human proteome mapping, human biofluids – urine, blood and cerebrospinal fluid metabolome screening has already been achieved hence profiling and quantitative applications of mass spectrometry are widening vertically and horizontally across the fields of science. It is necessary to generalize this approach for researching complex pharmacological and biochemical principles. Then only the processing cost comprising of sample preparation and data acquisition and analysis would become more economical. The contribution of findings to common online libraries and databases by working groups of scientists would assist budding researchers to begin their journey in this challenging, but interesting and promising field. The easy, free, and updated knowledge sharing would assist not only life science people but also engineers to upgrade the mechanical and technological aspects of the instrumentation.

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Chapter 9
Assessment of Anticancer Properties of Betelvine

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9.1 Introduction

Cancer is steadily gaining the status of a disease with high death rate worldwide, accounting for 8.8 million deaths in 2015, as per WHO report (WHO 2017). Researchers worldwide are reporting new advances in cancer treatment which have undeniably improved health and care of cancer patients. However, the available medication is too costly and harsh; besides, advanced metastasized cancer remains untreatable. Nevertheless, there is a continuous search for effective and safer chemopreventive molecules for the improvement of the drug efficiency and also to lower the treatment cost for cancer. Although breakthroughs are awaited, every research brings in new knowledge and insights that ultimately improves the vision for cancer patients (Burstein et al. 2017). Several ionic radicals produced in the cells such as superoxides, hydroxyl, and nitric oxide radicals, popularly known as reactive oxygen species (ROS) and reactive nitrogen species (RNS), act as pro-mutagens leading to cancer development (Pourahmad et al. 2016). Perceiving the role of ROS and RNS in cancer, mediation of complementary and alternative drugs is notable in the treatment of cancer (Fuchs-Tarlovsky 2013; Xu et al. 2017). Consequently, use of natural phytochemical compounds useful in prevention, retardation, delay, or cure of cancer is emerging as a backup strategy. These alternative methods preferably target the anti-inflammatory and antioxidant properties of the polyphenols, among the different classes of phytochemicals found in several medicinal plants. Nowadays, researchers are trying to formulate various plant-based purified compounds that have chemopreventive and chemotherapeutic properties. This chapter compiles the chemopreventive action of betelvine plant and its bioactive chemical components against cancer.

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Plants are nature's gift, known as the most reliable source for diverse bioactive compounds useful for mankind (Mahady 2001). The plants known to have medicinal or toxicological effects in man and animals are largely used as the source of these bioactive compounds. Some of these plants are rich sources of phytocompounds like phenolics, flavonoids, and complex alkaloids which are able to modulate several metabolic mechanisms and, therefore, can be used as therapeutic agents. Betelvine is one such plant, rich in bioactive molecules. Betelvine, religiously one of the most important plants of Southeast Asia, belongs to Piperaceae family and is mostly consumed for chewing purpose. It is consumed as *paan*, a preparation containing betel leaf and areca nut in India. It is also an economically important cash crop of India and many South Asian countries and is being promoted for its commercial cultivation owing to its nutraceutical properties (Das et al. 2016a). As this plant possesses antifungal constituents, it is also being promoted for use in agriculture against pest management and food spoilage (Pawar et al. 2017). In India, betelvine leaf is also associated with cultural and Ayurvedic importance since ancient times. Chewing of betelvine leaf was a common practice between 75 AD and 300 AD in India (Toprani and Patel 2013). Curative properties of betelvine leaf have been recognized for centuries. Evidence from Indian Ayurvedic literature such as Charaka, Sushruta Samhitas, and Kashyapa Bhojanakalpa shows the use of betelvine leaf in India since 400 BC. As per records of Traditional Knowledge Digital Diary, CSIR, India (TKDL), betelvine finds use in the medication of various illnesses such as acute diarrhea, osteoarthritis, diseases of the spleen, malaria, piles, flatulence, cough/bronchitis, hoarseness, pulmonary cavitation, and diseases of the abdomen to name a few. Several researchers have also revealed various medicinal properties of betelvine useful in the treatment of diabetes, cardiovascular, liver, and ulcer diseases and protection against inflammation, immune disorders, and bacterial, fungal, and parasitic infections (Kumar et al. 2010; Leesombun et al. 2016; Shah et al. 2016a).

Noncarcinogenic nature of Piper betel leaf (PBL) was proved as early as 1979. Water extract from betelvine leaf was used to induce tumor in Swiss mice and C17 mice, which failed showing that unlike believed, betelvine leaf was not carcinogenic (Bhide et al. 1979). However, the betelvine leaf chewing was for long time related to mouth cancer leading to categorizing this plant as a malignant one (Wang and Peng 1996). It is now evident that malignancy is not due to betelvine leaf, several studies have proved that betelvine leaf itself is not carcinogenic, and it is the tobacco and areca nut, promoted by slaked lime, which is responsible for the carcinogenesis (Jeng et al. 1994; Wang and Peng 1996; Lee et al. 2005; Liu et al. 2015). It is now known that the heart-shaped golden leaf of betelvine has considerable amount of antioxidant properties which imparts its anticarcinogenic properties (Choudhary and Kale 2002).

9.2 Antioxidant Property of Betelvine

Extracts of medicinal plants are invariably constituted of multiple chemical components. Betelvine leaf is a rich source of vitamins, ascorbic acid, thiamin, riboflavin, calcium, minerals, and carotenes. Besides, allylpyrocatechol, chavicol, hydroxychavicol, eugenol, and other essential oil components are also recorded in the plant betelvine (Yin et al. 2009; Das et al. 2016b; Sazwi et al. 2013; Syahidah et al. 2017). However, their activities in extracts are representative of an averaged "profile" of anti- and prooxidant behavior of the constituents. The activity of the extract may be additive, or there may exist a complex synergistic interaction among the individual components imparting the beneficial pharmacological effect. Such additive effect of betelvine extract has been reported to inhibit growth of the colon cancer cells, HT29 and HCT116 (Ng et al. 2014). PBL extracts could improve proliferation of human diploid fibroblasts by 143% in young cells, 127% in pre-senescent cells, and 157% in senescent cells (Durani et al. 2017). Combination of the PBL with 5-fluorouracil (5-FU), an antimetabolite-based chemotherapeutic drug commonly used in treating solid human tumors, was observed to be more effective than hydroxychavicol, the major active compound of PBL. Similarly, there are many conflicting reports on ROS-quenching antioxidant mechanisms and ROS-generating prooxidant activities which are believed to majorly drive the chemopreventive and chemotherapeutic effects (Turek 2005). The components like safrole and hydroxychavicol have been reported to produce hepatotoxic intermediates having genotoxic effects, and therefore, restrictions have been made by the Food and Drug Administration (FDA) and other health authorities on their use (Dietz and Bolton 2007). Therefore, there exists a question of ambiguity in defining a framework for an accurate assessment of the chemopreventive and/or chemotherapeutic contributions of these two-faced phytochemicals. The paradoxical roles of betelvine phytochemicals and the versatility of their phenolic functionality in curing disease need to be addressed.

In most of the leading health problems such as cancer, rheumatoid arthritis, Alzheimer's, and other neurological disorders and cardiovascular diseases, free radicals play a role of "key factor." These free radicals are the targets of cancer therapy. In late stages of cancer, when the cancer cells show high amounts of free radicals, prooxidants from plants, that is, the compounds which further enhance the free radicals, are used to kill the cancer cells. On the other hand, during the early stages and post chemo-/radiotherapy, antioxidants are preferred to lower the free radicals. Antioxidants are the compounds that have the capability to scavenge free radicals and prevent the possible damages against cell protein, lipid, and carbohydrate (Chakraborty and Shah 2011). Components in the betelvine leaf extract (PBL) have both prooxidant and antioxidant activities (Gundala and Aneja 2014). Some of the major antioxidant properties of PBL are given in Table 9.1.

Table 9.1 The antioxidant properties of *Piper betle* leaf extract

Scavenging effect/antioxidant properties	References
H_2O_2, superoxide radical and hydroxyl radical scavenging	Dasgupta and De (2004)
Prevention of hydroxyl radical-induced DNA strand breaks in the PUC18 plasmid	Young et al. (2007)
Scavenging of DPPH radicals	Rathee et al. (2006)
Methanolic PBL possess properties of reducing DPPH radical, scavenging superoxide anion and deoxyribose degradation activities	Manigauha et al. (2009)
Hydroalcoholic PBL leaf possesses nitrogen oxide scavenging effect, in vitro	Jagetia and Baliga (2004)
PBL increased the hepatic levels of vitamin A and vitamin C	Padma et al. (1989)
PBL increased the hepatic levels of GSH and SOD	Choudhary and Kale (2002)
Methanol-ethyl acetate of PBL fraction exhibited marked antitumor activity against Ehrlich ascites carcinoma in the mice by modulating lipid peroxidation and augmenting endogenous antioxidant defense systems	Alam et al. (2015)
PBL modulated the expressions of gene involved in antioxidant defense (SOD1, GPX1, and PRDX6), DNA damage, and cell cycle arrest (TP53, CDKN2A, PAK2, and MAPK14) signaling pathways during replicative senescence of human diploid fibroblasts	Durani et al. (2017)
The phytoconstituents in the betel leaves played a major role in the reduction of nanobioconjugates with silver nanoparticles. The anticancer properties of silver nanobioconjugates were higher in the conjugated nanoform than the nonconjugated forms	Preethi and Padma (2016)

9.3 Anticancer Activity and Mechanisms of Betelvine Leaf Extract and Its Constituents

Betelvine leaf is reported to prevent different types of cancer (Rai et al. 2011; Toprani and Patel 2013). Various mechanisms such as cytotoxic and antimutagenic properties of different bioactive constituents of betelvine have been studied for their anticancer properties. Betelvine leaf was observed to prevent mammary carcinogenesis induced by a laboratory immunosuppressor (DMBA), when administered during the initiation phase in rat (Rao et al. 1985). Administration of PBL orally in drinking water as well as feeding of betelvine leaf decreased tumor load and tumor frequency and delayed the onset of DMBA-induced tumors of mammary glands/cells in rat (Bhide et al. 1994). Betelvine leaf prevented tobacco-specific nitrosamine-induced carcinogenesis of tongues at lower dose of the carcinogen N′-nitrosonornicotine (Padma et al. 1989). Rao (1984) showed that topical application of betelvine leaf extract inhibited induced oral tumorigenesis in hamster. It is reported that supplementation of different concentrations of PBL significantly reduced the induced forestomach neoplasia in mice (Bhide et al. 1991). It is also shown that betelvine leaf extract constituents, β-carotene and α-tocopherol, are also effective in decreasing the tumor incidence, reducing tumor burden, and enhancing tumor dormancy period and

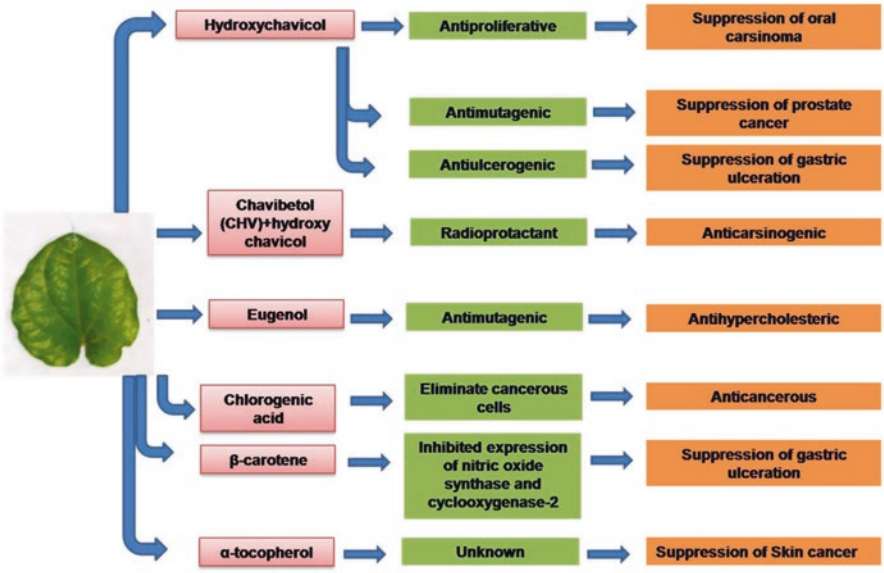

Fig. 9.1 Anticancer nature of different chemical components of *Piper betle*

regress the established frank tumors in Syrian hamsters (Chauhan et al. 2016). An account of different components of betelvine leaf which impart the anticancer activity is given in Fig. 9.1. The major chemical components of betelvine and their mode of action are listed in Table 9.2 and detailed further in the section.

9.3.1 Hydroxychavicol

Hydroxychavicol is the main active component of betelvine leaf which shows several chemotherapeutic and antiproliferative activities (Shah et al. 2016b). It is known as an antimutagenic agent and works as cyclooxygenase inhibiter (Chang et al. 2007). It is reported that hydroxychavicol prevented progression of cell cycle in oral carcinoma cells and prostate cancer (Chang et al. 2002, 2007; Paranjpe et al. 2013). Hydroxychavicol also played an important role in preventing stomach ulceration by its anti-nucleogenic activity. A study suggests that hydroxychavicol acts as APC (a tumor suppressor protein), possesses anti-ulcerogenic activity, and inhibits inflammatory response molecules (nitric oxide synthase and COX-2) which are known to enhance tumor growth by downregulation of the NF-kB pathway (Sarkar et al. 2008). It has been shown to alleviate indomethacin-induced stomach ulceration leading to gastric cancer (Bhattacharya et al. 2005). Rahman et al. (2014) have shown that hydroxychavicol acts with gamma-tocotrienol, an isomer of vitamin E, to modulate different cellular signaling, inducing apoptosis of human glioma cells so as to synergistically inhibit cell proliferation.

Table 9.2 The anticancer properties of the phytochemicals from *Piper betle*

Plant extract/ compound	Anticancer properties	References
Hydoxychavicol	Chemotherapeutic and antiproliferative activities	Shah et al. (2016b)
	Cyclooxygenase inhibiter	Chang et al. (2007)
	Anti-nucleogenic activity	Sarkar et al. (2008)
	Acts as APC (tumor suppressor protein)	
	Inhibits inflammatory response molecules	Rahman et al. (2014)
	Modulates different cellular signaling	
Chavibetol	Radio-protectant	Bhattacharya et al. (2005)
	Immunomodulatory	
	Free radical scavenging activities	Paranjpe et al. (2013)
	Antiproliferative activity	Chen et al. (2000)
	Prooxidant activity	
Safrole	Antimutagen	Nagabhushan et al. (1989)
Chlorogenic acid	Enhance the effects of chemotherapeutic drug	Yan et al. (2015)
Eugenol	Antitumor activity	Al Wafai et al. (2017), Dervis et al. (2017), Venkadeswaran et al. (2014), Manikandan et al. (2010), Pisano et al. (2007), and Kaur et al. (2010)
	Anti-inflammatory	
	Anti-hypercholesterolemic activity in cancer patients	
	Pro-apoptotic activity	
	Inhibits angiogenesis	
	Antioxidant and anti-inflammatory	
β-carotene	Antioxidant	Hosein et al. (2015)
	Nitric oxide synthase and cyclooxygenase-2 inhibitor	Rai et al. (2011)
	Reduces tumor formation	Azuine and Bhide (1992)
p-Hydroxybenzoic acid	Cytoprotective toward human gingival fibroblast cells	Sazwi et al. (2013)

9.3.2 Chavibetol

Chavibetol is a common component of betel leaf and is used as a short-term marker in betel-quid chewing people (Franke et al. 2016). Bhattacharya et al. (2005) have shown that chavibetol, along with hydroxychavicol, obtained in ethanolic extract of betelvine leaves acts as a radio-protectant and shows substantial immunomodulatory and free radical scavenging activities. It is reported that chavibetol synergizes with hydroxychavicol to exert antiproliferative activity against human prostate cancer cells (Paranjpe et al. 2013). The prooxidant activity of chavibetol is induced by high concentrations of hydroxychavicol causing oxidative damage in liver cancer cells (Chen et al. 2000).

9.3.3 Safrole

Safrole is another important component of betelvine leaf and inflorescence found in large amounts. In the human body, it is rapidly degraded into dihydroxychavicol and eugenol, which are antimutagenic agents and are readily excreted via the urine (Nagabhushan et al. 1989; Chang et al. 2002). However, safrole is listed as a potential human carcinogen (Patnaik 2004; Chen et al. 2007). Safrole is reported to suppress the defensive activity of neutrophils against oral pathogens, thus leading to compromised oral health (Hung et al. 2003). Chang et al. (2009) have reported inhibitory effects of safrole from *P. betle* inflorescence on phagocytic activity and release of superoxide anion (O_2^-) of polymorphonuclear leukocytes.

9.3.4 Chlorogenic Acid

Chlorogenic acid (5-caffeoylquinic acid) is a phenolic compound commonly found in coffee, apples, pears, and green tea. Due to its antitumor effect in multiple malignant tumors (Belkaid et al. 2006; Yan et al. 2015; Xue et al. 2017), these dietary products are often referred to as cancer preventive food. Chlorogenic acid has been reported to enhance the effects of chemotherapeutic drug 5-fluorouracil in human hepatocellular carcinoma cells by inhibiting extracellular signal-regulated kinases (Yan et al. 2015). Chlorogenic acid from betelvine leaves has been reported to eliminate cancerous cells without harming normal cells (Guha 2006; Rai et al. 2011).

9.3.5 *Eugenol*

Eugenol, a 4-allyl-2-methoxyphenol, is a common phytochemical and the major ingredient of clove oil, bay leaves, and cinnamon leaf which has been exploited for numerous pharmacological applications and is reported to possess antitumor activity against MCF-7, PC3, and Caco2 cancer cell lines (Al Wafai et al. 2017; Dervis et al. 2017). Eugenol is also a major constituent of betelvine leaf and consists of antimicrobial and anti-inflammatory properties. Eugenol from PBL was reported to possess a remarkable anti-hypercholesterolemic activity in mice induced by Triton Wr-1339. Importance of treating hypercholesteremia is to minimize the risk of heart disease and other cardiovascular complications especially in cancer patients (Venkadeswaran et al. 2014). In cancer prevention, eugenol plays a significant role by inducing apoptosis and inhibiting the angiogenesis and invasion in induced gastric carcinogenesis in rats (Manikandan et al.2010). Eugenol inhibited DMBA-croton oil-induced skin carcinogens in mice (Sukumaran and Kuttan 1995). Eugenol induced antiproliferative and apoptosis promoting activity in malignant melanocytes (Pisano et al. 2007). In mice eugenol prevented chemical carcinogenesis in cutaneous region by intercepting oxidative stress and inflammation. It downregulated c-Myc and H-ras and concomitantly activates p53-dependent apoptosis to eliminate the mutated cells (Kaur et al. 2010).

9.3.6 *β-Carotene*

Beta-carotene is a precursor of retinol (vitamin A) and occurs naturally in fruits and vegetables. It is a well-known antioxidant which inhibits DNA damage occurring due to free radicals and also exhibits potential antineoplastic and chemopreventive activities. Other anticancer activities of β-carotene include (i) induction of cell differentiation and apoptosis of some tumor cell types, in early stages of the disease, and (ii) stimulation of the release of natural killer cells, lymphocytes, and monocytes to enhance immune system (National Center for Biotechnology Information. PubChem Compound Database; CID = 5280489, https://pubchem.ncbi.nlm.nih.gov/compound/5280489). The antioxidant capability of β-carotene from betel leaf is reported to quench the mutagenic free radicals of betel quid and counteract the different pathology causing irritants. This was used to show that tobacco with green betel quid was less harmful than dried tobacco or "paan masala" due to the β-carotene activity (Hosein et al. 2015). β-Carotene extracted from betelvine leaf played an important role in inhibition of *Helicobacter pylori*-induced gastric disease. It inhibited the expression of *H. pylori*-induced nitric oxide synthase and cyclooxygenase-2 which play a critical role in cancer development (Rai et al. 2011). Several animal studies have also shown that topical application of betelvine leaf extract, α-tocopherol and β-carotene, shows effective results in reducing tumor formation (Azuine and Bhide 1992).

9.4 Conclusions and Future Prospects

Cancer is becoming a serious medical problem owing to the chemical pollution in food, air, and water and lifestyle and is increasing with a fast pace. Therefore, there is a need to discover new pharmacologically important compounds which can be used in cancer prevention, therapeutics, or postradiation/chemotherapy management. Plant sources which have nutraceutical properties and anti-malignant activities need to be identified and explored. Betelvine shows the potential to fight against cancer due to its rich antioxidant phytochemical compounds including phenolics, flavonoids, and alkaloids. Betelvine plant extract has proven anticancer role against oral cancer, mammary cancer, skin cancer, prostate cancer, and gastric cancer under lab conditions. However, in spite of the rich antioxidant activities, its use has been controversial owing to its role in oral carcinogenesis. Though it is now clear that the carcinogenic nature observed with betelvine is due to modifications brought about by other components (e.g., slaked lime in betel quid), it remains unacceptable in the international society. There is a need to explore basic mechanisms behind the anticancer properties of betelvine extract and its components to clearly understand the therapeutic role of compounds. Future studies to develop therapeutic or nutraceutical product and their use in cancer therapy can be of importance. There is a requirement of heavy antioxidants in cancer patients post chemotherapy and radiation. To begin with, a strong antioxidant formulation from betelvine leaves can be developed as a supplement.

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Chapter 10
Analysis of Patents Filed for the Herbal Therapeutics Against Cancer

Pooja Rawat and Pawan Kumar Singh

10.1 Introduction

World Health Organization (WHO) reported cancer as the second leading cause of mortality worldwide, which accounted for 8.8 million deaths in 2015. Cancer includes abnormal cell growth which proliferates and invades to other healthy body cells, making them diseased (Zaid et al. 2017). Uncontrolled proliferation of cancerous cells has been linked to mutations in several oncogenes and tumour suppressor genes. Several therapeutics designed to interfere with these mutated genes are known to target cancer cells. Besides oncogenes, disturbances in the metabolic processes of cancerous cells are closely associated with malignancy of cells (Wang et al. 2016). Major lines of treatment for cancer include surgery, chemotherapy and radiotherapy. Chemotherapy (synthetic drug) is the most widely adopted method of treatment in the majority of the cases. However, severe toxicity and side effects of synthetic drugs are generally associated with such treatment. Chemodrugs are categorized into different groups based on their mode of action and chemical structures. These mainly include alkylating agents such as altretamine, busulfan, carboplatin, carmustine, chlorambucil, cisplatin, cyclophosphamide, dacarbazine, lomustine, melphalan, oxaliplatin, temozolomide, thiotepa, etc. Alkylating agents cause damage to DNA of cancer cells and target different phases of the cell cycle. Antimetabolites are another class of drugs which substitute for the normal building blocks of RNA and DNA, thus interfere with DNA and RNA growth. These include 5-fluorouracil (5-FU), 6-mercaptopurine (6-MP), capecitabine (Xeloda®), cytarabine (Ara-C®), floxuridine, fludarabine, etc. Other categories of chemotherapeutic agents include topoisomerase inhibitors, mitotic inhibitors, corticosteroids, etc. The use of chemotherapeutic agents is mostly associated with several

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side effects. For example, use of alkylating agents involves dose-dependent risks to cells of bone marrow, which may also lead to leukaemia. Several other side effects, such as mouth and throat sores, headache, diarrhoea, constipation, nausea, vomiting, cognitive dysfunction, appetite loss, hair loss, etc., are associated with chemotherapy. Besides these, resistance to chemotherapeutic agents is a persistent problem making the therapy ineffective or less effective. Several mechanisms have been suggested to be involved in drug resistance including alterations in drug activation, drug degradation, target enzyme and membrane transport proteins, enhanced DNA repair, mutated cell cycle proteins such as p53, which leads to failure of cancer cell apoptosis (Luqmani 2005). These drawbacks associated with use of conventional therapeutics necessitate the need of safe alternative therapy or drugs. Discovery of natural products (obtained from plants, microbes, organisms, cell lines, etc.) are now gaining momentum and being relied upon as safe and effective alternatives for the cancer treatment (Newman and Cragg 2016; Zaid et al. 2017).

Anticancer activities of many plants are well reported in scientific literatures. Several studies have highlighted the potential of plant powder, extract, fraction or their compounds against different types of cancer cell proliferation (Sak 2012). Many compounds derived from plants have been used clinically as anticancer agents (Amawi et al. 2017). Based on the structure, four major classes of anticancer compounds isolated from the plants have been defined. These four classes include epipodophyllotoxin lignans, vinca alkaloids, taxane diterpenoids and derivatives of camptothecin quinolone alkaloid. Vinca alkaloids include vinblastine and vincristine obtained from *Catharanthus roseus* G. Don. (Apocynaceae). Several derivatives of vinca alkaloids such as vinorelbine and vinflunine with reduced toxicity have also been synthesized and used as anticancer agents (Okouneva et al. 2003). Epipodophyllotoxin lignan, an isomer of podophyllotoxin, is another class of plant-derived compound. Semi-synthetic analogs of epipodophyllotoxin lignans such as etoposide and teniposide have been found to be very effective in treating lymphomas and bronchial and testicular cancer. Paclitaxel, a taxane compound, is extracted from *Taxus brevifolia* bark. Docetaxel, an improved derivative of paclitaxel, is found to be effective against lung cancer, prostate cancer and also lymphoid malignancies. Other compounds with anticancer activity isolated from plants are berbamine, berberine, bruceantin, colchicine, cucurbitacin, curcumin, ellipticine, indirubin, salvicine, triptolide, etc. (Nirmala et al. 2011; Amawi et al. 2017).

The plants and their extracts are known to mediate their cytotoxic effect via a number of mechanisms, viz. alteration of mitochondrial metabolism, inhibition of P-glycoprotein and nuclear factor kappa-light-chain-enhancer of activated B cell (NF-kB) activation, inhibition of leucine transport in cancerous cells, apoptosis through epidermal growth factor (EGF) dependent pathways, etc. (Ossikbayeva et al. 2016; Pan et al. 2016; Singh et al. 2016; Wibowo et al. 2016). Besides the

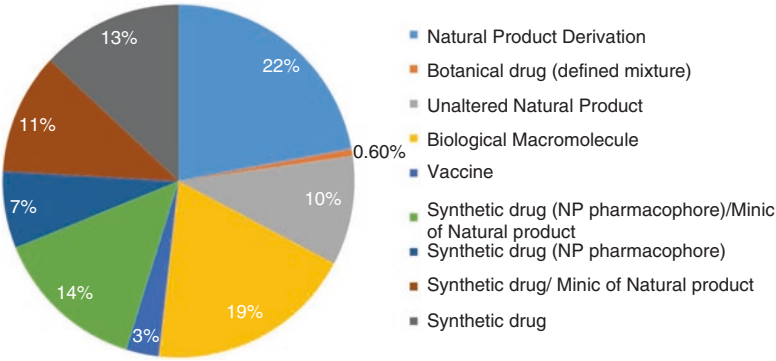

Fig. 10.1 Percentage distribution of anticancer drugs (1981–2014). (Source: Newman and Cragg 2016)

direct cytotoxic effects, herbal extract act as immunomodulators and are known to strengthen the immune system of the body leading to higher chances of survival. Natural products are, therefore, generally recognized as safer and reliable drug candidates against various types of cancers (Reddy et al. 2003). Several classes of natural compounds such as phenolics, alkaloids, triterpenoids, sesquiterpenoids, etc. exhibit a significant anticancer activity (Jakobs et al. 2016; Shanmugam et al. 2016; Ma et al. 2017a, b).

Globally, investigations are underway to evaluate and develop herbal-based therapeutics for the treatment of different cancer types. Out of 174 anticancer drugs developed during 1981–2014, 89 were either natural product derivatives, biological macromolecules or unaltered natural products, whereas 80 drugs were synthetic drugs including the natural product mimics (Newman and Cragg 2016). Percentage distribution of different categories of anticancer drugs developed during the above period is shown in Fig. 10.1. Recognition of plant-derived products as a potential and safer alternative for cancer treatment is evident by the increasing number of research papers being published across the world. However, rate of translation of herbal leads into drugs can be more precisely estimated from the number of patents being filed in the area. Compared to publications, patents may be considered better indicator of translation of researches into commercial products. In this chapter, we attempted to analyse and discuss the trends of patents being filed by different countries for innovative herbal solutions for cancer treatment. The main objective of this article is to identify trends of patents being filed in the area of herbal therapeutics for cancer. The reasons why few countries are leading, and the initiatives taken by these countries towards promotion of herbal medicines have also been highlighted. Key compounds isolated from the plants and claimed in many patents during 2012–2017 have been summarized along with their mode of action and extent of their protection. It will be interesting to investigate the occurrence of similar anticancer compounds in plants, not explored till date and to check their anticancer activity.

10.2 Methodology Used for Data Collection

Retrieval of patent data was done using licenced version of Thomson Innovation patent database (www.info.thomsoninnovation.com/; data accessed on 06 May 2017). For confirmation of patent trends, search was also carried out on various free databases, i.e. Patentscope, Espacenet, etc. Patents filed and published in the area of herbal therapeutics against cancer were searched using different combinations of keywords such as cancer, herbal, tumour, traditional Chinese medicines and TCM.

10.3 Global Scenarios in Terms of Patents Filed for Herbal Therapeutics Against Cancer

A total of 7967 patents were found on herbal therapeutics invented and innovated against cancer. These patents were filed by different countries across the globe. Among all, China is on the top in the area of inventions filed on herbal therapeutics against cancer followed by the United States. Out of 7967, 4657 patents (58%) were filed in China, whereas in the United States, 727 (9%) of the total patents were filed, which is very less as compared to China. The distribution of patent applications filed by different countries is shown in Fig. 10.2a. A shift in the focus towards herbal medicines for the treatment of cancer can be inferred from the number of patents being filed and published. During the last 15 years, a gradual increase of the number of patents published in the area has been observed. Published patents almost doubled in the number from 2010 to 2016 (Fig. 10.2b). Hu Anran, China; University of Yale, United States; Council of Scientific and Industrial Research (CSIR), India; and GW Pharmaceuticals Ltd., United Kingdom, were among the top 4 assignees of herbal patents for cancer filed across the world (Fig. 10.2c).

From the analysis of top technology areas in which patents related to herbal therapeutics for cancer were filed revealed that the technology categories A61K and A61P to be among the top, followed by A23L, C12N and C07D (Table 10.1). A single innovation/invention may be given only one or different categories/codes of international patent classification (IPC).

10.4 Analysis of Patents Filed by China, the Top Player in Herbal Therapeutics for Cancer

China was found among the top in terms of inventions/innovations filed (total 4657) for herbal therapeutics against cancer. Trends of patents published in China during the last 10 years are shown in Fig. 10.3a. Out of these, majority of herbal leads were directly or indirectly derived from traditional Chinese medicine (TCM). A total of 1530 patents filed in China involved the use of traditional Chinese medicinal herbs,

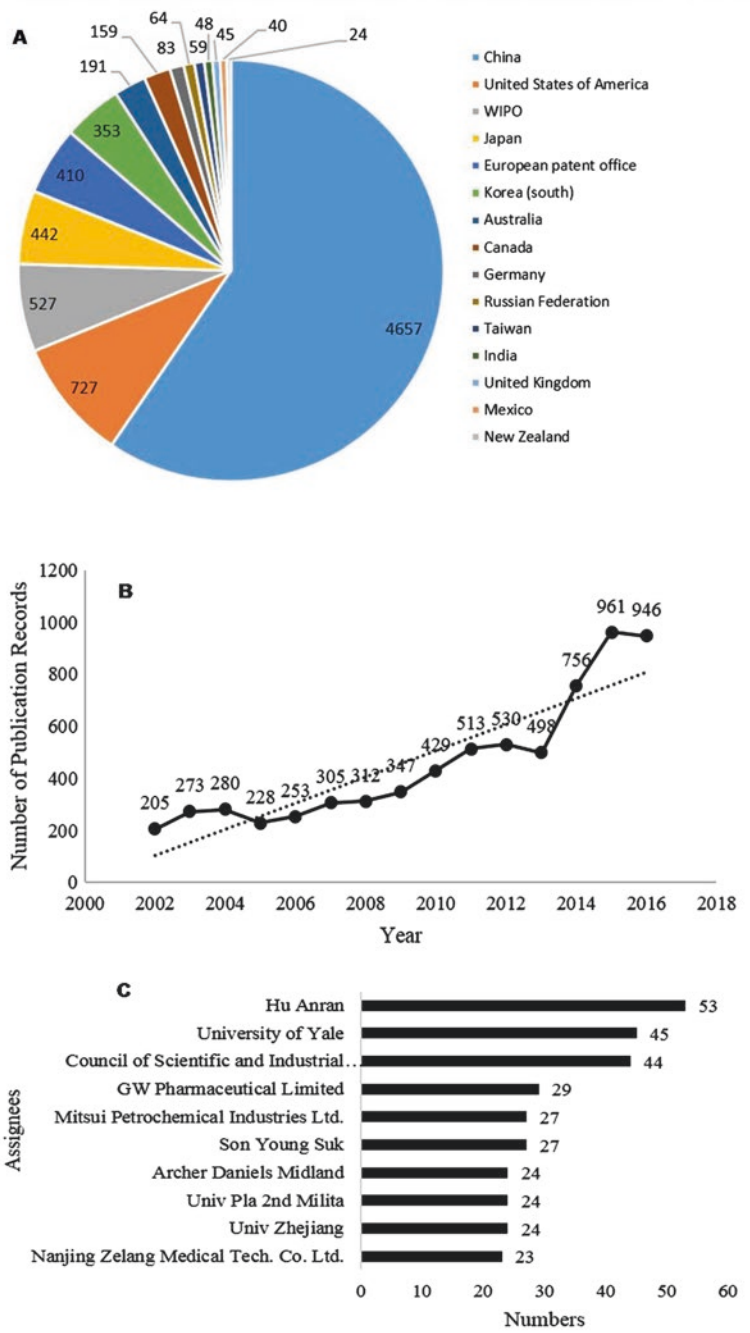

Fig. 10.2 (**a**) Top 15 countries filing patents for herbal therapeutics against cancer; (**b**) trends of publications across the globe during the last 15 years; (**c**) top 10 assignees across the world for herbal therapeutics of cancer

Table 10.1 Top technology areas where patents on herbal therapeutics for cancer were filed

IPC	Document count	Percentage	Details
A61K	5934	45.15	Preparations for medical, dental or toilet purposes
A61P	4996	38.01	Specific therapeutic activity of chemical compounds or medicinal preparations
A23L	1090	8.29	Foods, foodstuffs or non-alcoholic beverages; their preparation or treatment, e.g. cooking, modification of nutritive qualities, physical treatment; preservation of foods or foodstuffs, in general
C12N	660	5.02	Microorganisms or enzymes; compositions thereof; propagating, preserving or maintaining microorganisms; mutation or genetic engineering; culture media
C07D	463	3.52	Heterocyclic compounds

which accounted for about 99% of the patents filed globally on TCM. This data shows the focus of Chinese researchers, industries and policy makers towards the promotion and protection of their indigenous system of medicine, i.e. TCM.

The top assignee in China for TCM-based patents on cancer is Hu Anran. Other assignees include Qingdao Huangdao District Chinese Medicine Hospital, Qingdao Tumour Hospital, Qingdao Central Hospital, Chen Xin, Qingdao Xinlide Traditional Chinese Medicine Technology Research and Development Co. Ltd., Qingdao Chang'anda Pharmaceutical Co. Ltd., Shandong Provincial Hospital, etc. (Fig. 10.3c). Tasly Phar. International Co. Ltd., Tianjin, and Tong Ren Tang Chinese Medicine Co. Ltd, Beijing, are among some of the top emerging pharmaceutical companies, while as top brands of TCM in China are Yunnan Baiyao (a haemostatic powdered medicine), Pien Tze Huang (used for liver disease) and Dong'e Ejiao (DEEJ) (used for the treatment of gynaecological diseases, such as menoxenia and post-partum uterine bleeding). Most of the translational researches on cancer therapeutics based on TCM include substantial data from controlled clinical trials. In these studies, TCMs have been used either as adjunct therapy or as a sole therapy against cancer. A significant rise in the number of publications of controlled clinical studies on efficacy of TCM against cancer has been observed. Categories of cancer in these trials include melanoma and malignant neoplasms of the digestive organs, bone, eye, brain, central nervous system, endocrine glands, etc. About 25.51% out of total 2964 studies showed effectiveness of TCMs against cancer (Li et al. 2013). Kanglaite Injection, a novel broad-spectrum anticancer injection produced from traditional Chinese medicinal herb (the coix seed) by China's Zhejiang Kanglaite Pharmaceutical, was the first TCM drug approved by the US Food and Drug Administration (FDA) for clinical trials on humans. The key active ingredient of the drug is the purified oil in microemulsified form. Currently, phase III clinical trial of Kanglaite is under process.

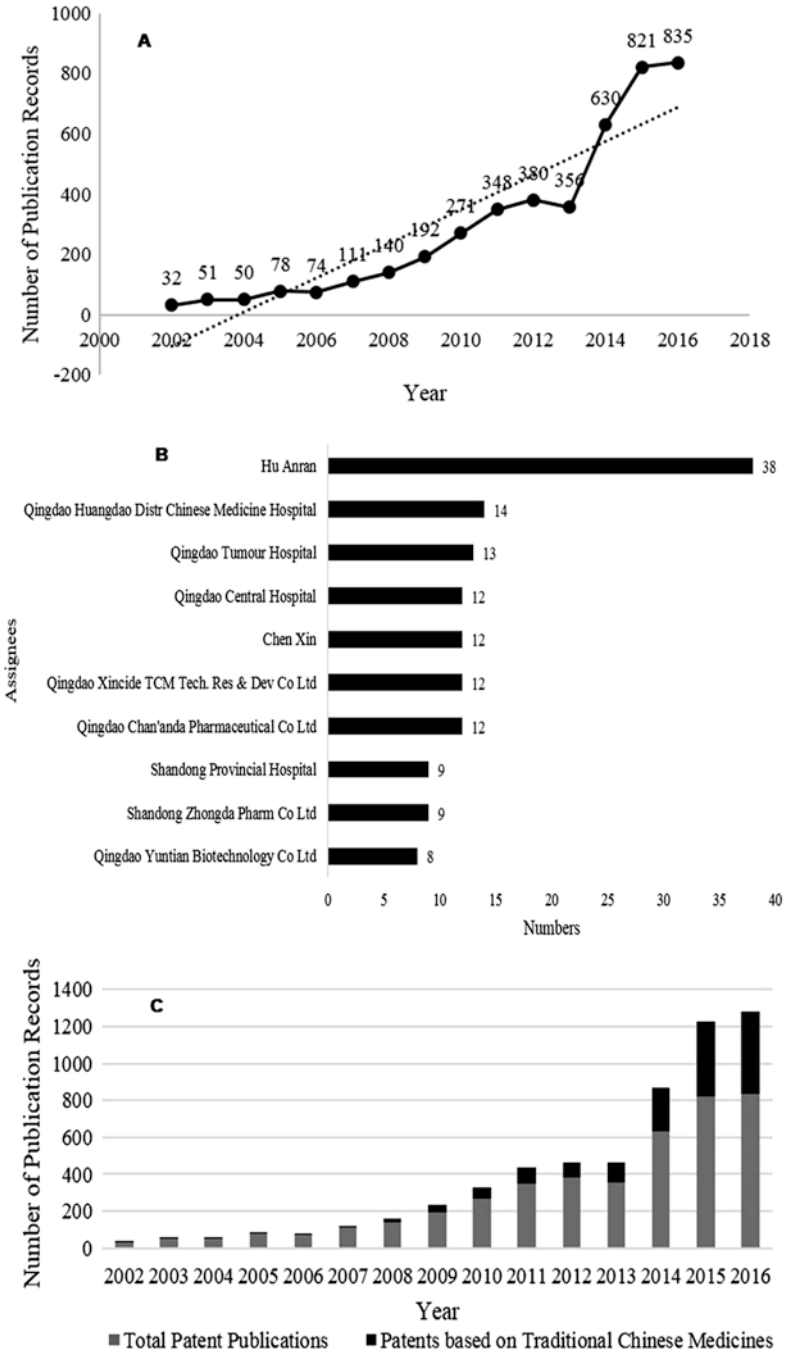

Fig. 10.3 (**a**) Trends of patent publications on cancer herbal therapeutics in China during the last 15 years; (**b**) top 10 assignees in China for TCM-based therapeutics of cancer; (**c**) comparison of total patents published in China and publications on TCM (2002–2016)

Table 10.2 Top 5 categories of patents filed in China and involving TCM herbs

Top technology areas	Numbers
A61K	1380
A61P	1362
A23L	102
A23F	39
C12G	16

10.4.1 Traditional Chinese Medicines

The patents filed on traditional medicinal herbs from China fall under A61K and A61P categories (Table 10.2). Thrust on TCM in China is evident from the parallel increment in the total number of patents filed in the country and the patents based on TCM. A sharp increase in the number of TCM-based patents published in China increased from 20 in 2008 to 448 in 2016 (Fig. 10.3b). This approximated to 95% increase in publications on traditional Chinese medicines. In China, government has taken initiatives and created national policy to include TCM in the process of drug development for various medications including cancer. History of integration of TCM in the national health policy of China dates long back to 1949, when TCM national office was established under the Ministry of Health. In China, a large portion of the national budget is allocated for TCM research and development, TCM hospitals and TCM research institutes. Several national plans such as development plan for Chinese medicine health services and the protection and development plan of Chinese Materia Medica (CMM) were implemented in 2015. For TCM promotion, China has focussed in all the relevant areas of management, markets, education, industries, human resources, research and development and international cooperation. In 2014, CMM (including 203 CMM formulated drugs and 1100 CMM slices) occupied 31% of total pharmaceutical Chinese markets with a sale value of more than US $120 billions. In comparison to 2011, budget allocation for CMM was double in 2014 (US $4.66 billion) (Dang et al. 2016).

10.5 Trends of Patent Publications Filed in the United States

A total of 727 patents were filed in the United States approximating to 9% of the total patents filed across the globe. After China, the United States was the second country where maximum patents in herbal therapeutics for cancer were filed. Analysis showed almost constancy in the number of patents published from the country during last 15 years (Table 10.3). Assignees for patents filed in the United States include the University of Yale (United States), Council of Scientific and Industrial Research (India), Lee Chen-Yu (Taiwan), Archer Daniels Midland Co. (United States), EcoSMART Technologies Inc. (United States), Macau University of Science and Technology (Macau, China), University of Southern California (United States), etc. (Table 10.4).

Table 10.3 Trends of patent publications in the United States during the last 15 years

Year	Number of publications
2016	35
2015	34
2014	38
2013	45
2012	37
2011	32
2010	34
2009	36
2008	40
2007	45
2006	40
2005	40
2004	46
2003	71
2002	48

Table 10.4 Top 10 assignees of patents published in United States, for herbal therapeutics of cancer

Top assignees in the United States	Numbers
University of Kaohsiung Medical	6
US Agriculture	6
US Health	6
University of Southern California	6
Macau University of Science and Technology	7
EcoSMART Technologies Inc.	7
Archer Daniels Midland	8
Lee Chen-Yu	8
University of Yale	10
Council of Scientific and Industrial Research	10

10.6 Trends of Patent Publications Filed in Japan

A total of 442 patents were filed in Japan. No major incremental shift in the patents published from Japan in the area of herbal therapeutics against cancer was observed (Table 10.5). Top assignees from the region include Mitsui Petrochemical Industries, Umezawa Kazuo, Ichimaru Pharcos Inc., Terumo Corp., Zenyaku Kogyo KK, etc. (Table 10.6).

Table 10.5 Number of patent publications in Japan during last 15 years

Year	Number of publications
2015	13
2014	11
2013	11
2012	18
2011	18
2010	16
2009	15
2008	0
2007	16
2006	0
2005	0
2004	17
2003	13
2002	19
2001	0
2000	14

Table 10.6 Top assignees in Japan for herbal therapeutics of cancer

Top assignees in Japan	Numbers
Zenyaku Kogyo KK	7
Terumo Corp.	8
Ichimarus Pharcos Inc.	8
Umezawa Kazuo	9
Mitsui Petrochemicals	21

10.7 Status of Herbal Therapeutics Claimed for Cancer Treatment in India

Globally, India represents only 0.6% of the total patents published related to cancer. This is despite the fact that India has a long heritage of codified and non-codified traditional system of medicines, which mainly comprises herbs. In India, the knowledge on various medicinal herbs and their uses in the treatment of various ailments including cancer and related problems are well codified in classical literatures and also being practised by traditional healers even today. However, unlike TCM, Indian traditional system of medicines has not been promoted at the national and global level. Trends of patents published in the area of herbal therapeutics for cancer in India is shown in Table 10.7. Despite the shift in attention of the world towards herbal medications, no major changes in the patent publication rate was observed across the years. No significant increment in the number of patents published across

Table 10.7 Number of patent publications in India during last 15 years

Year	Number of publications
2016	4
2015	8
2014	3
2013	1
2012	3
2011	1
2010	6
2009	7
2008	6
2007	6
2006	2
2005	1

Table 10.8 Top assignees in India for herbal therapeutics of cancer

Top assignees in India	Numbers
Piramal Life Sciences Limited	2
Queensland University of Technology	2
Zetiq Technologies Ltd.	2
Medsaic Pty Ltd.	2
Hexima Ltd.	2
Balmoral Australia Pty. Ltd.	2
Sterogene Bioseparations Inc.	2
Bui, Can V.	2
Bui, Cuong Q.	2
Council of Scientific and Industrial Research	4

the years could be seen; however dip was observed in the published patents during 2011–2013. Top assignees in India include the Council of Scientific and Industrial Research (CSIR), Piramal Life Sciences Ltd., Queensland University of Technology, Medsaicpty Ltd., Sterogene Bioseparations Inc., etc. (Table 10.8). Many other countries have filed patent applications in India, considering India as one of the prime markets for the herbal drugs for cancer.

The less number of patents from India may be due to the absence of streamlined efforts by the government towards promoting traditional Indian medicines (TIM) at the national and global level. Though in recent years, few initiatives were taken by the government such as establishment of department for Indian Systems of Medicine and Homoeopathy (ISM&H), presently known as AYUSH (Ayurveda, Yoga, Unani, Siddha and Homoeopathy), and drug testing laboratories for TIM, however a lot more needs to be done.

To promote the scientific research focussed on various areas of traditional medicines of India, five research councils were established under the Ministry of AYUSH, India, viz. Central Council for Research in Ayurvedic Sciences (CCRAS), Central Council for Research in Unani Medicine (CCRUM), Central Council for Research in Yoga and Naturopathy (CCRYN), Central Council for Research in Siddha (CCRS) and Central Council for Research in Homoeopathy (CCRH), with around 81 research institutes and centres. Efforts are under process to increase international collaborations thorough signing memorandum of understandings (MoUs), setting up of AYUSH Information Cells at several locations, viz. Cuba, Hungary, Indonesia (Jakarta and Bali), Mauritius and Russia for dissemination of AYUSH systems of medicine. Furthermore, focus has to be given in other areas such as integration of TIM in education, research and development, management of resources and industrial collaborations.

Another constraint in promoting herbal medicines is the stringent patent laws in India. In Indian Patent Act, 1970, u/s 3 (p), an invention which, in effect, is traditional knowledge or which is an aggregation or duplication of known properties of traditionally known component or components cannot be patented. Since TIM is part of traditional knowledge, the section 3 (p) presents itself as a bottleneck for the industries to shift their interest towards herbal drug development, and thus we can see lesser promotion of TIM at global front.

10.8 Active and Specific Patented Phytochemicals with Anticancer Properties

During years 2012–2017, we found 66 patents (24 granted) where active phytochemicals with anticancer properties were isolated from plants. Englerins and their derivatives were isolated from *Phyllanthus engleri* and used for the treatment of a number of cancers, particularly renal cancer. Biological activity of the compound fractions was assessed by cell growth assays using the human renal line U0-31. The compound was also found to be active against a number of leukaemia, non-small cell, colon cancer, melanoma, prostate, renal, breast, ovarian and CNS cancer cell lines (CA2711434 C). Moreover, indirubin derivatives were used as therapeutics for wild-type or T315I mutant Bcr-Abl-positive chronic myelogenous leukaemia patients. The compounds were also shown to be effective against prostate cancer and lymphoma. Inhibition of Bcr-Abl/Stat5 or Src/Stat5 signalling in human KCL-22 CML and imatinib-resistant human KCL-22 CML cells expressing the T315I mutant Bcr-Abl indicated the involvement of above signalling pathway (US9512076B2). A dimer ketone compound, fistulain B, was extracted from the bark of *Cassia fistula* and showed cytotoxicity against leukaemia cells (NB4), lung cancer cells (A549), human neuroblastoma cells (SHSY5Y), prostate cancer cells (PC3) and breast cancer cells (MCF7). Several other compounds, their mechanism of action and extent of their protection along with their natural source are listed in

Table 10.9. Identification of active ingredients of herbal medicines is important for understanding the mechanistic action and further improvement in the efficacy. Through structural modifications of natural phytomolecules, side effects may be reduced and in vivo efficacy may be enhanced. Alteration of structures of naturally derived phytomolecules through derivatization has been used for modernization of traditional medicines. This is also evident from the percentage distribution of anticancer drugs, where derivatized natural product constitutes the higher percentage (22%) in comparison to anticancer drugs based on natural products (11%) (Fig. 10.1).

Apart from single phyto-compound-based drugs, various standardized extracts or fractions are also effectively used as anticancer therapeutics. The single compounds isolated from plants are known to show anticancer activity through various mechanisms such as cytotoxicity, inhibition of cell growth and induction of apoptosis, inhibition of formation of new blood vessels of tumour as well as suppression of cancer invasion and metastasis. Extracts or fractions isolated from plants and claimed to have anticancer efficacy include single plant-based extracts as well as composite medicinal herbs. Some of the single herb-based extracts have been isolated from *Anemarrhena asphodeloides* (US20100009017), *Serenoa repens* (US6599540), *Gleditsia sinensis* (US20090258096), *Rhus verniciflua* (US7618661), *Solanaceae dulcamara* (US7250180), *Resina ferulae* (US20040043083), *Euphorbia antiquorum* (US20030165579), etc. Several patents granted on the extracts isolated from multiple herbs include herbal formulation based on *Tinospora cordifolia* and *Aloe barbadensis* (US6649185); herbal extracts consisting essentially of goldenseal, myrtle and *Centaurea* (US5876728); herbal composition based on six herbs, *Wu Bei Zi* sp., *Lonicera japonica*, *Astragalus membranaceus*, *Radix Rehmanniae*, *Radix glycyrrhizae* and *Panax ginseng* (US20070082072); and composition based on *Cinnamomi cortex* extract and a *Zizyphi fructus* extract (US20070160691) (Feng et al. 2011).

10.9 Conclusions and Future Prospects

Success of China in becoming a leader in the herbal therapeutics may be attributed to the recognition of TCM by the government and their inclusion in the national health policy. Like TCM, TIM is the traditional system of medicines from India and includes Ayurveda, Siddha and Unani medicinal system. This chapter reviewed various published patents on the isolation of compounds from plants, their extraction and their use against cancer disease. Many of these anticancer plants reported in the patents are also reported in TIM, and recent studies have validated their efficacy against many cancer cells. The extracts and compounds were claimed to mediate their anticancer action through a number of mechanisms including inhibition of nuclear factor kB pathway, inhibition of Bcr-Abl/Stat5 or SFK/Stat5 signalling pathway, anti-microtubule activity, inhibition of pro-inflammatory cytokines, etc.

Table 10.9 Patents (granted or under application) on compounds obtained from natural sources, with anticancer properties, mechanism of their action and the extent of their protection

Plant material	Active component	Extent of patent protection	Mechanism	Application number	Grant status
Phyllanthus engleri	Englerins and derivatives	Compound, use	–	CA 2711434	Granted
–	Flavone, flavanone and flavano	Compounds, method of isolation, use	–	US14835198	Granted
Plant of families Dioncophyllaceae and Ancistrocladaceae	Naphthoquinone derivatives	Compounds, use	–	EP 009707393A	Granted
Chimonanthus salicifolius	Total alkaloid extract and chimonanthine monomer compounds (1–4)	Extract, method of extraction, use	Anti-proliferative potential, cancer cell apoptosis	CN105985358 A	–
Gynostemma pentaphylla	Clerodane diterpenoid compound	Compound, use	Effect on proliferation, migration, invasion of cancer cells	CN105481873A	–
Cassia fistula	Fistulain A	Compound, process of extraction, use	Cytotoxicity effect	CN105294720A	–
Corymbose hedyotis	Hedyotiscone A	Method of extraction, use	–	CN105713005A	–
Traditional Chinese herbal medicine	Indirubin derivatives (IRDs)	Compounds, use	Inhibition of Bcr-Abl/Stat5 or SFK/Stat5 signalling pathway	US13758921	Granted
Cassia fistula	2-(2-hydroxy ethyl)-5-methyl-6-prenyl-iso indolin-1-one	Compound, method of preparation, use	Cytotoxicity	CN105481754A	–

Cassia fistula	Isoquinoline alkaloid compounds	Compound, method of preparation, use	Cytotoxicity	CN105348193A	–
Salvia roborowskii	Kauranditerpene compound	Compound, method of extraction, use	Effect on multiplication, migration, invasion of cancer cells	CN105384750A	–
Dysoxylum binectariferum	5,7-dihydroxy-6-(3-hydroxy-1-methylpiperidin-4-yl)-2-methyl-4H-chromen-4-one	Compound, use	Anti-proliferative effect, inhibits production of pro-inflammatory cytokines	US14783878	–
Sculellaria barbata	Leucothoe alkane type diterpene compound	Compound, method of preparation, use	Anti-proliferative effect	CN105566251A	–
Zhaoqing citrus	Zhaoqing hesperetin hydrazone compound	Compound, method of preparation, use	–	CN105693682A	–
Ainsliaea fulvioides	Ainsliatrimer B	Method of extraction, use	Anti-proliferative activity	CN104311572A	–
Cassia fistula	Fistulain B	Compound, process of extraction, use	Cytotoxicity effect	CN104860912	Granted
Wedelia prostrata	Enantiomer-dammara-16-alkene-19-acid	Compound, process of extraction, use	–	CN104529811	–
Ligularia pleurocaulis	Ligupleurolide A	Compound, method of preparation, use	–	CN201510410139	Granted

(continued)

Table 10.9 (continued)

Plant material	Active component	Extent of patent protection	Mechanism	Application number	Grant status
Desmodium oxyphyllum	Oxyphyllum flavone A	Compound, method of preparation, use	Cytotoxicity	CN104262316 A	Granted
Gardenia sootepensis	Gardenin A	Compound, method of extraction, use	–	CN104610207	Granted
Rabdosia rubescens	Kaurane diterpenoid compound	Compound, method of extraction, use	Anti-proliferative effect	CN105198897A	–
Annona bullata	Bullatanocin	Method of preparation, use	–	CN104910107A	–
Hedychium forrestii	Forrestiin A	Method of preparation, use	–	CN104926762A	–
Helenium scorzoneraefolium	Mexicanin I	Method of preparation, use	–	CN104910115A	–
Baphicacanthus cusia	Qingdainone	Method of preparation, use	–	CN104910160A	–
Psychotria rubra	Extract	Method of preparation, use	–	CN104926840A	–
Baliospermum montanum	*Baliospermin*	Method of preparation, use	–	CN104926758A	–
Anogeissus	Castalagin	Method of preparation, use	–	CN104926827A	–
Nitraria tangutorum Bobr Pomace	Anthocyanin	Process of extraction	–	CN104447663A	–

Inula britannica	Sesquiterpene lactone 1, sesquiterpene lactone 2	Compound, method of preparation, use	Anti-proliferative effect	CN201510002488	Granted
Andrographis paniculata	15-benzylidene substituted derivatives of 14-deoxy-11,12-didehydro-andrographolide and their 3,19-ester derivatives	Compounds, use	Inhibition of tumour growth, invasion, metastasis and angiogenesis of tumour cells	US13823841	Granted
Graptopetalum sp., *Rhodiola* sp. or *Echeveria* sp.	Compound from extracts	Extract, process of extraction, use	–	US13/249904	Granted
Caesalpinia mina	Cassane diterpenoid compounds	Compound, process of extraction, use	Cytotoxicity	CN103910633A	–
Manglietia fordiana	Total sesquiterpene lactone extract	Method of extraction, use	–	CN201410354084	Granted
Antitussive plant	Noscapine analogs	Compound, method of preparation, use	Anti-microtubule agent	US13488621	Granted
Chrysopogon aciculatus	Aciculatin	Method of extraction, use	–	CN104230951A	–
Neouvaria acuminatissima	Acuminolide	Method of extraction, use	–	CN104072458A	–
Asimina parviflora Dunal	Asimicilone	Method of extraction, use	–	CN104072417A	–
Angelica sinensis	Angelicin V	Method of preparation, use	–	CN104072508A	–

(continued)

Table 10.9 (continued)

Plant material	Active component	Extent of patent protection	Mechanism	Application number	Grant status
Radix fici simplicissimae	Psoralen	Compound, method of preparation, use	–	CN104230947A	–
Indian epimeredi herb	Ovatodiolide	Method of preparation, use	–	CN104072513A	–
Violet prairie clover	Petalopurpurenol	Method of preparation, use	–	CN104177322A	–
Verbascum sinaiticum	Sinaiticin	Method of preparation, use	–	CN104193733A	–
Chenopodium botrys	Sinensetin	Method of preparation, use	–	CN104193710A	–
Laurencia thyrsifera hook	Thyrsiferol	Method of preparation, use	–	CN104072506A	–
Uvaria tonkinensis Finet et Gagnep	Tonkinin A	Method of preparation, use	–	CN104072453A	–
Dysoxylum binectariferum	5,7-dihydroxy-6-(3-hydroxy-1-methylpiperidin-4-yl)-2-methyl-4H-chromen-4-one	Compound, use	Anti-proliferative effect, inhibition of production of pro-inflammatory cytokines	US20160046611A1	–
Parnassia	2-furoacridone-beta-dihydroagarofuran sesquiterpene compound	Compound, method of preparation, use	Anti-proliferative activity	CN201310023928	Granted
Phellinus	Inoscavin A	Compound, method of preparation, use	Anti-proliferative activity, anti-inflammatory activity, regulation of immune response	CN102977114A	–

Inula wissmannian	Gemma alkane-type sesquiterpene lactone inula wissmannian lactone methyl, ethyl, propyl, butyl and amyl or sheepear inula herb lactone	Compound, method of preparation, use	Anti-proliferative potential	CN103102336A	–
Aconitum hemsleyanum var. janyuanum	C19 diterpenoid alkaloid	Compound, method of extraction, use	–	CN102977019A	–
Tobacco	Phenylpropanoid compound	Compound, method of extraction, use	–	CN201210518939	Granted
Zedoary	Guaiane-type and selinane-type sesquiterpenoids	Compound, method of preparation	–	CN201310174267	Granted
Graptopetalum sp., *Rhodiola* sp. or *Echeveria* sp.	Plant extract	Extract, process of preparation	–	US13249904	Granted
Aconitum taipeicum	3-isopropyl-pyrrolidine-[1,2-alpha] pyridine-2,4(1H,3H)-diketone or 1-acetyl-2,3,6-triisopropyl-ectoin-4(1H)-ketone	Compound, method of extraction, use		CN201010216586	Granted
–	Bisindole alkaloid compound	Compound, method of preparation, use	–	CN201010101733	Granted
Zephyranthes candida	N-phenethylcrinidine alkali, zephyranthes candida alkaline A and zephyranthes candida alkaline B	Compound, method of preparation, use	Anti-proliferative potential	CN103641839 A	–

(continued)

Table 10.9 (continued)

Plant material	Active component	Extent of patent protection	Mechanism	Application number	Grant status
Arundina graminifolia	Furan-flavone compound	Compound, method of preparation, use	–	CN201210131014	Granted
Azadirachta indica	Triterpenoid 1 or 2 or 3	Compound, method of preparation, use	–	CN201010501091	Granted
Nitraria sibirica	Anthocyanin	Method of extraction, use	–	CN201010596684	Granted
Radix Clematidis	Lignans compounds	Method of extraction, use	–	CN102603763A	–
Fissistigma oldhamii	Lactam alkali compound	Method of extraction, use	Inhibition of neovascularisation	CN201010556477	Granted
Barbed skullcap herb	Neo-clerodane diterpenoid compound	Method of preparation, use	–	CN201010296002	Granted
Millettia pulchra Kurz var-laxior (Dunn) Z.Wei	Flavone compound and monomer compound	Compounds, use	–	CN102462727A	–
–	Hypoestoxide-related compounds	Compounds, use	–	JP2011535545A	–

In addition to TIM, a vast pool of non-codified traditional knowledge in herbal human health also exists across the nation, especially in the areas rich in plant biodiversity. Efforts are being put in place by the government for making presence of Ayurveda noticeable at global front. Though the demand of medicinal plants from India has increased, however most of the demand is for the raw herbal materials. In India, herbal drug development capability has not been achieved at the desirable scale. A relevant national policy, legislation on the laws and regulations of herbal drug manufacturing and its stringent implementation are required to promote TIM within and across the national boundaries. Categorization of herbal-based drugs under phytopharmaceuticals and release of separate guidelines as per the amendments done recently in the Drug and Cosmetic Act, 1940, is one such step forward in the direction. Flexibility of patent laws will provide better patenting opportunities to the industries. This will be an incentive for the industries to invest in research and development of herbal drugs. Use of in silico tools for prediction of compounds and plants of TIM database may be utilized as an excellent means for screening potential anticancer agents. Additionally, simple and effective IP policies can be framed to promote the intellectual property protection culture and support the researchers, scientists and knowledge holders working in herbal drug development process. The traditional knowledge validation, value addition and product development programme can be initiated in the nations, which are lagging behind in developing new products and filing patents in the area of cancer therapeutics.

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Chapter 11
Appraisal of Medicinal Plants with Anticancer Properties in South America

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11.1 Introduction

Cancer remains an important public health problem in both developed and developing countries, and the statistical numbers suggest that it is increasing steadily. It is well known that cancer is not a particular illness; it is considered more as a variety of diseases and many types of cancers can be manifested in various ways based on the organ or organs affected (Zaid et al. 2017). It is a leading cause of death worldwide, this explains the great amount of research in this area, making big efforts to identify compounds with anticancer activity that help to fight against this terrible disease. Historically, natural products have been used for treating various diseases, including tumor (Ekor 2013; Swamy and Sinniah 2015; Atanasov et al. 2015; Bailon-Moscoso et al. 2015). Plant- and marine-based natural products in the last few years have played a very crucial role as sources of anticancer compounds, and many of these drugs are in the clinical use at present. Thus nowadays, plant-based compounds remain as the principal source of drugs with anticancer potential. There are several approaches to drug discovery derived from plants. Some of them follow the strategy of ethnobotanical knowledge (Quiroga et al. 2012; Swamy and Sinniah 2016; Arumugam et al. 2016; Pinheiro-Ferreira et al. 2016; Mohanty et al. 2017). This approach is very common in South America and is based on the information derived from the medicinal use of plants by the population. This knowledge has been transmitted from generation to generation, from our ancestors, and it has been a guide to identify compounds derived from plants that may be of used in the cancer treatment.

South America is the fourth largest continent, which houses a huge biodiversity and contains a large fraction of the earth's forests. It is the region of the world's five most biodiverse countries: Brazil, Colombia, Ecuador, Peru, and Venezuela.

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Also, this region includes the Amazon rainforest, the Atlantic Forest, and the Andean region. On the other hand, there are vast zones of uncharted territory and some lesser known forests such as Guyana, French Guiana, and Suriname. The flora and fauna of this part of the world is unique, in addition to an immense cultural wealth with very old traditions in the knowledge of using plants to treat various illnesses (Jones 2003; Bailon-Moscoso et al. 2015; Pinheiro-Ferreira et al. 2016). It is coupled with the fact that many of the countries in this region are considered as developing countries with a very low income, meaning that the people do not have monetary resources to attend their health problems; thus they depend on the ancestral knowledge about medicinal plants that have allowed the survival of their ancestors. This is in agreement with the published by WHO, which estimates that 80% of the global population of developing nations mostly depend on the use of plant-based traditional medicines for their primary health-care requisites (Kumara et al. 2012; Swamy et al. 2016, 2017). Many of the modern medicines have been derived from plants, but only small ratio of them has been analyzed chemically. It is true that there is much more to discover, especially due to the rich flora of this continent. In this chapter, we present an appraisal of some studies that have been carried out with plants in different countries of South America emphasizing the ethnobotanical information reported for these plant species.

11.2 Medicinal Plants with Anticancer Potential from the South American Countries

11.2.1 Argentina

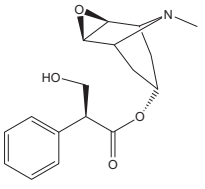
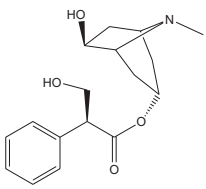
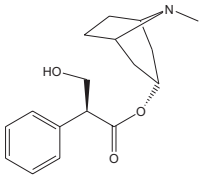
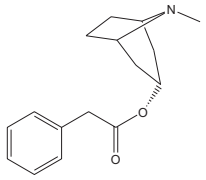
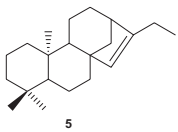
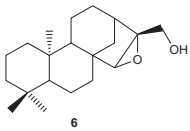
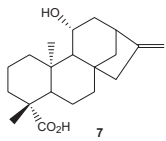
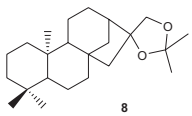
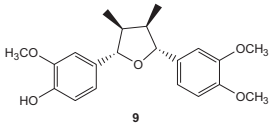
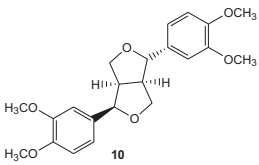
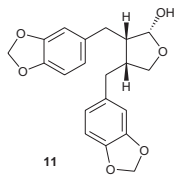
Argentina is a big country, which has an abundant and diverse flora ranging from subarctic to subtropical climates. The existing antecedents of ethnobotanical studies in the country indicate that many plant species are used by the inhabitants of this big country to help in the fight against ailments including cancer. Several studies related to the anticancer activity of medicinal plants used by Argentina inhabitants have been published, and some of them are mentioned here.

Medicinal plants of the central region of Argentina used in the domestic medicine were studied for their antiproliferative effect on breast cancer cells (MCF-7). The cell viability test using crystal violet staining allowed selecting plant extracts that inhibited tumor cell proliferation (Bongiovanni et al. 2006). A total of 17 indigenous species belonging to 9 different botanical families were evaluated: *Aspidosperma quebracho-blanco* Schlechtendahl, *Mandevilla pentladiana* (A. DC.) Woodson., and *Mandevilla laxa* (Ruiz and Pav.) Woodson. (Apocynaceae); *Aristolochia stuckertii* Speg. (Aristolochiaceae); *Eupatorium buniifolium* Hook. ex Hook. and Arn. (Compositae); *Baccharis* sp., *Gaillardia megapotamica* (Spreng.) Baker., *Thelesperma megapotamicum* (Spreng.) Kuntze, *Zexmenia bupthalmiflora*, and *Heterotheca latifolia* Buckley (Asteraceae); *Acalypha cordobensis* Müll. Arg.

and *Sebastiania commersoniana* (Baill.) L.B. Sm. and Downs (Euphorbiaceae); *Oxalis erythrorhiza* Gillies ex Hook. and Arn. (Oxalidaceae); *Lantana grisebachii* Stuckert ex Seckt. (Verbenaceae); *Larrea nitida* Cav. and *Larrea divaricata* Cav. (Zygophyllaceae); and *Monnina dictyocarpa* Griseb. (Polygalaceae). The authors have concluded that only eight species possess the antiproliferative activity. Among them, *T. megapotamicum*, *O. erythrorhiza*, and *L. divaricata* showed the higher values of inhibitory activity on MCF-7 cell line (Bongiovanni et al. 2006). In a similar work, 75 aqueous and methanol extracts of 45 Argentinean plants were evaluated on mammary adenocarcinoma cells (LM2), using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. From this wide screening, eight methanol extracts were considered for the second phase and evaluated against three more cancer cell lines, bladder (MB49), melanoma (B16), and lung (A549), and normal cell lines, keratinocytes (PAM212), mammary (Hb4a), and keratinocytes (HaCat). The results revealed that four plant species, namely, *Solanum chacoense* Bitter., *S. verbascifolium* L., *S. sisymbriifolium* Lam., and *S. amygdalifolium* Steud., showed higher values of cytotoxicity. These species belonging to the *Solanum* genera (Solanaceae) are a rich source of alkaloid metabolites, especially tropane alkaloids (Table 11.1-A). The other most promising species with anticancer potential included *Collaea argentina* Griseb (Fabaceae), *Iochroma australe* Griseb (Solanaceae), *Ipomoea bonariensis* Hook (Convolvulaceae), and *Jacaranda mimosifolia* D. Don. (Bignoniaceae). The phytochemical studies of some of these species indicated the presence of alkaloids; however some of these do not have phytochemical reports (Mamone et al. 2011). The species *Thelesperma megapotamicum* (Spreng.) Kuntze *(*Asteraceae), known by the population as Indian tea or Pampa tea, is generally used to treat various diseases in Argentina. The solvent fractions of this plant were found to be very active against MCF-7 cell lines, and the phytochemical studies revealed the presence of common flavonoids and phenylpropanoids reported from this species (Figueroa et al. 2012).

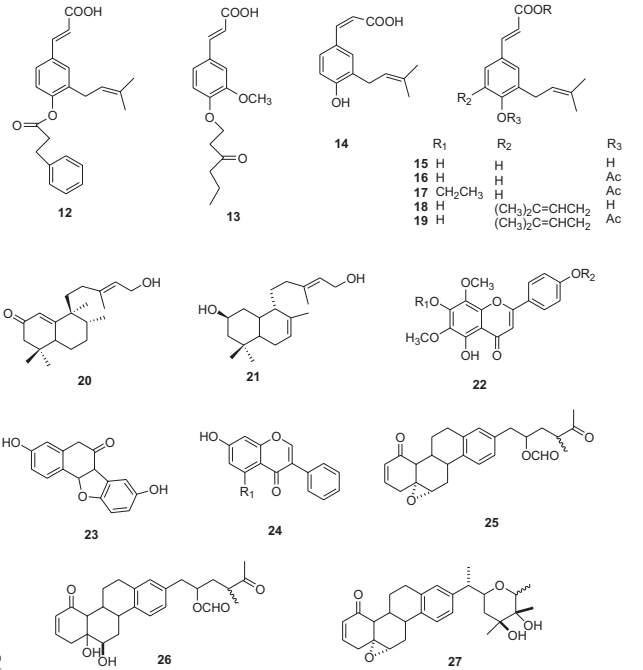
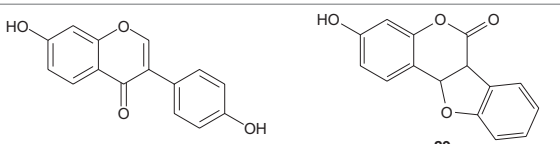
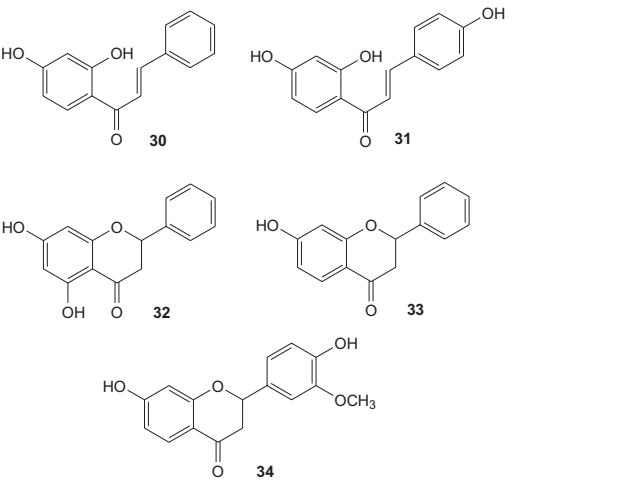
In a different research, eight species belonging to seven different families were investigated for their potential antiproliferative activity against the Hep G2 (human hepatocellular carcinoma) cell lines. The methanolic extracts obtained from these Argentinean medicinal plants such as *Schinus molle* L. commonly known as aguaribay, curanguay, and anacuita and *Lithraea molleoides* (Vell.) Engl. (Anacardiaceae) chichita and arbol malo; *Aristolochia macroura* B.A. Gomes (Aristolochiaceae) patito coludo and mil hombres; *Chenopodium ambrosioides* L. (Chenopodiaceae) paico and apazote; *Achyrocline satureioides* (Lam.) DC. (Compositae) marcela and marcela hembra; *Petiveria alliacea* L. (Phytolaccaceae) mapurite and ajillo; *Plantago major* L. (Plantaginaceae) llantén and torraja; and *Celtis spinosa* Spreng (Ulmaceae) tala and tala blanco were evaluated for cell proliferation using the non-radioactive 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2Htetrazolium (MTS) assay. The results showed the IC_{50} values between 50 and 237 μg/ml. The extracts of *S. molle* and *A. satureioides* were the most active. *S. molle* specially deserve considerations for further studies. Plants like *P. alliaceae* from other latitudes have been mentioned with potential anticancer activity; however the results obtained in this research are not conclusive for these

Table 11.1 Summary of chemical compounds extracted from the various plants

Plants	Extracted compounds
Solanum species	 Hyoscine (**1**)  Anisodamine (**2**)  Hyosciamine (3)  Liitorine (4) A
Aristolochia triangularis	 **5**  **6**  **7**  **8**  **9**  **10**  **11** B

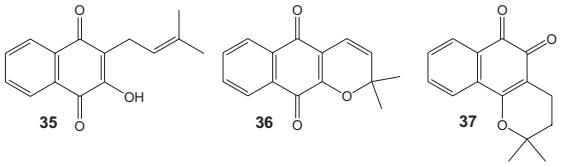
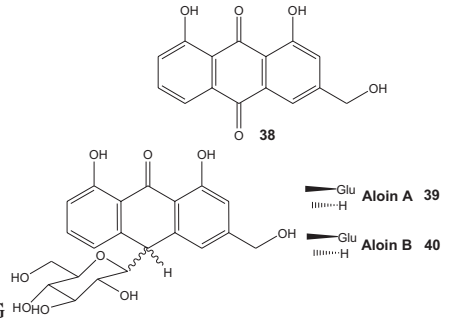
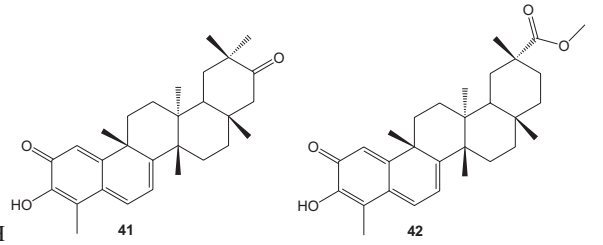
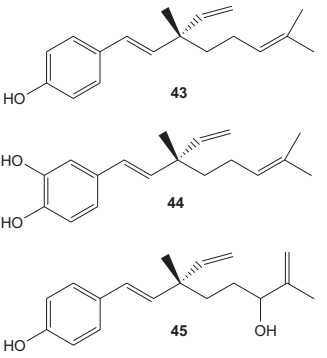
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
Argentinean plants	 C
Erythrina crista-galli	 D
Flourensia oolepis	 E

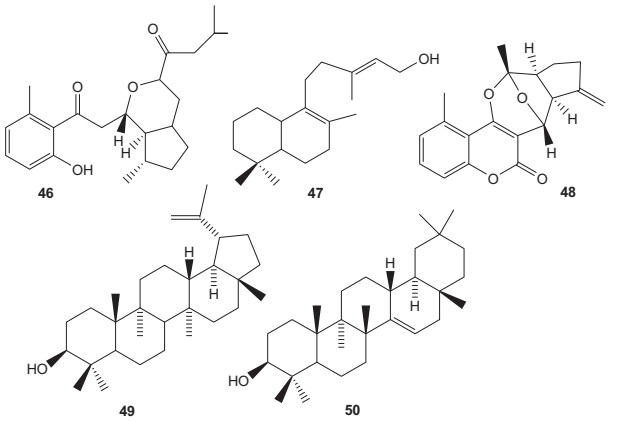
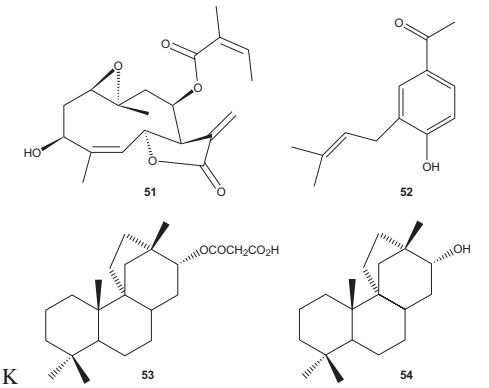
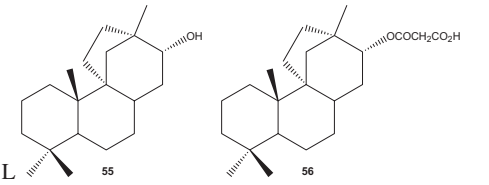
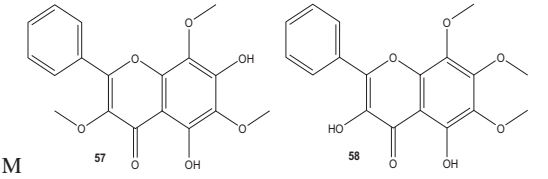
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
Tabebuia impetiginosa	 F
Aloe species	 G
Maytenus ilicifolia	 H
Psoralea glandulosa	 I

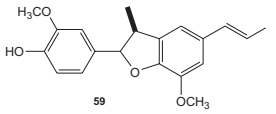
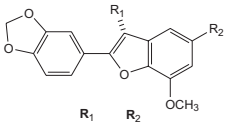
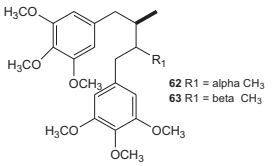
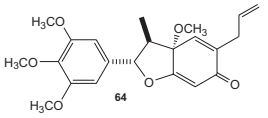
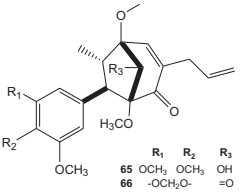
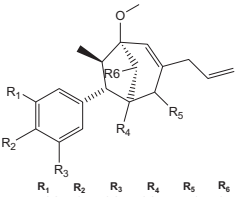
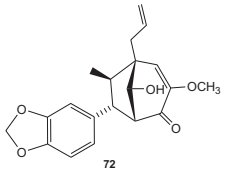
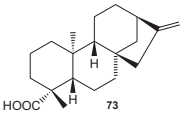
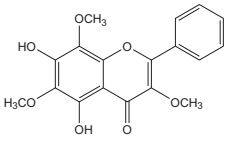
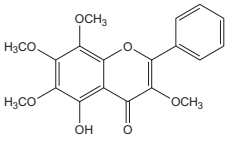
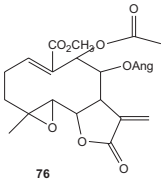
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
Gyphotamniun pinifolium	 J
Leptocarpha rivularis	 K
Calceolaria thyrsiflora	 L
G. elegans and *A. bogotensis*	 M

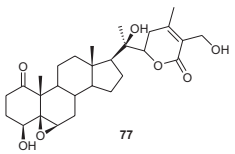
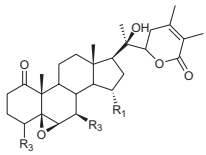
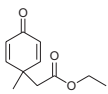
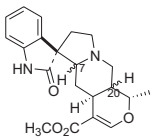
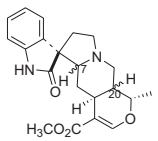
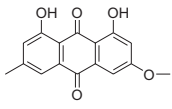
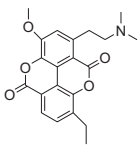
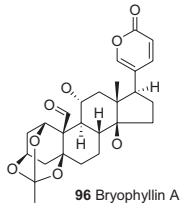
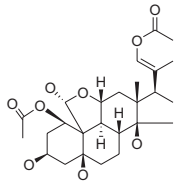
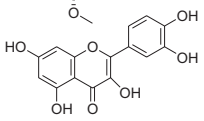
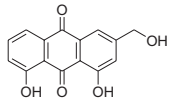
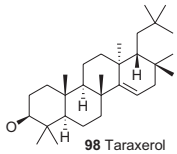
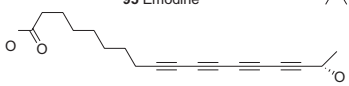
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
Colombian Lauraceae	 **59**  **60** H Allyl **61** CH_3 CHO  **62** R1 = alpha CH_3 **63** R1 = beta CH_3  **64**  **65** OCH_3 OCH_3 OH **66** $-OCH_2O-$ =O  **67** -OCH2O- OCH_3 OCH_3 =O OH **68** -OCH2O- OCH_3 OCH_3 b-OH =O **69** -OCH2O- OCH_3 OCH_3 =O =O **70** OCH_3 OCH_3 OCH_3 H =O OH **71** OCH_3 OCH_3 H H =O OH  **72**  **73**
Espeletia killipii	 **74**  **75**  **76**

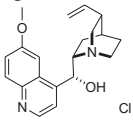
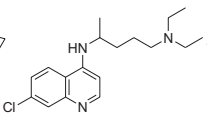
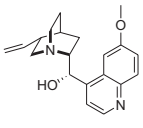
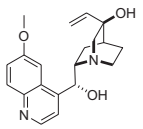
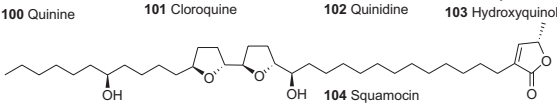
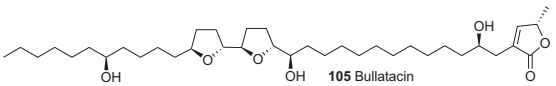
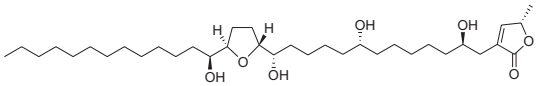
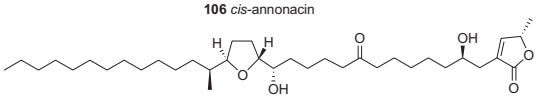
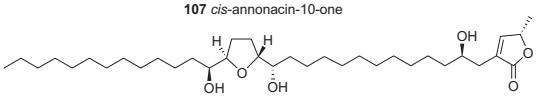
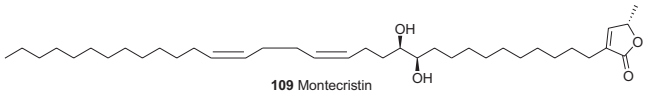
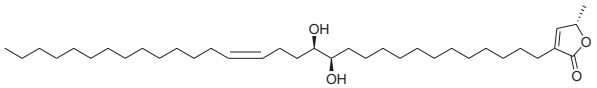
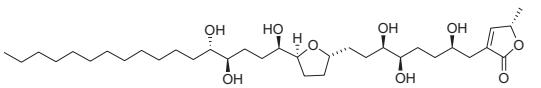
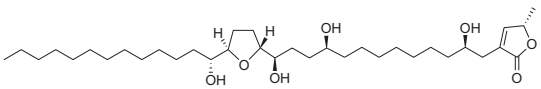
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
Acnistus arborescens	 **77**  **78** -Δ^2, R_1 = H, R_2 = β-OH,α-H, R_3 = OAc **79** -Δ^2, R_1 = OAc, R_2 = β-OH,α-H, R_3 = OAc **80** -Δ^2, R_1 = OAc , R_2 = H2, R_3 = OAc **81** -Δ^2, R_1 = H, R_2 =β–OH, α-H, R_3 = H **82a** -Δ^2, R_1 = H, R_2 = β -OAc, α-H, R_3 = OAc **82b** -Δ^2, R_1 = H, R_2 = O, R_3= OAc **82c** -Δ^1, R_1 = H, R_2 = β-OH α-H, R_3 = OAc **83a** -Δ^2, R_1 = OAc, R_2 = OAc, R_3 = OAc **83b** -Δ^2, R_1 = OAc, R_2 = O, R_3 = OAc  **84**  **85** P
U. tomentosa, *C. lechleri*, *M. condurango*, *S. multiglandulosa*, and *M. guianensis*	Q  **86** Isopteropodine: H7, H20, Me-18 **87** Isomitraphylline: H7, H20, Me-18  **88** Pteropodine: H-7, H-20 **89** Uncarine F: H-7, H-20 **90** Mitraphylline: H-7, H-20  **91** Physcion  **92** Tapsine  **96** Bryophyllin A **93** Condurango glycoside A  **97** Bryophyllin B  **94**Quercetin  **95** Emodine  **98** Taraxerol  **99** Minquartynoic acid

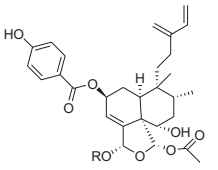
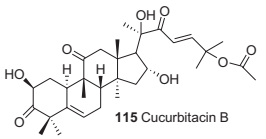
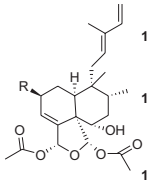
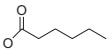
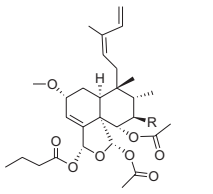
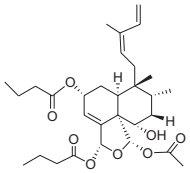
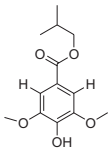
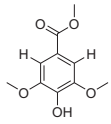
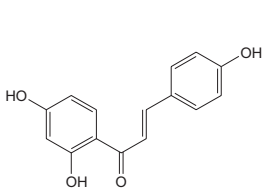
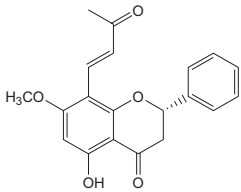
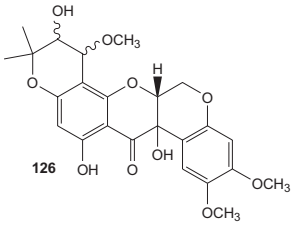
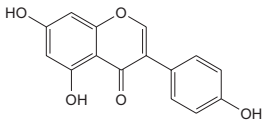
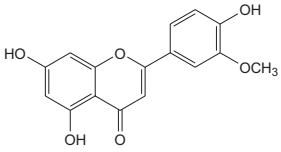
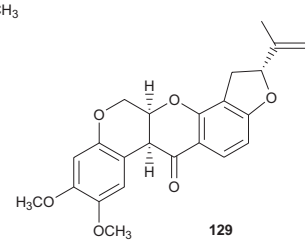
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
C. officinalis and *A. squamosa*	Q  **100** Quinine  **101** Cloroquine  **102** Quinidine  **103** Hydroxyquinoline  **104** Squamocin  **105** Bullatacin  **106** *cis*-annonacin  **107** *cis*-annonacin-10-one  **108** Murisolin
A. muricata	R  **109** Montecristin  **110** Cohibin A  **111** Muriexocin  **112** Adrianacin

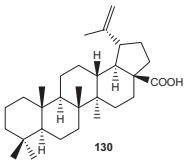
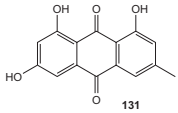
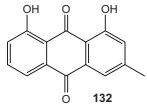
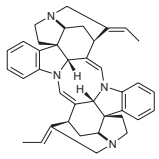
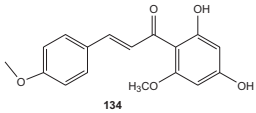
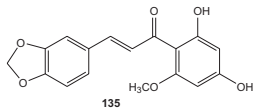
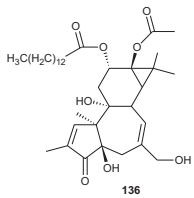
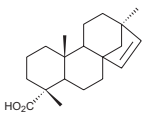
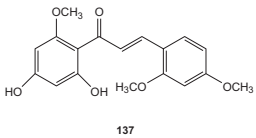
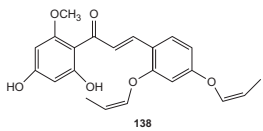
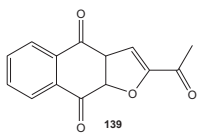
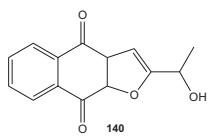
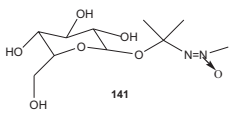
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
L. intrapetiolaris and *C. sylvestris*	 **113** Intrapetacin A: R = Me **114** Intrapetacin B: R = Ac  **115** Cucurbitacin B  **116** Casearvestrin A: R =  **117** Casearvestrin B: R =  **118** Casearvestrin C: R =   **119** Casearin L: R = OH **120** Casearin O: R =   **121** Casearin X  **122** IGDE  **123** MGDE S
Tephrosia toxicaria and *Lonchocarpus* species	 **124**  **125**  **126**  **127**  **128**  **129** T

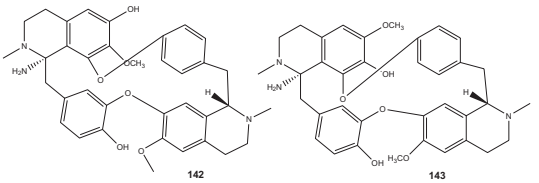
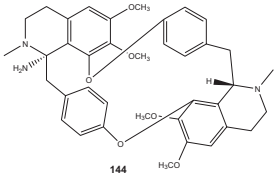
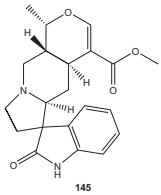
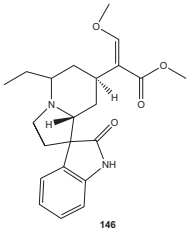
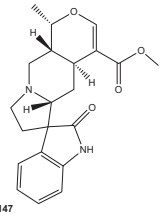
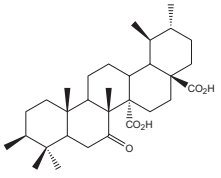
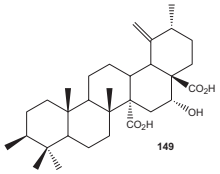
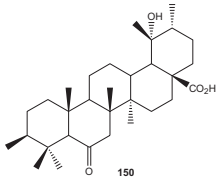
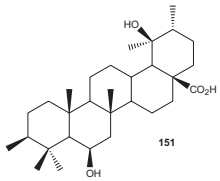
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
D. dentatus, *P. sellowii*, *S. mitscherlichii*, *I. juruensis*, and *C. alnifolius*	 130  131  132  133  134  135  136  137 U
Peruvian plants	 137  138  139  140  141 V

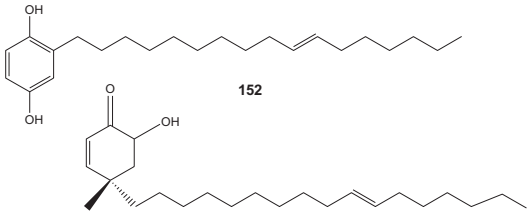
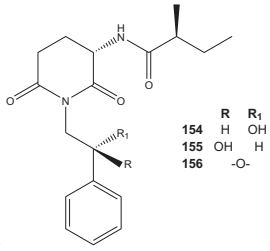
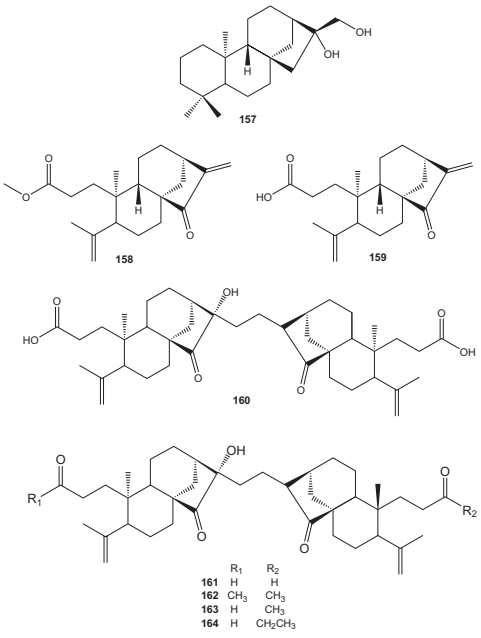
(continued)

Table 11.1 (continued)

Plants	Extracted compounds
Chondrodendron tomentosum	  W
Uncaria tomentosa	   X
Uncaria tomentosa	    Y

(continued)

Table 11.1 (continued)

Plants	Extracted compounds
Tapirira guianensis	 Z
Croton cuneatus	 AA
Croton species	 BB

(continued)

plants. Hence, they can be further considered for phytochemical analysis and also new assays in different cancer cell lines (Ruffa et al. 2002). Seven Argentine plants with cancer-related ethnobotanical uses were screened in a study to detect cytotoxic activity (Mongelli et al. 2006). The plants studied were *Aristolochia triangularis* (DC.) (Aristolochiaceae), *Baccharis grisebachii* Hieron (Asteraceae), *Bolax gum-*

mifera (Lam.) Spreng. (Apiaceae), *Eupatorium hecatanthum* (DC.) (Asteraceae), *Erythrina crista-galli* L. (Faboideae), *Pterocaulon polystachyum* (DC.) (Asteraceae), and *Salpichroa origanifolia* (Lam.) Baill (Solanaceae). DNA interaction, crown gall tumor inhibition, and the KB cell inhibition were evaluated by the use of the DNA-MG (DNA-methyl green), the potato disc, and the KB cell toxicity bioassays, respectively. The observations revealed that *A. triangularis*, *B. gummifera*, and *E. hecatanthum* could contain the KB cells inhibiting phytocompounds. Interestingly, all the evaluated plants exhibited the inhibition of the crown gall tumor growth. The results confirm the traditional use of these plants against tumors. In addition, the extracts of *E. hecatanthum* and *P. polystachyum* were reported to possess compounds which interact with the DNA (Mongelli et al. 2006). Nevertheless, other plants have been studied for their phytochemicals, for example, the phytochemical examination of *A. triangularis* led to the isolation of lignans and ent-kaurane-type diterpenes (Lopes et al. 1990) (Table 11.1-B). Likewise, *S. origanifolia* contains withanolides (Tettamanzi 1999), and *B. grisebachii* constitutes diterpenes, flavonoids, and coumaric acids (Feresin et al. 2003). Flavonoids from *E. hecatanthum* (Clavin et al. 2013), coumarins from *P. polystachyum* (Vera et al. 2001; Medeiros-Neves et al. 2015) (Table 11.1-C), and isoflavonoids and pterocarpanes from *E. crista-galli* (Redko et al. 2006; Tjahjandarie et al. 2015) have been reported (Table 11.1-D). *Flourensia oolepis* S.F. Blake (Asteraceae), commonly known as chilca, a species collected from the central region of Argentina, showed antibacterial activity. A group of investigators isolated five compounds present in an antibacterial extract that were evaluated against the CML (chronic myeloid leukemia) and ALL (acute lymphoblastic leukemia) cell lines including their multidrug-resistant (MDR) phenotypes. Among the isolated flavonoids (Table 11.1-E), the best cytotoxic activity was induced by the compound, 2,4-dihydroxychalcone. In addition, the compound showed a strong and selective cytotoxicity against CML and ALL cells and their MDR phenotypes (Joray et al. 2015).

11.2.2 Bolivia

Bolivia is located in the central zone of South America. With different climatic conditions and variations of soils, it has a wide range of environments and ecosystems. This country has a rich biodiversity, with a large number of plant species, being many of them used in folk medicines. Besides this, Bolivia has a great cultural diversity with a mainly indigenous population distributed in different ethnic groups. The indigenous communities have practiced herbal medicine therapy for hundreds of years, and these knowledge about medicinal plants have passed on through generations. Even today, Bolivians still prefer the use of folk medicines over modern medicine (Fernandez et al. 2003). In spite of this, there are only limited studies focused on ethnobotanical inventory of these plants used by Bolivians inhabitants to treat their health problems (Cussy-Poma et al. 2017). In these studies, we found that

the medicinal plants reported are used to treat pain, fever, inflammation, urological problems, diseases of the respiratory system, skin affections, and mainly gastrointestinal disorders. The species reported with use against cancer by Bolivians inhabitants are presented below.

Bourdy et al. (2000) reported a list of plant used as medicinal for the Tacana, an Amazonian Bolivian ethnic group. Among them, *Copaifera reticulata* Ducke (Fabaceae) and *Piper* sp. (Piperaceae) are used to treat uterine cancer. It is important to mention that the genus *Piper* contains various species that have been used in traditional medical practices in many countries for the treatment of cancer or cancer-related symptoms. Investigations have witnessed the occurrence of some cytotoxic compounds in the extracts of few *Piper* species. Among them are the amide alkaloids, the major active principles (Wang et al. 2014). In Bolivia, species of the genus *Piper* are widely distributed in the tropical regions, and the antecedents show their extensive use in the folk medicines for the treatment of parasitic diseases. Despite this, there are no phytochemical studies directed toward the search for bioactive compounds.

Uncaria guianensis (Aubl.) J.F. Gmel (Rubiaceae) and *Uncaria tomentosa* (Willd. ex Roem. and Schult.) DC., both known as "uña de gato," are distributed in different countries such as Bolivia, Brazil, Colombia, Peru, Guyana, Venezuela, and Surinam of South America. They are used in Bolivia to treat various health conditions. The stems and bark of *U. guianensis* are used to treat coughs, colds, rheumatism, arthritis, diabetes, cirrhosis, conjunctivitis, gastric ulcers, prostate cancer, as anti-inflammatory, anticonceptive, and antitumoral, while the roots of *U. tomentosa* are used as anti-inflammatory, anticonceptive, and anticancer. Other species reported with anticancer activity are *Baccharis trimera* (Less.) known in Bolivia as carqueja and *Curcuma longa* known as curcumina (Terceros et al. 2007). A study by Quiroga et al. (2012) reported the use of three plants, namely, *Acacia aroma* (Fabaceae), *Coronopus didymus* (L.) Sm. (Brassicaceae), and *Sambucus peruviana* Kunth (Caprifoliaceae), against cancer and tumors in the traditional medicine of Huacareta. On the other hand, some studies of the evaluation of diverse biological activities of Bolivian plants have also been performed, but to our knowledge, only one is related to the antiproliferative activity of extracts from plants frequently used in Bolivian folk medicine. Rodrigo et al. (2010) investigated the antiproliferative activity against Caco-2 cell lines (colon cancer) using the extracts of 26 plant species which are common in Bolivia. They found that the ethanolic extracts of *Schkuhria pinnata*, *Piper longestylosum*, *Parastrephia lepidophylla*, and *Erodium cicutarium* showed inhibitory activity against cell proliferation. The most potent of them was the ethanolic extract from *Schkuhria pinnata* showing 53% inhibition of growth, followed by *Piper longestylosum* with 43% inhibition, *Parastrephia lepidophylla* with 19% inhibition, and *Erodium cicutarium* with 10% inhibition. No significant effects could be observed for the rest of the ethanol extracts of the tested plant species.

11.2.3 Brazil

Brazil, the largest country of South America, has the highest plant diversity. Various medicinal plants are found in different ecosystems (Amazon forest, Atlantic forest, Caatinga, Cerrado, Pampas, and Pantanal). Many of these plants are used as natural medicines by the people living in these areas to treat diverse diseases, including cancer. The traditional therapists have more understanding on these herbs growing in their territory (Agra et al. 2007). Besides the great biological diversity, Brazil has a vast cultural diversity and, hence, influences in the diverse forms of plants being utilized in health-care needs. Many exotic species were introduced by both Europeans and Africans during the times of colonization, and the knowledge about their uses was mixed with those of the indigenous people. This has favored a large popular pharmacopeia based on medicinal plants (de Melo et al. 2011). Diverse ethnobotanical studies have been carried out in the different ecosystems in Brazil, and some plants or their metabolites have been studied for having activity against cancer. The results of some of these studies are presented below.

Various medicinal plants of Brazil with anticancer properties were reviewed by de Melo et al. (2011). Their analysis included research outcomes published between 1980 and 2008; however, the authors found that the highest numbers of ethnobotanical and ethnopharmacological studies citing plants with antitumor activity were published between 2000 and 2008. About 84 anticancer plant species distributed among 42 families were reported in their review. The plant families Euphorbiaceae (nine spp.), Fabaceae (seven spp.), Apocynaceae (six spp.), and Vitaceae and Asteraceae (four spp. each) had the largest number of species represented. On the other hand, *Aloe arborescens*, *Aloe vera*, *Tabebuia impetiginosa*, and *Euphorbia tirucalli* were the plants most frequently cited. However, *T. impetiginosa* (Mart. ex DC.) Standl. is the only native species of Brazil (Martins et al. 2011). It is a tree with rosy or purple flowers belonging to the family Bignoniaceae and native to the Amazon rainforest and few parts of Latin and South America including Bolivia, Brazil, Ecuador, Colombia, Argentina, French Guinea, Peru, Paraguay, Surinam, Tobago, Trinidad, and Venezuela (Castellanos et al. 2009; Ferreira et al. 2015). *T. impetiginosa* is commonly known as Ipê, Ipê cavatan, Ipê roxo, Ipê comum, Ipê de São Paulo, Aipê, Ipeuva, Lapacho, Guiraiba, Pau d'Arco, Pau d'Arco velmelho, Paud'Arco roxo, Peuva, Piuva, or Upeuva in Brazil; Lapacho Rosado in Argentina and Paraguay; Cortez negro and Ipé in Costa Rica; Lapacho negro in México; Tajibo morado in Bolivia; and Puy in Venezuela (Roman et al. 2012). Traditionally, the stem-bark and/or inner bark of this tree has been used to treat health problems such as arthritis, bacterial and fungal infections, eczemas, inflammation of the prostate, fever, pain, dysentery, ulcers, dermatitis, syphilis, malaria, trypanosomiasis, and cancer (Kim et al. 2006; Agra et al. 2008; de Melo et al. 2011; Castellanos et al. 2009). *T. impetiginosa* is one of the most common plants used in the traditional

medicines of Brazil to cure cancer (Castellucci et al. 2000; Gazzaneo et al. 2005; Botsaris 2007; de Albuquerque et al. 2007a; Negrelle and Fornazzari 2007; Agra et al. 2008). The bark of *T. impetiginosa* contains a large number of phytocompounds including anthraquinones and naphthoquinones (Manners and Jurd 1976), quinones (Sharma et al. 1988), benzoic acids and derivatives of benzaldehyde (Wagner et al. 1989), flavonoids (Blatt et al. 1996), cyclopentene dialdehydes (Koyama et al. 2000), and furanonaphthoquinones (Zani et al. 1991; de Oliveira et al. 1993; Diaz and Medina 1996). Among the quinones, lapachol (35), α-lapachone (36), and β-lapachone (37) (Table 11.1-F) have been extensively studied for their interesting activity against cancer cells (de Almeida 2009; Hussain and Green 2017). The investigations of the therapeutic effects of lapachol began in the 1960s (Hussain et al. 2007), but after some research on the antineoplastic activity of lapachol, studies were not continued because high doses were needed to achieve the anticancer effect, which produced many side effects. These results motivated the researchers to perform the synthesis of lapachol derivatives including the naturally occurring quinone β-lapachone (Hussain et al. 2007; de Almeida 2009). At the beginning of this year, Hussain and Green (2017) published a review that summarizes the large number of interesting patents published on the therapeutic potential of quinones lapachol, β-lapachone, and α-lapachone. In some publications, *T. impetiginosa* is considered a synonym species of *T. avellanedae* Lorentz ex Griseb, which is also popularly used against cancer in Brazil (Agra et al. 2007, 2008).

Aloe vera L. (synonym *Aloe barbadensis* Miller) is a tropical plant member of Liliaceae family and well adopted to dry and hot climatic conditions especially in the regions of Africa, Asia, and other tropical countries. In Brazil, a cultivated species locally known as "babosa" is widely used in customary medicines to treat pain in the bones, rheumatism, eczema, hair loss, gastritis, hemorrhoids, inflammation, wounds, cough, burns, and cancer (Soares et al. 2004; de Souza and Felfili 2006; Pilla et al. 2006; de Albuquerque et al. 2007a, b; Negrelle et al. 2007; Calábria et al. 2008; dos Santos et al. 2008). Many biological properties associated with *Aloe* species are contributed by inner gel of the leaves. In studies performed, the plant has exhibited many pharmacological activities such anti-inflammatory, antioxidant, antimicrobial, immune boosting, antitumor, hypoglycemic, hypolipidemic, antiulcer, wound healing, hepatoprotective, and antidiabetic (Radha and Laxmipriya 2014; Rahmani et al. 2015). This plant contains different biologically active substances, including vitamins, minerals, saccharides, amino acids, and anthraquinones, such as aloe-emodin (38), aloin A (barbaloin) (39), and aloin B (isobarbaloin) (40) (Table 11.1-G). *Aloe vera* and its anthraquinones have attracted much the attention as agents against cancer. In a study performed by Saini et al. (2010), the antitumor activity of *Aloe vera* against stage 2 skin tumorigenesis induced by *Croton tiglium* (croton) oil and 7,12-dimethylbenz[a]anthracene (DMBA) was investigated. They found that compared to 100% incidence of tumor development in group I (DMBA + croton oil only), the incidence of tumors decreased to 50% in group II (DMBA + croton oil + topical *Aloe vera* gel), to 60% in group III (DMBA + croton oil + oral *Aloe vera* extract), and to 40% in group IV (DMBA + croton oil + *topical*

Aloe vera gel + oral *Aloe vera* extract). Another study indicated that the ethanolic extract (50%) of *Aloe vera* exhibited antitumor effect against Ehrlich ascites tumor in mice (Naveena et al. 2011). Later, Chandu et al. (2012) evaluated the in vitro antitumor activity of *A. vera* extract against the B16F10 melanoma cell line. *A. vera* showed good cytotoxic activity, and it had less toxic effects to the normal blood lymphocytes, as compared to that of standard anticancer drug. Aloe-emodin is a subtype of anthraquinones isolated from *A. vera* leaves. This compound has been able to prevent the growth of several cancer cells including human lung carcinoma (CH27, H460) (Lee 2001; Lee et al. 2001; Yeh et al. 2003), hepatoma (Hep G2, Hep 3B) (Kuo et al. 2002, 2004), bladder (Lin et al. 2006), breast (Huang et al. 2013), human tongue squamous carcinoma (SCC-4) (Chiu et al. 2009), colon carcinoma (Pecere et al. 2003; Lin and Uen 2010; Suboj et al. 2012), and leukemia (HL-60, U937) (Chen et al. 2002, 2004; Tabolacci et al. 2011). Furthermore, aloe-emodin was found very effective against the neuroectodermal tumor cells (Pecere et al. 2000; Ahirwar and Jain 2011). In some of these studies, it has been determined that the mechanism of cytotoxic action of this compound is through the induction of apoptosis (Lin et al. 2011). Aloin (aloin A or barbaloin) is a natural anthraquinone glycoside derived from *A. vera* leaves that has the anticancer potential (Pan et al. 2013). Aloin has showed activity against various human cancer cells, including gastric (Guo et al. 2007), ovarian, breast (Esmat et al. 2005, 2006), uterine carcinoma (Niciforovic et al. 2007), human Jurkat T lymphocyte cells (Buenz 2008), and B16-F10 murine melanoma (Tabolacci et al. 2013), and moreover, some of these studies showed that this compound induced the arrest of cell cycle and apoptosis. Pan et al. (2013) found that aloin treatment inhibited VEGF-stimulated angiogenesis in human endothelial cells. VEGF is one cytokine that stimulates angiogenesis; thus, they suggest an antiangiogenic effect of aloin. In a recently study, aloin acted as a chemopreventive agent against preneoplastic lesions in the colon of Wistar rats induced by 1,2-dimethylhydrazine (Hamiza et al. 2014). They found that aloin might inhibit the cancer-stimulating effects of 1,2-dimethylhydrazine via activating anti-inflammatory and antioxidant responses.

Aloe arborescens Mill. is another species widely used in the traditional medicines of Brazil for the treatment of cancer (Dorigoni et al. 2001; Garlet and Irgang 2001; Soares et al. 2004; Vendruscolo et al. 2005; Vendruscolo and Mentz 2006; de Barros et al. 2007). *A. arborescens* is also commonly used to treat burns and other skin-related diseases (Di Luccia et al. 2013). In a recent study, Ceccarelli et al. (2012) evaluated the antiproliferative properties of the leaf extract of *A. arborescens* on murine myeloma cell lines using the MTT method, and they found the cell inhibition rate up to 80%. On the other hand, they carried out a bioassay-guided fractionation by thin-layer chromatography (TLC) that allowed the identification of a spot showing antiproliferative activity. Further exploration by high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) spectra showed that the TLC spot consisting of aloenin A (41) and aloins A (39) and B (40) (Table 11.1-H). In a clinical study performed by Lissoni et al. (2009), a total of 240 patients with metastatic solid tumor were randomized to receive chemotherapy with

or without aloe (*A. arborescens*). The drugs etoposide, cisplatin, vinorelbine, oxaliplatin, gemcitabine, and 5-fluorouracil (5-FU) were used in the chemotherapy. Aloe was given orally at 10 ml thrice/daily. The patients treated with chemotherapy and aloe showed higher percentage of tumor regressions as well as higher disease control compared to the patients treated with chemotherapy alone. These results seem to suggest that aloe may be supplemented with chemotherapy to increase the efficacy of a cancer treatment. Likewise, Furukawa et al. (2002) evaluated the effect of freeze-dried aloe leaves powder in the course of *N*-nitrosobis (2-oxopropyl) amine (BOP)-induced tumorigenesis in hamsters was evaluated. The study showed that the rates of pancreatic cancer and hyperplasias were significantly reduced when treated with BOP and aloe (5%). In another study, aloenin was compared with its synthetic compound against Hep G2, HCT 116, and HCT 116/VCR 100-1-1 cells and found that aloenin isolated from *A. arborescens* had no distinct influence with IC_{50} values of more than 100 μM (Jin et al. 2005).

Ferreira et al. (2011) evaluated the antiproliferative prospects of the seed extracts of Brazilian plant species against HCT 8 (colon), HL-60 (leukemia), SF-295 (glioblastoma), and MDA/MB-435 (melanoma) cells. The results indicated that seed extract (ethanol) of only one plant (*Myracrodruon urundeuva*) showing potential anticancer activity and inhibited the cell proliferation up to 90%. This extract was more active against the HL-60 cells (IC_{50} = 12.5 μg/ml). Mans et al. (2000) have mentioned the importance of *Maytenus ilicifolia*, *A. vera*, or *A. arborescens* to evaluate their potential antineoplastic properties and represent a lead in finding novel anticancer compounds. Likewise, *Tabebuia* and *Hypericum* genera are also mentioned. *M. ilicifolia* belonging to the Celastraceae family is a native of south Brazil (especially of the forests of the Mato Grosso do Sul, São Paulo, and Rio Grande do Sul), Paraguay, Bolivia, Uruguay, and Northeastern of Argentina (Alonso and Desmarchelier 2007). In Brazil, it is popularly known as cancerosa, espinheira-santa, espinheira-divina, or maiteno (Cordeiro et al. 2006). It is used in folk medicine to alleviate nausea and stomach pain and mainly to treat ulcer and inflammation of the stomach lining (Camparoto et al. 2002), cancer, skin cancer, flu, rheumatism, pain, and inflammation (Soares et al. 2004; Tagliati et al. 2004; dos Santos et al. 2008; Santos et al. 2014). Espinheira-santa has pharmacological effects proven by the Ministry of Health of Brazil, approving its use as antiulcerogenic (de Moraes and da Cunha 2012). Furthermore, this plant presents activity against cancer, skin cancer, flu, rheumatism, pain, and inflammation (Tagliati et al. 2004; Santos et al. 2014). In Paraguay, this plant is used by the local people as an emmenagogue and a contraceptive, while in Argentina, it is used an emmenagogue, anti-abortive, and anticancer drug (Arenas and Moreno Azorero 1977). Many phytocompounds have been isolated from this plant including flavonoids (Leite et al. 2001; Cipriani et al. 2006; Baggio et al. 2007; Tiberti et al. 2007), tannins (de Souza et al. 2008; Pessuto et al. 2009), sesquiterpene pyridine alkaloids (Shirota et al. 1994a), triterpenes (Itokawa et al. 1991; Shirota et al. 1994b), and quinonemethide triterpenes (de Lima et al. 1971; Pereira and Borges 1960), of which maytenin (41) and pristimerin (42) have been reported with anticancer activity.

The antimutagenic activity of the infusion of *M. ilicifolia* against known mutagenic substances was determined by Horn and Vargas (2003). Of the doses of the infusion tested, 75% showed high and significant inhibition of the mutagenicity induced by aflatoxin B1, 2-aminofluorene, and 2-aminoanthracene. Araújo Junior et al. (2013) demonstrated that the leaf extract of *M. ilicifolia* induces apoptosis in Hep G2 (human hepatocellular cells) and HT-29 (colorectal carcinoma) cells by downregulating the expression of Bcl-2 and activating caspase-3-dependent signaling pathways. The compound pristimerin has shown antiproliferative activity against a series of cancer cells such as breast (MDA/MB-231, MCF-7, MDA/MB-435) (Chang et al. 2003; Wu et al. 2005; Costa et al. 2008), prostate cancer (PC-3) (Yang et al. 2008), leukemia (HL-60, K562), glioblastoma (SF-295), colon (HCT 8) (Costa et al. 2008), and glioma (Chang et al. 2003; Yan et al. 2013). Likewise, Wang et al. (2012) and Deeb et al. (2014) have demonstrated the antiproliferative activity of pristimerin against pancreatic cancer cells such as BxPC-3, AsPC-1, MiaPaCa-2, PANC-1, and PANC-1 by inducing apoptosis. Other species of the *Maytenus* genus reported for their use in Brazilian folk medicines are *Maytenus rigida* Mart. and *M. obtusifolia* Mart. A decoction of leaves from *M. obtusifolia* is used to treat common inflammations and tumor, while stem-bark powder is used for the treatment of skin ulcers. *M. rigida* is known as bom-nome, and the stem-bark of this plant is used against inflammations of ovaries, infections of kidneys, skin ulcers, and tumors (Agra et al. 2007, 2008).

Other species reported for their ethnobotanical use against cancer in Brazil are *C. multijuga* Hayne (Fabaceae), *Cissus decidua* J. A. Lombardi (Viscaceae), *Himatanthus articulatus* (Vahl) Woodson (Apocynaceae), *Marsdenia altissima* (Jacq.) Dugand (Apocynaceae), *Morinda citrifolia* L. (Rubiaceae), *Cnidoscolus urens* (L.) Arthur (Euphorbiaceae), *Crocus* sp. (Iridaceae), *Psychotria ipecacuanha* (Brot.) (Rubiaceae) (de Albuquerque et al. 2007b), *Bauhinia forficata* Link (Fabaceae), *Costus spicatus* (Jacq.) Sw. (Costaceae), *Hymenaea courbaril* L. (Fabaceae) (Santos et al. 2014), *C. decidua* Lombardi (Vitaceae) (de Melo et al. 2011), *Croton urucurana* (Euphorbiaceae) (de Souza and Felfili 2006), *C. spiralis* (Jacq.) Roscoe (Costaceae) (Pilla et al. 2006), *Symphytum officinale* (Boraginaceae) L., and *Stachytarpheta cayennensis* (Rich.) Vahl (Verbenaceae) (Merétika et al. 2010).

11.2.4 Chile

The Chile territory is a long and narrow strip land between the Pacific Ocean and the Andes mountains. All kinds of geographic conditions from deserts until rain forest extremes can be observed in this country. It is clear that this geographic diversity also offers floral diversity. The native inhabitants from Chile have a rich tradition of using plants to alleviate and fight diseases including cancer. This revision pretends to give information of some studies realized with native and endemic plants from Chile territory, against cancer.

One of the more recently published works on anticancer plants of Chile is *Psoralea glandulosa* L. (Papilionaceae) called as culen and hualhua. From the resinous exudate of this plant, the compounds such as bakuchiol (43), 2-hydroxy-bakuchiol (44), and 12-hydroxy-iso-bakuchiol (45) were isolated (Table 11.1-I). These metabolites and the resin showed a positive response against the A2058 (human melanoma) cells. However, the evaluated metabolites were not effective as that of the resin, which can indicate that synergistic effects gave the best results with the resin (Madrid et al. 2015). Various metabolites such as coumarins, labdane diterpenes, and sterols (Table 11.1-J) have been reported in *Gypothamnium pinifolium* Phil (Asteraceae), a native Chilean species (Zdero et al. 1988). In a study, a group of these isolated metabolites were evaluated against MCF-7 cells, and the observations showed that the compound 2-nor-1,2 secolylcoserone (46) (Table 11.1-J) had the highest cytotoxic effect and concluded it to be a good candidate for advanced anticancer research investigations (Simirgiotis et al. 2015).

Leptocarpha rivularis DC (Asteraceae), known as "palito negro" is traditionally used by the inhabitants of Chile. The plant has been demonstrated to decrease of cell viability in cancer cells (Martinez et al. 1995, 1998, 2006). A compound, named leptocarpin (51) (Table 11.1-K), the major metabolite, previously isolated from this plant was investigated for the cytotoxic mechanism on different cancer cell lines such as PC-3 (prostate cancer cell line), HT-29 (colon cancer cell line), MDA-MB-231 and MCF-7 (breast cancer cell lines), HDF (human dermal fibroblasts), and CCD 841 CON (human colon epithelial) cells. The outcomes revealed that leptocarpin treatment decreased the viability of cancer cells by inhibiting the NF-κB factor and inducing caspase-dependent apoptotic pathways (Bossio et al. 2015).

A different strategy in the way to find cytotoxic metabolites from plants is the use of synthetic methods to obtain better biologically active compounds from the natural one. This procedure is exemplified with the compound demalonyl thyrsiflorin A (55) which is a semisynthetic compound obtained for modification of natural one diterpene (56) isolated, together with other compounds from the Chilean species *Calceolaria thyrsiflora* Graham. (Scrophulariaceae) known with the common name capachito (Table 11.1-L). Traditionally, this plant is used as antidiuretic agent, for treating digestive problems, and is recognized with antibacterial properties (Betancur-Galvis et al. 2001). Further, the compound 55 was investigated for the cytotoxic effect on KB (oral squamous carcinoma) and Du-145 (androgen-insensitive prostate) cancer cells. Also, the study showed an apoptotic response and a necrosis effect at higher doses (Gabarino et al. 2007).

Senecio graveolens Wedd (Asteraceae) is a species belonging to one of the largest genera, *Senecio*, present in the mountains of the Andes, Chile. It is locally known as chachacoma and used for the ailments related to altitude. In a study, the antiproliferative effect of *S. graveolens* alcoholic extract and its most copious constituent, 4-hydroxy-3-(3-methyl-2-butenyl)acetophenone, were tested against MCF-7, ZR-75-1, MDA-MB-231 (breast cancer), and MCF-10F (non-tumorigenic) cells (Echiburú-Chau et al. 2014). The results of this study suggest a specific activity of the ethanolic extract on the breast cancer cells. However, the assayed phytocompound was not effective against the tested cancer cells. *Geum quellyon* Sweet

(Rosaceae), called by the Chilean inhabitants as hierba del clavo, is used for treatment of a series of health problems such as gastric inflammations and prostatitis as diuretic and also is endorsed with aphrodisiac activities (Muñoz et al. 2004). The anticancer potential of its methanolic extract was proved against Caco-2 (colon carcinoma), KB (oral squamous), and Du-145 (androgen-insensitive prostate) cancer cell lines. But, a necrosis effect was also observed on the Caco-2 and KB cells. Further studies are necessary to find the active metabolites from the extract to justify these results (Russo et al. 2007).

11.2.5 Colombia

Colombia owns a big territory under the South America and is one of the top 10 countries with the highest biodiversity worldwide. With a diverse climate and geography, this territory is the home of many unique flora and fauna. Under the Colombian population, the use of plants to treat diseases and ailments is common and practiced even now. Many plant species are used to fight against cancer; however the literature regarding the studies of anticancer plants is scarce, and some of the reported studies are considered in this review.

Plants belonging to Asteraceae family, such as *Gnaphalium elegans* H.B.K. and *Achyrocline bogotensis* (Kunt) DC., were isolated with two isoflavones, 57 and 58 (Table 11.1-M). These compounds were evaluated on colon (Caco-2, HCT 116), breast (MCF-7, SK-BR-3), prostate (PC-3, LNCaP), and pancreatic (MIA PaCa, Panc 28) cancer cells using the MTT assay. The results indicated the apoptotic effects of these two compounds as revealed from the terminal deoxynucleotidyl transferase (TdT) dUTP nick-end labeling (TUNEL) assays. Both compounds displayed a potent activity against the pancreas (Panc 28) and colon (Caco-2) carcinoma cells, whereas the cytotoxic activity of flavone 58 was observed in breast (SK-BR-3), colon (HCT 116), and pancreas (Mia PaCa) cancer cells (Thomas et al. 2012). Ten species of the *Euphorbia* genus (Euphorbiaceae) such as *E. heterophylla* L., *E. cyathophora* Murray, *E. graminea* Jacq., *E. tirucalli* L., *E. cotinifolia* L., *E. arenaria* Kunth, *E. pulcherrima* Willd. ex Klotzsch, *E. cotinifolia* L., and *Euphorbia* sp. are used by the Colombian traditional therapists to treat ailments such as cancer, ulcer, and warts. These plants were tested for their potential antitumor activity using the MTT colorimetric assay (Betancur-Galvis et al. 2002). About 47 different solvent extracts (petroleum ether, ethanol, dichloromethane, water, and water-methanol) from these 10 species were screened. Among them, the dichloromethane extracts of *Euphorbia* sp., *E. graminea*, and *E. cotinifolia* showed the highest activity. Later, these extracts showing positive response were further investigated for their antiproliferative effects against few cancer cells, and the results revealed the highest toxicity of the dichloromethane leaf extract of *E. cotinifolia* against Hep 2 and CHO cancer cells with an IC_{50} value of 35.1 and 18.1 μg/ml, respectively. Considering these results, *E. cotinifolia* deserves further studies to identify its antitumor compounds. In this chapter, the information about its phytochemical

composition is ignored. In a different study, where the inhibition of topoisomerase enzyme was the target to search for plants with anticancer activity, *Myriocarpa stipitata* Benth (Urticaceae) showed a good result, and the authors have suggested that this topoisomerase inhibitory activity is due to the presence of alkaloids in the dichloromethane extract of *M. stipitata*. The alkaloids are common metabolites in this genus, but there is no information regarding the alkaloid structures of this specific species (Niño et al. 2011). In another study, 14 phytocompounds isolated from 3 native Colombian plants, namely, *Pleurothyrium cinereum*, *Ocotea macrophylla*, and *Nectandra amazonum*, were tested against tumor cell lines A-549, HeLa, Hep 2, MCF-7, and PC-3 (Cuca et al. 2011). The results revealed that bicyclooctanoids and kaurenoic acid showed cytotoxicity against all tumorous cells, whereas benzofuranoids exhibited a selective action against the HeLa cells. In addition, (−)-cinerin A demonstrated a complete lethality against PC-3 and Hep 2 cells, while kaurenoic acid (73) completely inhibited A549 cell lines (Table 11.1-N). These plant species were reported to contain lignans, neolignans, diterpenes, and alkaloids (Coy and Cuca 2008, 2009a, b; Cuca et al. 2011). Likewise, the ethanolic extracts of leaves and fruits of *Cucumis dipsaceus* C.G. Ehrenb. ex Spach (Cucurbitaceae) from Colombia, called by the people as pepino diablito, were evaluated on the cancer cells (K562 and Hep 2). The phytochemical study of this plant only showed the presence of triterpenes and flavonoids and cucurbitacins, but their structures were not identified (Salama et al. 1999). The organic extracts of the species *Espeletia killipii* Cuatr. (Asteraceae) were investigated for cytotoxic activity, showing an interesting activity on few cancer cells (Jaimes et al. 2006). Further investigations showed that the cytotoxic principle of the *Espeletia killipii* (Asteraceae) was identified as the sesquiterpene lactone longipiline acetate (76) (Table 11.1-O). This compound was evaluated by the MTT method on Colombian cancer cell lines from thyroid gland, testicle, mouth, myeloid leukemia, Hodgkin's lymphoma, and K562 tumoral cell. The compound was active against the Hodgkin's lymphoma and the myeloid leukemia with IC_{50} value of 3.0 μg/ml. The results indicate that this molecule is an important target that deserves to be considered in further studies (Jaimes et al. 2006). The species *Acnistus arborescens* (L.) Schltdl. (Solanaceae), well known as guitite, is used in Colombia for inflammation, treatment of diseases of the liver and spleen, and treatment of cancerous growths. This plant was subjected to phytochemical and cytotoxic activity studies. Different organic fractions of butanol, methanol, and dichloromethane were screened against MCF-7, MKN-45, HT-29, SiHa, Hep 2, HeLa, and U937 cancer cell lines. The results indicated the highest activity of the dichloromethane extract with IC_{50} value of 50 μg/ml (Morantes et al. 2006). Later, withanolides (Table 11.1-P) with cytotoxic activity were isolated from the same plant (Minguzzi et al. 2011).

In an interesting work, the cytotoxic activity of extracts from six species including *Annona* sp. (Annonaceae), *Aristolochia cordifolia* Mutis ex Kunth. (Aristolochiaceae), *Crescentia cujete* L. (Bignoniaceae), *Callisia gracilis* (Kunth) D.R. Hunt (Commelinaceae), *Beta vulgaris* L., and *Chenopodium ambrosioides* L. (Chenopodiaceae) was evaluated against MDBK (bovine kidney) and Hep 2 (human larynx epidermoid) cancer cells. From the results, *Annona* sp. was shown to possess

a good cell toxicity and, thus, can serve as a good source for the identification and isolation of various active anticancer principles (Betancur-Galvis et al. 1999). Likewise, it has been reported that species from the *Annona* genus are rich in acetogenins, a kind of compounds with proven anticancer activity (Moghadamtousi et al. 2015; Gupta et al. 2011; Schlie-Guzman et al. 2009, Colman-Saizarbitoria et al. 1996, 1998).

The compounds extracted from three species (*Espeletia*, *Pentacalia*, and *Ageratina*) of the Asteraceae family, *Stemmadenia* from the Apocynaceae, and *Curatella* belonging to Dilleniaceae were evaluated against Hep 2, MCF-7, and four more breast cancer cell lines (CBC-1170, CSC-1595, CSC-3322, and CSC-3325) obtained and characterized at the National Cancer Institute in Colombia. The sesquiterpene lactone longipiline acetate (76) isolated from the species *E. killipii* and the quinol jacaranona (84) (Table 11.1-P) isolated from *P. corymbosa* exhibited very high cytotoxicity with IC_{50} values below 5 μg/ml. It is also important to indicate that these two compounds were very active against the Colombian tumoral breast cancer cell lines assayed (Téllez et al. 2004). Similarly, *P. abietina* containing two jacaranone compounds and a quercetin glycoside and kaurene diterpenes are considered as the metabolites with the cytotoxic activity (Santana and Varela 2013). Two very common flavonoids, quercitrin and quercetin isolated from *Brownea ariza* Benth. (Fabaceae), were effective against the VERO cells and Myeloma Murino SP2/0-Ag14 cells (Gil et al. 2008). The plant *Gnaphalium meridanum* Aristeg. (Compositae) is used in the folk medicines of Colombia to treat anti-inflammatory diseases and skin infections and also used as a hemostatic and anticancer agent. Interestingly, a scientific study has shown that flower and leaf extracts of this plant possess a potent anticancer activity against the cell line, J-774 (murine macrophages). Interestingly, the ethyl acetate extract from the flowers showed a strong activity against the cancer cell line and, thus, indicates a possible candidate for further exploration to isolate anticancer metabolites from this plant (Torrenegra et al. 2016).

11.2.6 Ecuador

Ecuador is one of the most biodiverse countries in the world and is well known for its old tradition of medicines. Importantly, indigenous communities of Ecuador present a good ethnomedical information about several medicinal plants (Bailon-Moscoso et al. 2015). In this chapter, we update the latest information on traditional plants of Ecuador which are proven scientifically as well as yet to be explored for their anticancer properties. *Uncaria tomentosa* (Will.) DC. is widely distributed in South America. It is traditionally used by Ecuadorian, Peruvian, Bolivian, and Asian population to treat ulcers, warts, intestinal problems, body pain, and microbial infections (Bourdy et al. 2000; Pohle and Reinhardt 2004; Heitzman et al. 2005; Tene et al. 2007). Plant extracts of *U. tomentosa* are reported to possess several biologically important phytocompounds including proanthocyanidins, alkaloids, terpenes, sterols, and flavonoids. The major alkaloids include isopteropodine (86),

isomitraphylline (87), pteropodine (88), and uncarine F (89) (Table 11.1-Q), which are known to exhibit antiproliferative activity against the acute lymphoblastic leukemia (CCRF-CEM-C7H2) cells. Moreover, uncarine F and pteropodine compounds also have the capability to induce apoptosis (Bacher et al. 2006). However, mitraphylline (90) (Table 11.1-P) failed to inhibit the CCRF-CEM-C7H2 cells but was found effective against neuroblastoma and glioma cells (IC_{50} of 12–40 μM) (Garcia Prado et al. 2007). Alkaloids such as pteropodine and isopteropodine inhibited the growth of medullary thyroid carcinoma cells by inducing caspase-associated cell death mechanisms (Rinner et al. 2009). Various organic extracts of *U. tomentosa* are capable of inducing apoptosis in the human promyelocytic leukemia (HL-60) cells (Sheng et al. 2000; De Martino et al. 2006; Cheng et al. 2007; Pilarski et al. 2010, 2013). In addition, the alkaloid-supplemented plant extracts are capable of inhibiting the growth of xenografts of HeLa (cervical cancer), HCT 116, and SW480 (colon cancer) cells by affecting the Wnt signaling pathways (Gurrola-Díaz et al. 2011). Likewise, the aqueous extract of this plant induced cytotoxicity mediated by apoptosis in both HL-60 and K562 leukemia cell lines (Sheng et al. 2000). Further, the quinovic acid glycosides were isolated from the ethanol/water bark extract. In another study, this plant was shown to inhibit the T24 growth of human bladder cancer cells with IC_{50} of 78.36 μg/ml. The cytotoxicity was mediated by activating the caspase-3-dependent apoptosis signaling pathways and through a mechanism that involves translocation of NF-κB to the nucleus (Dietrich et al. 2014). In addition, several studies have shown that the solvent extracts of this plant and its isolated pure compounds exhibit anti-inflammatory activity which might be involved in stimulating the immune system and prevent the proliferation of tumor cells (Heitzman et al. 2005; Allen-Hall et al. 2010).

A red sap, also identified as Dragon's blood, is produced from the bark of ≥3-year-old *Croton lechleri* Müll. Arg. (Euphorbiaceae) plants (Salatino et al. 2007). This sap has been used by the Amazon ethnic people to cure wounds and treat gastrointestinal illnesses and tumor (Cerón 2006; Gupta et al. 2008). Also, the sap has the capability to inhibit myeloid leukemia cells and K562 and SK23 melanoma cells at 1 mg/ml (Rossi et al. 2003). However, at higher concentrations (tenfold), both colon cancer cells HT-29 and LoVo were inhibited. Likewise, an alkaloid called tapsine (92) (Table 11.1-P) was shown to effectively inhibit both SK-23 and HT-29 cancer cells (Montopoli et al. 2012). The sap contains mainly flavonols and oligomeric proanthocyanidins. Interestingly, proanthocyanidin coded as SP-303 is reported to possess antiviral and antidiarrheal activity; however no cytotoxic activity is reported yet (Jones 2003). In contrast, catechin, epigallocatechin, epicatechin, and gallocatechin occurring in *C. lechleri* sap are shown to exhibit cytotoxicity against human cancer cells. They induce antiproliferative activity through various ways such as apoptosis, inhibiting protein kinases, activating caspases, and modulating cell cycle regulations (Fujiki et al. 2002; Forester and Lambert 2014). Previously, clerodane-type diterpenes were isolated from its sap (Cai et al. 1993). In another study, A431 epidermoid carcinoma cell line was inhibited by taspine (an alkaloid) by the induction of apoptosis by regulating the ratio of Bax/Bcl-2 and activating caspase-3 enzymes (Montopoli et al. 2012). Similarly, taspine has been reported to

prevent the proliferation of HUVEC (human umbilical vein endothelial) cells (Zhang et al. 2010). A derivative of taspine, HMQ1611, showed an antiproliferative effect in breast cancer (MCF-7, ZR-75-30, SK-BR-3) cells and xenografts in mice. Interestingly, HMQ1611 presented a cytotoxic effect against breast cancer cells, by activating the EGFR/MAPK, ERa, and EGFR/PI3K/AKT (vascular endothelial growth factors, EGFRs) pathways (Zhan et al. 2012).

Marsdenia condurango Rchb. f. (Apocynaceae), also well known as *Gonolobus condurango*, is used in the customary medications for treating syphilis, inflammatory diseases, and cancer (Tene et al. 2007). The bark of *M. condurango* is reported to contain few pregnane glycosides (Hayashi et al. 1980, 1981; Berger et al. 1988). A glycoside-rich extract of *M. condurango* was shown to inhibit NSCLC (non-small cell lung cancer) cells (IC_{50} = 0.22 μg/μl) through inducing apoptotic pathway. Further, DNA damage and the arrest of cell cycle at sub G0/G1 phase were confirmed (Sikdar et al. 2014). Similarly, condurango glycoside A (93) (Table 11.1-P), present as the major constituent of its bark extracts, was capable to inhibit HeLa cells via p53 activation and induction of reactive oxygen species, damaging DNA resulting in apoptosis-mediated cell death (Bishayee et al. 2013). The occurrence of coumarins and quercetin in the bark of *M. condurango* (94) (Table 11.1-P) has been reported to possess antitumor activity (D'Agostini et al. 2005; Gurib-Fakim 2006; Ji et al. 2009). The quercetin compound induces cytotoxicity in HeLa, HT-29, and A431 (human vulva carcinoma) cell lines through apoptosis by the modulation of tyrosine kinase EGFRs (Xavier et al. 2009). Further, it can be noted that quercetin has a higher affinity against wild-type EGFRs as well as two mutated EGFRs as evidenced by the molecular docking studies (Singh and Bast 2014). Likewise, many studies have reported their wide applications in various phytodrugs (Banerji and Campbell 2008).

Senna multiglandulosa (Jacq.) Irwin and Barneby (Fabaceae), a medicinal shrub native to Ecuador is spread alongside the Andean mountains. Emodin (95) (Table 11.1-P) and other bianthraquinones have been isolated from this species (Abegaz et al. 1994). Emodin, a natural anthraquinone, has been shown to effectively inhibit the growth of several cancer cells such as human proximal tubular epithelial (HK-2), prostate (LNCaP), and cervical cancer (Ca Ski, HeLa, Bu 25TK, and ME-180) cell lines (Zhang et al. 1998; Srinivas et al. 2003; Wang et al. 2007; Yu et al. 2008; Yaoxian et al. 2013) by inducing apoptosis. Emodin is a tyrosine kinase inhibitor and naturally induce ROS. Hence, it increases the anticancer activity when used together with along with other therapeutic agents (Ko et al. 2010; Dave and Ledwani 2012; Qu et al. 2013). Likewise, another cytotoxic molecule, physcion (91) (Table 11.1-P), an anthraquinone derivative has been reported to exert inhibitory property against HeLa cells by apoptosis mediated by the generation of ROS (Wijesekara et al. 2014). However, it failed to inhibit MCF-7 and SW620 (human colon adenocarcinoma) cell lines (Almeida et al. 2010).

Minquartia guianensis Aubl. (Olacaceae), a native tree of Ecuador, is used against lung cancer in ethnomedical preparations. Phytocompounds such as betulinic acid, erythrodiol, myristic acid, palmitic acid, and stearic acid have been isolated from the bark and roots of this plant. However, minquartynoic acid (99)

(Table 11.1-P) (El-Seedi et al. 1994) is the major compound with a proven anticancer ability. It was found effective against the colon (Col2), oral epidermoid (KB), and KB-V+ (multidrug-resistant KB) cancer cells when treated together with vinblastine (1 μg/ml). In contrast, it inhibited KB-V– cells even in the absence of vinblastine with an IC_{50} value ranging from 1.6 to 5.5 μg/ml (Ito et al. 2001). Further, few chemical derivatives are developed from minquartynoic acid against cancers, viruses, and parasites (Gung and Dickson 2002; Dembitsky 2006; Gachet et al. 2010). The anti-inflammatory compound taraxerol is also documented from this plant. Triterpenes, namely, taraxerol (98) (Table 11.1-P), lupin-3-one, squalene, and lupeol, occur in the leaves of *M. guianensis* (Cursino et al. 2009).

A native bush of Ecuador, *Monnina obtusifolia* H.B.K. (Polygalaceae), finds its application as antitumor agent. The metastatic spread of cancer involves the growth of new blood vessels, i.e., angiogenesis (Bailón-Moscoso et al. 2014). The butanol extract of *M. obtusifolia* leaves was shown to function as antiangiogenic agent by inhibiting vascular endothelial growth factors (VEGFs) and play a crucial role in regulating angiogenesis (Lepore et al. 2011). A recent study has shown that phytocompounds (Table 11.1-P; Q and 18) of Ecuadorian traditional plants are effective against various cancers. The bark of *Cinchona officinalis* L. (Rubiaceae) is known for antipyretic activity since ancient times, and it is an introduced plant to Europe (Ferreira Júnior et al. 2012). The bark was used mainly for treating malaria, and later, the antimalarial compound quinine (100) (Table 11.1-Q) was isolated. Quinine and its derivative, chloroquine (101) (Table 11.1-Q), were widely used to treat malaria. The bark mainly constitutes large number of alkaloids (5–14%) (Kacprzak 2013). Studies have witnessed the cytotoxic ability of many of these alkaloids against Hep G2 hepatoma, MCF-7 breast adenocarcinoma, HL-60 leukemia, and SH-SY5Y neuroblastoma cells with IC_{50} values ranging from 0.75 to 89 μM (Károlyi et al. 2012). In specific, chloroquine effectively inhibited many cancer cells by inducing autophagocytosis and apoptosis (Solomon and Lee 2009). Another major alkaloid is the quinidine (102) (Table 11.1-Q). It was shown to prevent the proliferation of MCF-7 cells by interrupting the cell cycle process (Solomon and Lee 2009). From phase I and II clinical trial studies, it has been proved that chloroquine and 3-hydroxyquinoline (103) (Table 11.1-Q) compounds when employed in the combinational treatments with other drugs showed synergistic and selective induction of apoptosis in the breast, lung, and glioblastoma cancer cells (Solomon and Lee 2009).

Traditionally, the tree species such as *Annona squamosa* L., *A. muricata* L., and *A. montana* Macfad. (Annonaceae) have been used for treating rheumatism. About 400 acetogenins (ACGs) have been isolated from these plant species (Liaw et al. 2010). ACGs possess a wide array of pharmacological properties including immunosuppressive, antimalarial, and anticancer activities (Rupprecht et al. 1990; Liaw et al. 2010). Interestingly, most of these ACGs inhibit various cancer cells with an IC_{50} value ranging between 10^{-6} and 10^{-14} M. The compounds squamocin (104) and bullatacin (105) (Table 11.1-Q) isolated from *A. squamosa* induce apoptosis in many cancer cells (Zhu et al. 2002; Chiu et al. 2003; Derbré et al. 2006; Yuan et al. 2006). Other ACGs such as montecristin (109), cohibin A (110), murihexocin (111),

and arianacin (112) (Table 11.1-R) isolated from *A. muricata* are demonstrated to possess antitumor properties (Rieser et al. 1996; Alali et al. 1999; Ragasa et al. 2012). In addition, the extracts of *A. muricata* induce necrosis, suspend the cell cycle, and reduce the viability of pancreas cancer cells (Torres et al. 2012). ACGs of *A. montana* seeds including annomonysvin, annonacinone, annomontacin, and annonacin were shown effective against L1210 (murine leukemia), MDA-MB231 (human breast adenocarcinoma), and MCF-7 cancer cells (Jossang et al. 1991). Likewise, ACGs such as *cis*-annoreticuin; montalicins A–E; montalicins G and H; montalicins F, I, and J; montalicins A and B; and monhexocin (+)-monhexocin isolated from *A. montana* seeds showed selective cytotoxic activity against 1A9 and Hep G2 cancer cells (Liaw et al. 2004a, b).

Licania intrapetiolaris Spruce ex Hook. f. (*Chrysobalanaceae*) is a tree from the Ecuadorian amazon rainforest. For the first time, intrapetacin A (113) and intrapetacin B (114), the clerodane-type diterpenoids were isolated from this plant. These two compounds showed cytotoxicity against KB cells (human oral epidermoid carcinoma) with IC_{50} of 2 and 0.8 μg/ml, respectively (Oberlies and Burgess 2002). Later, a very active triterpene, cucurbitacin B (115) (Table 11.1-S), was isolated (Mukherjee et al. 2013) and proved its cytotoxic ability against BEL-7402 (human hepatocellular carcinoma cells), osteosarcomas (MG-63 and SAOS-2), and Hep 2 laryngeal cells (Liu et al. 2010; Chan et al. 2010b; Lee et al. 2011). Also, cucurbitacin B inhibited several leukemia cells such as CCRF-CEM, MOLT-4, K562, SR, and RPMI-8226 with an IC_{50} between 15.6 and 35.3 nM. The cell toxicity was due to the cell cycle arrest (Chan et al. 2010b) and by mediating many signaling pathways (Chan et al. 2010a).

The plant, *Casearia sylvestris* Sw. (Flacourtiaceae), a native plant of Ecuador is traditionally used against tumors (Graham et al. 2000). The aqueous-ethanolic and chloroform extracts of *C. sylvestris* leaves were reported to inhibit the multiplication of MCF-7 cells with an IC_{50} value of 141 μg/ml for aqueous-ethanolic extract and 66 μg/ml for the chloroform extract. Moreover, *C. sylvestris* extract treated animals showed a reduced proliferation of tumor cells (Felipe et al. 2014). *C. sylvestris* containing casearvestrins A (116), B (117), and C (118) (Table 11.1-S) were shown to possess antitumor activity against LX-1 (lung cancer), HCT 116 (colon cancer), and A2780 (ovarian cancer) cell lines with IC_{50} values ranging between 0.12 and 0.89 μM (Oberlies and Burgess 2002). Likewise, casearins isolated form this plant have the antitumor activity (Gonzaga dos Santos et al. 2010; Felipe et al. 2014). Casearins L (119), O (120), and X (121) (Table 11.1-S) showed a strong anticancer activity against leukemia cells such as HL-60, CEM, and K562 (Ferreira et al. 2010). Another bioactive compound, casearin X, showed a greater inhibitory potential against HL-60 and CEM cell lines with an IC_{50} of 0.4 μM. This compound induced cell death through apoptotic pathways (Pinheiro-Ferreira et al. 2014, 2016). The leaves of *C. sylvestris* contain isobutyl gallate-3,5-dimethyl ether (IGDE) (122) and methyl gallate-3,5-dimethyl ether (MGDE) (123) (Table 11.1-S). Both these compounds were shown to exhibit antitumor activity in xenograft models of Lewis lung and Ehrlich tumor cell lines (Da Silva et al. 2009).

11.2.7 Guyana

Guyana has a rich flora biodiversity with 87% of its land area covered by forests (Tewari and Gomathinayagam 2014), but unlike many other tropical countries, most of these forests remain unexplored, and the ethnobotanical information is scarce, especially because in the few anthropological studies carried out, the scientific names of the species are not included. Austin and Bourne (1992) conducted a research on the uses of medicinal plants in the region of Guyana in order to improve ethnobotanical information. They found that the common names used on coastal Guyana are different from the neighboring English and non-English speaking areas, but the same plants are usually used for similar purposes in different countries.

Guyana's original inhabitants were the Amerindians (descendants of the indigenous people of Guyana), and they are often the only ones who know the properties of plants and how they should be used. Nevertheless, with the passage of time, the influences from the outside world, and the process of "civilization," indigenous language has been lost, and it implies the loss of ethnobotanical knowledge, because many of the species used in this region are known only by their indigenous names (van Andel 2000a). Amerindians have used the plants for the treatment of diseases and magic rituals and as a source of foods. In addition, many of these species are used as poisons for fish as a hunting practice to obtain food although this method is prohibited by law. For this reason local Amerindians have cultivated ichthyotoxic plants for a long time, and many of these plants are also used as medicine, such as the species of *Lonchocarpus* and *Tephrosia sinapou*, which have been attributed activity against cancer and acquired immune deficiency syndrome (van Andel 2000b).

Tephrosia sinapou (Buc'hoz) A. Chev. (Fabaceae) is a shrub known in Guyana as kunali, Surinam poison, yarro-cunali, aiari (Guyana Akawaio), yaurokonan (Guyana Arawak), and ai (Guyana Wapishana). The roots of this plant are used for treating cancer in the northwest Guyana, and a decoction of leafy branches is drunk to treat snakebite and as an antisyphilitic (DeFilipps et al. 2004). In South America, it is found in Bolivia, Brazil, Colombia, Ecuador, Guyana, Guyana Francesa, Peru, Surinam, and Venezuela. The main classes of compound isolated from this species include flavonoids and rotenoids (Jang et al. 2003; Vasconcelos et al. 2009). This species exhibits a cancer preventive potential (Jang et al. 2003), larvicidal activity (Ribeiro et al. 2006; Vasconcelos et al. 2012), antioxidant activity, anti-inflammatory and antinociceptive properties (Martinez et al. 2012, 2013, 2016; do Val et al. 2014). Jang et al. (2003) determined the potential cancer chemopreventive properties of the compounds isolated from the stems of *T. toxicaria* induced quinone reductase in cultured Hepa 1c1c7 (mouse hepatoma cells). The induction of quinine reductase reflects the inhibition of cancer initiation. They found that the chalcone, isoliquiritigenin (124), exhibited the most potent activity. Furthermore, the compounds (2*S*)-5-hydroxy-7-methoxy-8-[(E)-3-oxo-1-butenyl]flavanone (125), 4′,5′-dihydro-11,5′-dihydroxy-4′-methoxytephrosin (126), genistein (127), and chrysoeriol (128) (Table 11.1-T) significantly induced quinine reductase activity.

The genus *Lonchocarpus* (Fabaceae) is known as haiari. Various species of *Lonchocarpus* are used as fish poison in northwest Guyana, and of these, *L. chrysophyllus* Kleinh. (black haiari), *L. martynii* A.C. Smith, *Lonchocarpus* sp. (red haiari), and *L. martynii* A.C. Smith are associated with cancer treatment (DeFilipps et al. 2004; van Andel 2000b). The most known active ingredient of *Lonchocarpus* species is the rotenone (129) (Table 11.1-T), which has been shown to display anticancer activity through the induction of apoptosis in various cancer cells (Isenberg and Klaunig 2000; Armstrong et al. 2001; Deng et al. 2010; Siddiqui et al. 2013; Hu et al. 2016). However, studies have witnessed that rotenone induces many adverse side effects including neurodegeneration (Emmrich et al. 2013).

11.2.8 Paraguay

The Paraguay River running through Brazil, Bolivia, and Paraguay divides the country, Paraguay into the western region (Chaco) and the eastern region. Chaco is inhospitable, semiarid, and infertile with scrub forests. Only about 3% of the population live in the Chaco. However, the eastern Paraguay constitutes fertile soil with rolling hills, lavish semitropical forests, and grassy savannas. The consumption of medicinal and aromatic herbs in Paraguay is traditional and widespread; it is a custom that comes from the Guaraní Indians, who had extensive knowledge about the use of plants for medicinal purposes. Nowadays, the use of these plants by the Paraguayans is a mixture of the knowledge of the Guaraní Indians and the Spanish people, who introduced their own healing plants progressively. To treat the health problems, the Paraguayans use herbal remedies simultaneously with pharmaceuticals. Most people have knowledge about the medicinal uses of common plants and use them to treat many of their diseases, as for many, health services in clinics and hospitals are inaccessible, especially in rural areas. Many different species of medicinal plants are commercialized in Paraguayan markets but many of them with minimal studies that support their use in the population. In general, medicinal plants of Paraguay are basically unknown to the scientific community. In view of the great use of medicinal plants by the population of Paraguay, some ethnobotanical studies have been carried out, but species plants used for cancer have been mentioned sporadically in research works and compilations of the Paraguayan pharmacopeia (Schmeda-Hirschmann and Bordas 1990; Basualdo et al. 1991, 1995; Cáceres and Machain 2001; González et al. 2013; Basualdo and Soria 2014; Degen de Arrúa and González 2014; Soria and Ramos 2015).

In 1995, Basualdo et al. identified 17 species (12 families) that are being sold as medicinal plants in the market #4 of Asunción known as “Pettirossi” to treat ulcers, gastritis, cough, respiratory tract diseases, syphilis, amenorrhea, and rheumatism. They are also used as abortives, hemostatics, hypotensives, expectorants, diuretics, and refreshing beverages. In this work the only species used to treat cancer was *Maytenus ilicifolia* Reiss (Celastraceae), known as “kangorosa” in Guaraní for the

rhizome, and "Kangorosa rapo piré" in Guaraní for the bark of the rizhome. Suárez and Mereles (2006) collected verbal information about the uses of 35 species of medicinal trees, belonging to 18 families in Paso Jovái District of the Guairá Department, Paraguay. *Erythrina crista-galli* L. (Fabaceae) known as seibo was reported to treat uterus cancer and other types of cancer. In another ethnobotanical research, *Annona muricata* L. (Annonaceae), *Couepia grandiflora* (Mart. and Zucc.) Benth. (Chrysobalanaceae), and *Croton urucurana* Baill. (Euphorbiaceae) were reported used against cancer (Basualdo and Soria 2014). In a recent paper, Soria and Ramos (2015) identified 56 species used in the IV Health Region of Guairá, Paraguay, with diverse medicinal purposes, of which the most common were *Mentha x piperita* L. (Lamiaceae), *Eugenia uniflora* L. (Myrtaceae), *Lippia alba* (Mill.) N. E. Brown. (Verbenaceae), *Allophylus edulis* (St. Hil.) Radlk. (Sapindaceae), *Scoparia dulcis* L. (Plantaginaceae), and *Chenopodium ambrosioides* L. (Chenopodiaceae); nevertheless, the one species reported to prevent cancer was *Croton urucurana* Baill. (Euphorbiaceae), known in Paraguay as Sangreado.

11.2.9 Peru

Peru is a mega-diversity country, where the conventional medicine coexists with traditional, complementary, and alternative medicine. These kinds of medicines have been institutionally recognized by the Peruvian government, through the implementation of safety procedures and encouragement of research in this area. In a recent research, a multidisciplinary group from Peru, Czech Republic, and Belgium published an interesting work, where the phenolic composition and the antioxidant and antiproliferative activity of a group of edible and medicinal plants of Peruvian-Amazon were assayed (Tauchen et al. 2016). The selection of plants was done, considering the use for the treatment of ailments associated with oxidative stress. The extracts of different parts, fruits, leaves, and barks, of the group of plants were screened on the liver carcinoma cell line Hep G2, colon carcinoma cell line HT-29, and normal fetal lung cells MRC-5 by modified MTT method. The most active plants include *Annona montana* Macfad. (Annonaceae), Inga edulis Mart. (Fabaceae), *Myrciaria dubia* (Kunth) McVaugh (Myrtaceae), *Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum. (Malvaceae), *Mauritia flexuosa* L. f. (Arecaceae), and *Oenocarpus bataua* Mart. (Arecaceae). Many known compounds have been characterized from these plants (Tauchen et al. 2016). In a similar work, an antiproliferative effect of bioassay-guided fractions of five Peruvian plants such as *Doliocarpus dentatus* (Dilleniaceae), *Picramnia sellowii* (Picramniaceae), *Strychnos mitscherlichii* (Loganiaceae), *Iryanthera juruensis* (Myristaceae), and *Croton alnifolius* (Euphorbiaceae) is reported. The study included few cancer cell lines such as A31 (embryonic mouse fibroblast), ME180 (human cervical), H460 (human large cell lung), DU145 (human prostate), M-14 (human melanoma), MCF-7 (human breast), HT-29 (human colon), PC-3 (human prostate) cancer cells,

Vero cells, and normal African green monkey kidney epithelial cells. Betulinic acid (130) (Table 11.1-U), a very well-known metabolite with many biological activities including cytotoxicity, was isolated from *D. dentatus*. The isolated compound, naftaloemodin (131), from *P. sellowii* was considered the cytotoxic principle of this plant. This compound and other metabolites such as chalcones isolated from this plant are shown in the Table 11.1-U.

In general, the *Strychnos* genus is rich in alkaloids. In a study, the bioassay-guided fractionation of *S. mitscherlichii* resulted in the isolation of a dimeric alkaloid 133 (Table 11.1-U) which showed good results against the HT-29 and K562 cell lines with a growth inhibition, GI_{50} <1.0 μg/ml.

Two chalcones, 134 and 135 (Table 11.1-U), isolated from *I. juruensis* were considered as the active principles in the cytotoxic evaluation on human cancer cell lines (Aponte et al. 2008a). Likewise, two different chalcones isolated from this plant also showed good cytotoxic activity (Table 11.1-U) (Aponte et al. 2008b). The *Croton* genus is well recognized with many species, which has been demonstrated with anticancer activity. A series of diverse structures, especially diterpenes, had been isolated from its species. The species *C. alnifolius* possess the phorbol ester 12-*O*-tetradecanoylphorbol-13-acetate (136) (Table 11.1-U), as the cytotoxic compound, especially active against the K562 cell line. In a similar study, 34 extracts from 8 ethnopharmacologically selected Peruvian plants were screened against leishmania, trypanosomiasis, and cell viability. The species included in the study were a representative of eight different families of the Peruvian Amazonia: *Aristolochia pilosa* L. (Aristolochiaceae), *Brunfelsia grandiflora* L. (Solanaceae), *Cedrela odorata* L. (Meliaceae), *Chondodendron tomentosum* Ruiz and Pavón (Menispermaceae), *Paullinia clavigera* Schltdl (Sapindaceae), Tabebuia serratifolia (Vahl) G. Nicholson (Bignoniaceae), *Tradescantia zebrina* (Rose) D.R. Hunt (Commelinaceae), and *Zamia ulei* Dammer (Zamiaceae). The cell viability was studied by using the modified MTT assay against the CHO (mammalian Chinese hamster ovary) cells (Gonzalez-Coloma et al. 2011). The results indicated that mostly all extracts showed at least some small activity against the cell viability, except few extracts from *C. odorata* bark, *C. tomentosum* bark, *P. clavigera* bark, and the hexane extract from leaves of *B. grandiflora*. Some known compounds including alkaloids and naphthoquinones were isolated and identified from these plants (Table 11.1-V) (Gonzalez-Coloma et al. 2011). In a study based on interviews of 88 patients and 117 noncancerous individuals who participated in the survey related to the use of herbal medicines to treat liver cancer in the Peruvian population, the plants *A. vera* and *M. citrifolia* were significantly associated with the treatment of liver cancer-related symptoms in the patient group (Rojas-Rojas et al. 2016).

Other big group of 341 Peruvian medicinal plants, from the northern part of the country, was screened using the brine shrimp lethality assay to determine the cytotoxicity of plant extracts (Meyer et al. 1982; Coe et al. 2010). The toxicity values obtained with this bioassay were later considered to obtain prospective candidates for further investigations. The authors mention in their conclusions that about 75%

of the evaluated species showed cytotoxic potential. However, the species showing higher levels of cytotoxic activity included *Bejaria aestuans* L. (Ericaceae), *Erodium cicutarium* (L.) L'Her (Geraniaceae), *Brachyotum naudine* Triana (Melastomataceae), *Miconia salicifolia* (Bonp. ex Naud.) (Melastomataceae), *Cuscuta foetida* Kunth (Convolvulaceae), *Caesalpinia spinosa* (Molina) Kuntze (Fabaceae), and *Phyllactis rigida* Humb. and Bonpl. (Valerianaceae) (Busmann and Glenn 2011).

The antitumor effect of aqueous extract of *Bomarea cornigera* Herb. (Alstroemeriaceae) from Peru was investigated in Swiss albino mice strain, inoculated with tumor cell line TG-180. The results demonstrated an inhibitory effect of the extract in the development of solid tumor in mice, where the TG-180 sarcoma was transplanted. The inhibition rates were 87.44% and 8.52% after 17 days of treatment. These results shows that this plant deserves further studies to identify the compounds responsible of this antitumor activity (Suárez et al. 2010). Likewise, *Uncaria tomentosa* (Willd. ex Schult.) DC. is another famous plant of Peru endorsed with anticancer activity. Several studies of anticancer assays had been reported on this species. The activity of *U. tomentosa* preparations on cancer cells was studied using in vitro and in vivo models; Lewis lung carcinoma (LL/2), cervical carcinoma (KB), colon adenocarcinoma (SW707), breast carcinoma (MCF-7), and lung carcinoma (A-549) cells were used in this study. Oxindole alkaloids, isolated from this plant, were screened to demonstrate the cytotoxic activity (Table 11.1-W) (Rojas-Duran et al. 2012; Pilarski et al. 2010). The antiproliferative and pro-apoptotic effect of fractions obtained from *U. tomentosa* was screened on medullary thyroid carcinoma (MTC) given interesting results (Rinner et al. 2009). Extracts of *U. tomentosa* bark were also investigated on B16/BL6 melanoma cells (Fazio et al. 2008). Today, it is a recognized species with good anti-inflammatory activity, but still the anticancer studies are not conclusive. Polyhydroxylated triterpenes and oxindole alkaloids are the most important metabolites reported from the phytochemical studies of *U. tomentosa*. Some examples of these triterpenes are shown in tabular form (Table 11.1-Y; Z) (Heitzman et al. 2005). Though many medicinal plants used in folk medicines are effective against several human ailments, however, therapeutical potential of these plants still requires the scientific validation. In a work of Bussmann and Glenn (2011), 47 plant species were documented and identified as anticancer and antidiabetic herbal remedies. From this study, 17 species were identified with anticancer remedies. In vitro studies of *Thevetia peruviana* (Pers.) K. Schum (Apocynaceae) (Haldar et al. 2015) and *Arctium lappa* L. (Asteraceae) (Ishihara et al. 2006) have shown good and interesting results against cancer cells.

11.2.10 Uruguay

Uruguay is the second-smallest country of the South America, after Suriname. It is a country sandwiched between Brazil, Argentina, and the Atlantic Ocean. In spite of this, it is a country with a distinct culture from its neighbors and has a population of

basically European origin; the original indigenous peoples have disappeared. The majority of the population are urban based and resides in the southern half of the country in or around the capital Montevideo. Most of the Uruguay is grassland, no forest. The largest natural area of Uruguay is tall-grass savanna, originally covered with many species of grasses (Burford 2014). During the colonization, native plants were merged with the European origin plants and formed the basis of Uruguay's popular medicine. Further, innate vegetation used in the neighboring countries was added over time to this basic pharmacopeia by adopting their use (González et al. 1993). Herbal/traditional products are a small category, with little use in the treatment of the health of Uruguayans since they still have certain distrust regarding the effectiveness of herbal/traditional products. For these reasons in Uruguay, a few published works on medicinal plants, their use and/or their chemical composition. The following are the results of two research studies carried out.

González et al. (1993) screened some selected medicinal plants traditionally used in Uruguay for their biological activities. They used two bioassays, namely, the *Artemia salina* toxicity test and the wheat rootlet growth inhibition (WRGI) assay. They found that seven plants (*Jaborosa runcinata*, *Dodonaea viscosa*, *Psidium incanum*, *Acanthospermum australe*, *Baccharis trimera*, *Anagallis arvensis*, and *Muehlenbeckia sagittifolia*) exhibited substantial dose-dependent growth inhibitions in the WRGI test. Among these, only three plants, namely, *D. viscosa*, *A. arvensis*, and *B. trimera*, inhibited up to 50% at 0.5% concentration. Three plants, namely, *Achyrocline satureioides*, *B. trimera*, and *Equisetum giganteum*, showed a strong concentration-related toxic activity on brine shrimps. *A. satureioides* showed toxicity even at the lowest concentrations tested. In other work, Barneche et al. (2010) reported a list of plants present in the gallery forest of the Uruguay River and its ethnobotanical information. Of these plants, the only species mentioned to treat cancer was *Maytenus ilicifolia*.

11.2.11 Venezuela

Venezuela is a tropical country located in the north of South America and is characterized by its great variety of ecosystems ranging from perpetual snow in the Andes to desert or semidesert areas in Falcón. Venezuela is a country that possesses an extraordinary biodiversity, being one of the ten countries with the highest biodiversity in the world. In general, it is usually divided into four major ecological regions, namely, the coastal zone, the Llanos (plains), the Andean mountain range, and the Guiana Highlands. Venezuela possesses an enormous wealth of useful and medicinal plants. In Guiana, where most natives live, there is a high percentage of the utilization of medicinal plants. Even in regions where there is a great rural population, people use medicinal plants in higher percentages. However, the urban people do not use many of these plants to treat their illnesses.

It is estimated that in Venezuela there are more than 20,000 plant species, of which over 1500 are being used by the native communities. Various ethnobotanical

studies on medicinal plants have been carried out; however, these have been concentrated mainly in indigenous communities of the Orinoco (Castillo 1998, 2001; Narváez et al. 2000). In the last years, several investigations have been carried out in other communities of the country (Játem-Lásser et al. 1998; Hidalgo-Báez et al. 1999; Bermúdez and Velásquez 2002; Cumana 2002; Gil et al. 2003, 2006; Aranguren 2005; Carrillo-Rosario and Moreno 2006; Lezama et al. 2007; Carmona et al. 2008; Jaramillo et al. 2014). In these studies, few species with anticancer activity used by the population are reported. Among the most mentioned are *Petiveria alliacea* (Phytolaccaceae) "mapuritico" (Gil et al. 2006; Jaramillo et al. 2014; Játem-Lásser et al. 1998), *Moringa oleifera* Ben. (Moringaceae) "ben," *Aloe vera* (L.) Burm.f. (Liliaceae) "zábila," *Roupala mollis* Pittier (Proteaceae) "mapurite" (Lezama et al. 2007), *Acanthospermum australe* (Loefl.) Kuntze (Asteraceae), *Porophyllum ruderale* (Jacq.) Cass. (Asteraceae) "mapurite," and *Argemone mexicana* L. (Papaveraceae) "Cardo Santo" (Játem-Lásser et al. 1998). On the other hand, some studies related to the anticancer activity of plant species collected in Venezuela have been published, and some of them we mention here.

Taylor et al. (2006) selected 17 species based on their ethnobotanical usage in Venezuela and other Neotropical countries to explore their potential anticancer activity. These plants were collected from the Yutaje area in the northern part of Amazon state, Venezuela, between 1999 and 2002. About 40 plant extracts were evaluated for their cytotoxicity at different concentrations (10, 100, and 1000 mg/ml) against lung (A549, CALU-6), colon (HT-29, Caco-2), pancreas (PANC-1), and breast (SKBR3, MCF7, MDA-MB231) carcinoma cells. Also, the extracts were tested on the proteases as they are known to be involved in the cancer induction. The results obtained indicated that 13 extracts from 10 species showed a cytotoxic effect at 100 mg/ml on more than one tumor cells. The species such as *Jacaranda copaia*, *Tapirira guianensis*, *Gnetum nodiflorum*, *Protium unifoliolatum*, *Protium heptaphyllum*, *Costus scaber*, and *Croton cuneatus* presented activity against some of the cancer cell lines tested. Among all extracts, *T. guianensis* bark and leaf extracts showed a higher cytotoxic activity at 100 mg/ml concentration against Caco-2, PANC-1, and CALU-6 cells. Besides, these extracts also exhibited protease inhibitory activity.

T. guianensis, a member of the Anacardiaceae family is known in Venezuela as jobillo and tapaculo. It is used in Venezuelan's folk medicine to treat measles and warts and as antidiarrheic agent (Taylor et al. 2006). This species has been previously studied to evaluate its anticancer activity. David et al. (1998) reported two compounds from the chloroform extract of their seeds with cytotoxic activity against BC1 (human breast cancer) with IC_{50} of 1.3–4.3 μg/ml and Col2 (human colon cancer) with IC_{50} of 0.8–1.8 μg/ml. These compounds correspond to 2-[10(*Z*)-heptadecenyl]-1,4-hydroquinone (152) and (4R,6R)-dihydroxy-4-[10(*Z*)-heptadecenyl]-2-cyclohexenone (153) (Table 11.1-Z). In a recent work, the effect of this species was tested on a panel of head and neck squamous cell carcinoma (HNSCC) cell lines. The extract showed a significant cytotoxicity effect, as well as an ability to inhibit tumor migration and invasion (Silva-Oliveira et al. 2016).

Villasmil et al. (2006) evaluated the antitumor activity of ethanolic extracts from 11 species collected in Amazon state, Venezuela. They screened the anticancer activity both in vitro and in vivo against (a) five tumor cell lines, (b) primary tumor growth and metastasis in the B16/BL6 melanoma/C57BL/6 mouse model, and (c) NF-κB inhibitory activity in HeLa cells transfected with an NF-κB/luciferase reporter gene plasmid. They found that *Byrsonima crassifolia* (Malpighiaceae), *T. guianensis* (Anacardiaceae), and *Vismia cayennensis* (Clusiaceae) were cytotoxic for more than two of the cell lines at lower concentrations. In in vivo assays, the treatment with the *Jacaranda copaia* (Bignoniaceae) extract delayed tumor growth by up to 40%, while *Piper marginatum* (Piperaceae) showed a pronounced inhibitory effect on tumor growth. *Xylopia aromatica* (Annonaceae) only inhibited tumor growth to a small degree, and the other plants were not inhibitory.

Continuing with this line of research, Taylor et al. (2012) evaluated the effect of 308 plant extracts from 102 different species (78 genera and 48 families) against 6 tumor cell lines. They found that extracts from *Annona squamosa*, *Heliotropium indicum*, *Hamelia patens*, *Jacaranda copaia*, *Clavija lancifolia*, *Physalis cordata*, and *Piper san-vicentense* were the most active with mean LC_{50} values of 750 mg/ml. Of these, the leaf and fruit *C. lancifolia* extracts exhibited the highest cytotoxicity against all cells except the Raw 264.7. In the literature, there are few reports on its use and phytochemistry. However, other plants such as *Calotropis gigantea*, *Chromolaena odorata*, *Hyptis dilatata*, *Jacaranda obtusifolia*, *Siparuna guianensis*, *Protium heptaphyllum*, *Piper arboretum*, *Tapirira guianensis*, and *Xylopia aromatica* extracts recorded a lower cytostatic activity.

Croton cuneatus Klotz. belonging to the Euphorbiaceae is widespread in tropical regions of the world. Several species of the genus *Croton* have a long role in the traditional use of medicinal plants in Africa, Asia, and South America. Generally it is used for the treatment of cancer, inflammation, hypertension, diabetes, hypercholesterolemia, malaria, constipation, indigestion, dysentery, pain, wounds, fever, intestinal worms, ulcers, and weight loss. Some species of this genus present in Venezuela have been studied, and their extracts or metabolites have presented cytotoxic activity. From the dichloromethane extract of the aerial parts of *C. cuneatus* were isolated three glutarimide alkaloids, julocrotol (154), isojulocrotol (155), and julocrotone (156) (Table 11.1-AA), along with other known compounds. The in vitro cytotoxic activity of these compounds was evaluated against five human tumor cell lines (MCF-7, X-17, Hep G2, Skhep-1, and LoVo) using the MTT bioassay. Compound 154 the most potent and selective on MCF-7 with an IC_{50} of 21.0 μg/ml (Suárez et al. 2004). Likewise, *C. malambo* H. Karst., a small tree that grows in the western region of Venezuela, was isolated with an *ent*-kaurane (157) (Table 11.1-BB) that showed activity against a human mammary carcinoma cell line (MCF-7), and it was determined that its anticancer effect is through the induction of apoptosis (Morales et al. 2005). From the flowers of *C. micans* Sw. (which was erroneously identified as *Croton caracasana* Pittier), two *ent*-3,4-*seco*-kauranes were isolated, caracasine (158) and caracasine acid (159) (Table 11.1-BB) (Suárez et al. 2008). Both compounds showed potent activity against a series of cancer cell lines (Suárez et al. 2009). In a later study, five new *ent*-3,4-*seco*-kaurane dimers, micansinoic acid

(160), isomicansinoic acid (161), and the dimethyl (162), monomethyl (163), and monoethyl ester (164) of micansinoic acid, were isolated from the stems of *C. micans* Sw. (Mateu et al. 2012). The compounds caracasine acid (monomer) and micansinoic acid (dimer) were evaluated against the prostate cancer cell line PC-3 and human dermis fibroblasts (control cells). In addition, the combination of the monomer and dimer was realized with the antitumoral drugs taxol and adriamycin. These two compounds exhibited practically the similar cytotoxic activity against human tumor cells but not to normal cells. Caracasine acid combined with adriamycin or taxol resulted in synergistically enhanced growth inhibitory activity to a 1:1 ratio in tumor cancer cell line PC-3. The synergistic effects on PC-3 were also demonstrated when micansinoic acid was combined with adriamycin or taxol. This result indicates that the sensitivity of tumor cells to drugs (adriamycin-taxol) was increased by natural products (Vivas et al. 2013). In a recent work, a series of *ent*-3,4-*seco*-kauranes derived from the natural diterpene caracasine acid (159) were prepared and evaluated for antibacterial, leishmanicidal, trypanocidal activities and against cancer cells. The synthesized derivatives exhibited moderate activities against PC-3 cancer cells, although less effective than caracasine acid. However, the breast cancer cells MCF-7 were less sensitive to all compounds evaluated (Chávez et al. 2015).

11.3 Conclusions and Future Prospects

Cancer is a terrible disease that greatly affects the world's population. At present, there is no effective treatment, since the drugs used produce numerous side effects and are toxic. Therefore, there is a need to seek new therapies to treat and prevent this disease, which is one of the leading causes of death worldwide. This need has led to an increase in research in the field of natural products and has increased the interest in the compounds of natural origin and/or their synthetic derivatives. In this sense, South America is a region that includes five of the countries with the greatest biodiversity in the world; in addition, many of the inhabitants in this region preserve the traditions of our ancestors in relation to the medicinal use of plants. This vast knowledge however has been losing over the years, due to several causes including urbanization, technology advancement, deforestation, population increase, etc. In this way, ethnobotanical knowledge is threatened, and many species of plants are at risk of disappearing if we do not do our best to conserve these resources. This chapter has provided the ethnomedicinal and scientific evidences on various South American medicinal plant species used against cancer. Also, some results of phytochemical and biological studies on plant species of this region are presented. In general, ethnobotanical knowledge of the inhabitants of these countries is broad, especially for the treatment of many diseases, such as those affecting the respiratory tract, skin, kidney, and digestive system; however knowledge of plants to treat cancer is smaller, which could be related to the absence of symptoms in the first stages of this disease. Even so, studies of many plants present in South America have contributed to the search for new treatments for cancer. There are many areas of virgin

forest and a large number of unknown species, which means a great opportunity to continue researching in this area without ignoring the traditional knowledge of the plants in these countries.

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Chapter 12
Scientific Validation of the Usefulness of *Withania somnifera* Dunal in the Prevention and Treatment of Cancer

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12.1 Introduction

Cancer has demoralizing breakthrough event which happens in the life of the individuals which is the typical reason for death over the world. The prevalence of this bug is because of the changing lifestyle behavior of the humans. It has become a considerable burden on the communal health organization. The numerical analysis data showed that the cancer affects a third of the human population (Zaid et al. 2017). The studies on the cancer incidence in the body involve initiation, promotion, and progression, whereas reverting this process through therapeutic approaches by means of drugs is classically known as chemotherapy. The chemotherapeutic molecules currently employed in the treatment of cancer have the effects on normal characteristics like immune suppression, hair loss, vomiting, nausea, and impotence, and importantly the procedure is expensive which is not handy to the poor people and also interferes the metabolic events needful for the cell division. To overcome the above said issues and to maintain a better medical practice, the use of natural drugs is highly encouraged. The standardization and maintaining consistency of the synthetic drugs is a risky job, however the natural agents are safer with negligible or no side effects and easily available in the dietary procedure, and they had an ancient customary in disease regulations which are scientifically proven (Sak 2012; Amawi et al. 2017).

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Dietary food-based chemoprevention is gaining more importance recently because of the fact that it is reasonably an inexpensive approach and can improve the quality of life universally. Further, correct identification of chemopreventive agents from foods might be more beneficial in combating the problem of cancer. Traditional medicine is revalued by an extensive activity of research on different plant species and their therapeutic principles all over the world (Mohanty et al. 2017). More recently, medicinal plants as a source for discovering novel drug molecules has become a topic of global importance (Swamy and Sinniah 2016). The presence of various bioactive compounds and the mode of actions of these phytocompounds have further witnessed the significance of medicinal plants for therapeutical applications (Swamy and Sinniah 2015; Arumugam et al. 2016; Swamy et al. 2017). Certainly, some parts of these medicinal plants or phytocompounds have been used as nutraceuticals or dietary supplements to overcome various human health problems including cancer. In Ayurveda medicines, *Withania somnifera* (Ashwagandha) has been validated for rejuvenating potential, and the plant plays an essential role in improving the quality as well as prolonged life in human beings. Considering the above facts, this chapter highlights the importance of Ashwagandha as a perspective for treating and preventing cancer.

12.2 Botanical Aspects of *Withania somnifera*

Withania somnifera, colloquially known as Ashwagandha or Indian winter cherry or Indian ginseng, is a plant found in the tropical areas of Asia (Singh et al. 2010). Ashwagandha is a member of the Solanaceae plant family, and its name is supposed to be originated from Sanskrit meaning "horse's smell" principally because of the roots (Ashwa = horse; Gandha = smell). The plant prefers growing in variety of soil but favorable in sandy loom or literate soil with pH range of 7.5–8 (Verma and Kumar 2011). The plant is a perennial small woody shrub found growing in dry climate and belongs to family Solanaceae. The plants are found to be growing in summer vocational parts in the South Asian countries along the regions of Mediterranean belt and the zone of canaries. In India, the plants are found in the higher altitudes of Himalayas and as in other hotter parts. The plant grows up to a height of 30–170 cm in optimal conditions and is a stout shrub with central stem, star-shaped branching, and thin fine hairy structure. The plant bears yellow-colored flowers and red berries with long fleshy tap root system (Uddin et al. 2012; Verma and Kumar 2011).

12.3 Traditional Uses

Ashwagandha is arguably known as the most important medicinal herb in the Indian pharmacopeia and has been used in the various traditional customary of medicines like Ayurveda, Siddha, Unani, Tibetan, Sri Lankan, Arabic, and many folk systems

in the Asian subcontinent. In the Ayurvedic system of medicine, Ashwagandha is considered to be a *Rasayana herb* and to rejuvenate the body (Ovadje et al. 2015; Verma and Kumar 2011). The roots, which are the most important plant part, have been reported to possess myriad benefits in various literature. In practice, the fresh root system of the herb is used in cheese making for the coagulation as a substitute for rennet before drying; the constituents were added to the milk and boiled for coagulation. Due to all these benefits, Ashwagandha is known as *Queen of Ayurveda* and an important medicinal agent in the Indian pharmacopeia (Davis and Kuttan 2000; Mir et al. 2012).

12.4 Ethnomedicinal Effects

In ancient medical science practiced on *W. somnifera* and declared as Ramayana or rejuvenative, adaptogenic herb nourishes and tones the entire body with their aphrodisiac, sedative, and life-delaying properties. It is also used in geriatric problems and in Medhya Rasayana which promotes learning and memory as a general energy-reserving and enhancer tonic. The plant extracts from different sources are used in folk, Ayurvedic, Unani, and Siddha systems of medicine and the biological activities associated with different extract systems. The leaf part of the plant tastes like bitter and is used as an anthelmintic. The combination is given in cold and fever. Bruised leaves and fruits are typically used to treat tumors and tubercular glands by local application and also for carbuncles and ulcers. The fruit berries of the plant have a milk-coagulating activity which attributed to the pulp and husk of the fruit, used in the preparation of vegetable rennet during the fermentation for cheese production. The fruits are reported to be sedative, emetic and stomachic, blood purifier, and febrifuge (fever reduction), as an alternative, diuretic, and bitter tonic in dyspepsia as well as a growth promoter in infants. The root part extracts are also useful in constipation, depression, nervous exhaustion, loss of memory, loss of muscular energy, and spermatorrhoea (Singh et al. 2010; Mir et al. 2012).

12.5 Phytochemistry

Ashwagandha is one of the most well-investigated plants, and numerous studies have shown it to possess more than 35 chemical components. Pharmacognostic studies confirm that the plant contains alkaloids, steroidal lactones, steroids, salts, flavonoids, and nitrogen-containing compounds extracted from different parts of the plant. Each plant part bears an endless medical value in it as shown in the Table 12.1.

Table 12.1 Phytochemistry of *Withania somnifera*

Nature of compounds	Phytochemicals present in the herb	References
Alkaloids	Withanine, withaninine, somniferine, tropeltigloate, somniferinine, somninine, nicotine, visamine, withasomine	Kaur et al. (2013), SaiduluCh and GangadharRao (2014), and Chaurasia et al. (2013)
Salts	Cuscohygrine, anahygrine, tropine, pseudotropine, anaferine	
Steroidal lactones	Withaferin A, withanone, WS-1, withanolide E, withanolide F, withanolide G, withanolide H, withanolide I, withanolide J, withanolide K, withanolide L, withanolide M	
Nitrogen-containing compounds	Withanol, somnisol, somnitol	
Steroids	Cholesterol, β-sitosterol, stigmasterol, diosgenin, stigmastadien, sitoinosides VII, sitoinosides VIII, sitoinosides IX, sitoinosides X	
Flavonoids	Kaempferol, quercetin	
Other components	Resins, fat, coloring matters, a reducing sugar, phytosterol, ipuranol, and saturated and unsaturated organic acids	

12.6 Scientifically Validated Pharmacological Properties

Ashwagandha is arguably one of the highly employed plants in Ayurveda and in various folk systems of medicine. In the traditional system of medicine, this plant is claimed to have adaptogenic, immune stimulatory, and life-prolonging properties. The plant decoction (especially of roots) is a proven rejuvenator and is used to treat nervous exhaustion, memory-related conditions, insomnia, tiredness potency issues, skin problems, and coughing. Scientific studies carried out in accordance to the modern system of medicine have shown that the plant extracts do indeed possess anti-inflammatory (Mishra et al. 2000; Giri 2016), antioxidant (Chaudhuri et al. 2012), anti-stress (Kaur et al. 2001), antimicrobial (Mir et al. 2012), cardioprotective (Ojha and Arya 2009), antidepressant (Bhattacharya et al. 2000), immunomodulatory (Davis and Kuttan 2000), and antidiabetic (Jena et al. 2016) effects. In addition to the various extracts of the plant, studies have also shown that the major phytoconstituent of Ashwagandha, withaferin A, is extracted from the leaves and roots of plant (Verma and Kumar 2011). Chemically, withaferin A is a member of steroidal lactone containing cycloalkane rings and lactone rings and has been reported to possess myriad benefits including in cancer. Various pharmacological properties of this plant are given and summarized in tabular form (Table 12.2).

Table 12.2 Pharmacological activities of *Withania somnifera* phytocomponents

Phytocomponents	Pharmacological activity against various disorders	References
Withaferin A	Anti-inflammatory, antiarthritic, antidepressant, antibiotic	Singh et al. (2010) and Kaur et al. (2013)
Sitoinosides VII-X	Antioxidant, nootropic activity	
Withanoside IV	Alzheimer's disease	
Glyco-withanosides	Parkinson's disease	
Withanolide E	Antifeedant	
Withanolide 5	Immunomodulatory	
Withanolide D	Anticancer	
Sitoinosides IX, X	Immunomodulatory, CNS effects	

12.7 Effectiveness of Ashwagandha Against Cancer

The process of neoplasia is extended and involves myriad overlapping events. The process initiates with mutation that with time leads to cellular transformation and hyperproliferation and culminates in the acquisition of invasive and angiogenic properties that ultimately leads to metastatic lesions (Aggarwal et al. 2006). In Indian traditional system of medicine the Ayurveda, it is a well-known potential herb which fights against cancer and in addition to that helps in the maintenance of normal diet in the quality of life. Ashwagandha has been investigated for its anticancer effects, and studies have shown it to be effective (Tables 12.3 and 12.4). In the subsequent section, the anticancer effects of Ashwagandha will be addressed in detail against various cancer types.

12.7.1 Ashwagandha in Breast Cancer

The second foremost reason of the death in the world is because of breast cancer. On an average, out of ten, one woman will progress with this disease in their lifetime. Cause for the breast tumor is dependent on several issues like gender, diet, hereditary, lifestyle, and hormonal imbalance due to endocrine aspects. There are some other important factors that lead to breast cancer, like previous benign and mammographic density, but still it is not confirmed which factor is the most important in breast carcinogenesis (Abdulkareem 2013). Several studies involving Ashwagandha against the breast cancer show potent inhibitory activities in various cell lines (MCF-7 and MDA-MB-231). These studies have established that the cell cycle arrest at G2/M phase leading to the apoptosis as the chief mechanism of action (Maliyakkal et al. 2013). Similar experimental had been done against breast cancer cell lines with Ashwagandha extract which inhibited cell proliferation at both in vitro and in vivo with significant reduction in the cytokine and CCL2 expression; thereby it reduces the migration and invasiveness in MDA-MB-231 cells (Khazal and Hill 2015).

Table 12.3 Effect of *Withania somnifera* extracts on various type of cancers

Type of study	Type of cancer	Cell lines and IC50	Type of extract and dosage	Parameters studies	Inference	References
Cell culture studies	Breast	MCF-7	Ethanol extract	Cytotoxicity, apoptosis	Cell cycle arrest	Maliyakkal et al. (2013)
		22.33 µg/ml				
		MDA-MB-231				
		31.99 µg/ml				
		MCF-7	Aqueous extract			
		388.0 µg/ml				
		MDA-MB-231				
		471.40 µg/ml				
	Colorectal	HCT 116	Methanol extract	Cytotoxicity, antiproliferative effect	Caspase 3 activation	Alfaifi et al. (2016)
		2.19 µg/ml				
	Liver	HepG2	Methanol extract	Cytotoxicity, antiproliferative effect	Caspase 3 activation	Alfaifi et al. (2016)
		1.89 µg/ml				
	Laryngeal	Hep2	Chloroform and aqueous extract	Cytotoxic activity, anti- angiogenesis activity	Cell cycle regulation, anticancer activity. VEGF targets and inhibits neovascularization	Mathur et al. (2006)
		25 µg/ml	5–100 µg/ml			
	Human T leukemia	Human T-lymphoblastoid cells	Root powder in DMSO	Cytotoxicity, DNA damage, ROS production, Ca^{2+} and oxidative stress induction	Proapoptotic with intercellular Ca^{2+} accumulation, causes immunogenic cell death, genotoxicity	Turrini et al. (2016)
			0–1.6 mg/mL			
	Melanoma	A375:	Deionized water (aqueous) extract	Cytotoxicity, morphological analysis	DNA fragmentation, induction of nuclear blebbing and apoptotic body formation	Halder et al. (2015)
		350 µg/ml/24 h,	6.25–400 µg/ml			
		250 µg/ml/48 h and 200 µg/ml/72 h				
	Neuroblastoma	IMR-32, TGW, SH-SY5Y, Neuro-2a	Water (aqueous) extract	Cell proliferation, morphological analysis, gene expression studies, wound healing assay	Antiproliferative, anti-migratory, modulation of survival	Kataria et al. (2013)
			0.01–1.0% in media		Markers inducing cell death	
	Prostate	PC-3	Ethanol extract	Cytotoxicity, genomic analysis	Antiproliferative	Aalinkeel et al. (2010)
			0.5–1 µg/ml		Involves in the modulation of gene expression and signaling markers to stimulate apoptosis	

Animal studies	Transplantable tumor lymphoma	Dalton's lymphoma ascites (DLA)	Ethanol extract, 200 mg/kg	Hematological parameters	Antitumor	Christina et al. (2004)
	Transplantable tumor carcinoma	Ehrlich ascites carcinoma(EAC)	Petroleum ether and ethyl alcohol extract conjugated with gadolinium III oxide nanocomposite	Cytotoxicity, biochemical parameters	Radiosensitization, antitumor, ROS-mediated apoptosis, DNA fragmentation	Abdallah et al. (2016)
			227 mg/kg			
	Melanoma	B16F10	Methanol Extract	Biochemical parameters	Anti-metastatic	Leyon and Kuttan (2004)
			20 mg/dose/animal	Histopathology		
	DMBA induced Skin cancer	–	Methanol extract,	Antioxidant	Chemopreventive	Davis and Kuttan (2001)
			20 mg/dose/animal	Enzyme activation		
	Benzo(a) pyrene-induced forestomach papillomagenesis	–	Root powder mixed with food pellets	Enzyme activities, histopathological examination	Chemopreventive	Padmavathi et al. (2005)
			2.5% and 5%			
	Azoxymethane-induced colon	–	Ethanolic extract	Immune function test, histopathologic evaluation	Chemopreventive	Muralikrishnan et al. (2010)
	Carcinogenesis		400 mg/kg			

Table 12.4 Active component Withaferin A effects on various cancer cells and cancer models

Type of study	Type of cancer	Cell lines	Parameters studies	Inference	Reference
Cell culture studies	Endothelial cells	HUVECs	Cytotoxicity, vessel formation studies	Anti-angiogenic activity	Mohan et al. (2004) and Kumar et al. (2009)
	Prostate cancer	PC-3, LNCaP, PzHPV-7, CWR22Rv-1, DU-145,	Cytotoxicity, immunocytochemistry, ELISA, xenografts, transfection, and PCR	Proapoptotic through Par-4 and caspase activation	Srinivasan et al. (2007) and Das et al. (2016)
	Leukemia	U937, Caki, AMC-HN-4, HT-29	Cytotoxicity, gene expression studies	Apoptosis inducer through AKT dephosphorylation	Oh et al. (2008)
		SEM, REH, RS4	Cytotoxicity, Western blot analysis	Antileukemic activity, cell death	Shi et al. (2015)
	Colorectal cancer	HCT-116	Cell proliferation, migration, and docking studies, wound healing assays, xenografts	Suppressing AKT-induced tumor growth and stimulates cell death	Suman et al. (2016a) and Choi and Kim (2015)
	Uveal melanoma	OMM2.3, MEL290,	Cell proliferation and migration studies	Antiproliferative activity	Samadi et al. (2012)
	Fetal fibroblast cells	MRC-5			
	Breast cancer	MCF-7, MDA-MB-231,	Cytotoxicity, cell cycle analysis, transient transfection studies, ROS detection	Cell cycle arrest, stimulates apoptosis	Stan et al. (2008) and Zhang et al. (2011)
	Osteosarcoma	U2OS, MG-63	Cell proliferation, PCR	Induces antiproliferation with cell cycle arrest at G2/M phase	Ting-Zhuo and Wang (2015)
	Ovarian cancer	A2780, CAOV3	Cytotoxicity, ROS determination, Western blotting, xenografts, immunohistochemistry	Enhancement of ROS production, DNA damage, autophagy, antitumor activity	Fong et al. (2012)
	Oral cancer	HSC-3, HSC-4	Cytotoxicity, immunocytochemistry	Chemotherapeutic, apoptosis inducer	Yang et al. (2013)
	Thyroid cancer	DRO81–1	Xenograft, Western blot analysis	Antitumor activity	Samadi et al. (2010)

Animal studies	Prostate cancer in C57BL/6	–	Immunohistochemistry	Anticarcinogenic activity	Suman et al. (2016b)
	Transplantable carcinoma in Balb/c	Ehrlich ascites carcinoma	Irradiation by ^{60}CO gammatron	Radiosensitization, antitumor, anti-angiogenic activity	Sharada et al. (1996) and Kumar et al. (2009)
			Teletherapy, tumor toxicity		
	Melanoma in nude mice	92.1	Cell proliferation and migration studies	Antitumor, apoptosis inducer	Samadi et al. (2012)
	DMBA-induced oral cancer in hamsters	–	Biochemical parameters	Chemoprevention	Manoharan et al. (2009)

The major phytoconstituent of Ashwagandha, withaferin A, has revealed a promising antitumorigenic effectiveness in the carcinoma of breast cell lines MCF-10A and MDA-MB-231 owing to their anti-invasive and anti-metastatic activities by inhibiting epithelial-mesenchymal transition (EMT) stimulated by tumor necrosis factor-α (TNF-α) and transforming growth factor-β1 (TGF-β), and also it regresses the pro-metastatic intermediate filament protein which is part of EMT, vimentin program to promote metastasis. The activity of withaferin A was revealed by pathway enrichment investigation that specifically targets carcinogenesis resulting in the apoptosis; cell cycle and proliferation could be functionally evaluated through the flow cytometry and cell proliferation analysis. Withaferin A also plays a role in the invasion inhibition as determined by single-cell collagen invasion assay supported by lower gene expression of extracellular matrix-degrading proteases like uPA, PLAT, ADAM8, cell adhesion molecules such as integrins, laminins, pro-inflammatory mediators which involved in metastasis-promotion present within the tumor microenvironment and also involved in the colonization of the tumor such as TNFSF12, IL6, ANGPTL2, CSF1R and metastasis suppressor gene that had concomitant increased expression of BRMS1 were investigated (Szarc vel Szic et al. 2014; Lee et al. 2015; Yang et al. 2013).

12.7.2 Ashwagandha in Lung Cancer

Lung cancer is the leading cause of cancer death in the United States and around the world. Oncogenesis in the lung is due to the mutation caused by the carcinogens leading to the transition of epithelial cells that results in the metastasis through the activation of cellular signals by the cancer cells. The steroidal lactone, withaferin A, found in the Ashwagandha showed a promising effect in non-small cell lung cancer (NSCLC; A549 cell line) cells through the involvement of reactive oxygen species (ROS)-induced cellular toxicity (Liu et al. 2017). Experimental evidences of *Withania somnifera* have shown it to be a potent anticancer activity in lung carcinoma induced by chemical carcinogen benzo(a)pyrene in male Swiss albino mice studies. The molecular inhibitory mechanism along with paclitaxel involves chemotherapeutic activities like cellular damage mediated through free radicals, and the treatment showed the defending role of these molecules through reducing ROS-mediated cellular damages; thereby the extract of Ashwagandha in conjunction with paclitaxel affords the stabilization of membrane-bound enzyme levels and decreases the lipid peroxidation in animal studies (Senthilnathan et al. 2006).

12.7.3 Ashwagandha Targets in Gastric Cancer

Gastric cancer is one of the five most leading cancers that commonly causes death which is associated with less survival rate. The chronic gastric cancer that mainly occurs as part of syndromic disease in that 90% of the cancer is associated with the adenocarcinoma ultimately involves the long-standing mucosal inflammation consequences (Rugge et al. 2017). More than 50% of the world's population are chronically infected with gastric cancer due to the colonization of gram-negative bacteria *Helicobacter pylori* due to excess secretion of IL-1β, and it is the main risk factor for gastric cancer, and progression is linked with chronic inflammation and recruitment of immune cells (Kim et al. 2015). The phytochemical studies showed that the major withanolide, withaferin A (WA) component present in the Ashwagandha, exerted persuasive anticancer effect on *H. pylori*-linked gastric tumor. The molecular mechanism involves the regression in the secretion of IL- IL-1β in bone marrow-derived dendritic cells and the underlying cellular signals. WA showed the low release of IL-1β results due to NF-κB activation inhibition. Additionally it targets the NLRP3 inflammasome mediated by ATP and MSU activators. These analysis of WA against gastric cancer suggested that it can inhibit IL-1β production and secretion via dual cellular mechanisms (Kim et al. 2015).

12.7.4 Ashwagandha in Other Cancers and in Experimental Animals

Ashwagandha is also shown to possess anticancer properties against prostate cancer (Aalinkeel et al. 2010), colon and liver cancer (Alfaifi et al. 2016), leukemia (Turrini et al. 2016), skin cancer (Halder et al. 2015), and head and neck cancer (Mathur et al. 2006) of various human carcinomas. Mounting evidence from research aspects on cell culture and animal studies suggested that Ashwagandha has potent roles in cancer prevention (Palliyaguru et al. 2016). Ashwagandha showed a promising antitumor effect on transplantable tumor models DLA and EAC (Christina et al. 2004. Abdallah et al. 2016), metastatic skin cancer cell lines in mice (Leyon and Kuttan 2004), and chemopreventive potential of Ashwagandha has been analyzed against forestomach and colon cancer in murine model system (Padmavathi et al. 2005; Muralikrishnan et al. 2010) indicating that the anticancer effect observed in cell culture was replicating in the animal models.

12.8 Preclinical and Clinical Studies of Ashwagandha

Queen of Ayurveda (*Withania somnifera*) is a medicinal traditional plant of Solanaceae family in which it has potent phytoconstituents which play an essential role in various disorders which are clinically proven such as hypnosedative, immunomodulation, fertility enhancement, anti-inflammatory, antiarthritic, anticarcinogenic and angiogenesis inhibitor in the prevention of cancer, anticholinesterase activity, antioxidant, and antibacterial. Among the diverse phytocompounds of Ashwagandha, withaferin A is one of the best well-studied steroid lactone agents as much as pharmacological investigations are carried out. And for further evaluation in this vicinity, more research is essential and very much needed for future progression in the field of medicine. The detailed summary of the preclinical and clinical evidence has been shown in Table 12.5.

Table 12.5 Pre-clinical evaluation of *Withania somnifera* against multi-cancer models

Preclinical evaluation	Part of the plant extract/ phytocompound used	References
Chemo-protective activity	Herbal extract	Davis and Kuttan (2001), Padmavathi et al. (2005), Muralikrishnan et al. (2010), and Krutika et al. (2016)
Effective source for L-asparaginase	Root	
Azoxymethane-induced colon cancer and their immune dysfunction	Root extract/withaferin A	
Breast cancer with lung metastasis mouse model	Withaferin A	
Breast carcinoma and colon cancer inhibition	Withaferin A	
Benzo(A)pyrene-induced lung carcinoma in albino mice	Root extract	
Nontoxic normal lymphocytes for control proliferation	Pure herbal isolated nutraceutical; withanolide D	
Skin cancer mice model and UV-exposed rats as skin carcinogenesis model	Phytocomponents isolated from the root of Ashwagandha	
Skin carcinogenesis in Swiss albino mice by DMBA (7,12-dimethylbenz[a] anthracene)		
Cytotoxic efficacy on human cancer cells (MCF-7, A549)	Isolated from leaf	
Swiss albino mouse model of fibrosarcoma induced by 20-methylcholantrene	Phytochemical isolated from leaf of the herb	
In vitro and in vivo studies on various cancer types	Withaferin A and other steroid lactones of Ashwagandha	

12.9 Conclusion and Future Prospects

Ashwagandha is arguably one of the most researched medicinal plants, and studies have shown that various plant extracts as well as the principal phytochemical, withaferin A possess myriad benefits. Experiments have shown that these pharmacological effects are due to antioxidant, free radical scavenging, anti-inflammatory, antimutagenic, and induction of apoptosis in neoplastic cells. Mechanistic studies have shown that the apoptotic effects are mediated by induction of free radicals, accumulation of Ca^{2+}, cell cycle arrest, activation of caspase 3, DNA fragmentation, induction of nuclear blebbing, and apoptotic body. Moreover, Ashwagandha is also shown to decrease VEGF and to inhibit neovascularization indicating its usefulness as an anti-metastatic agent. In addition to the crude extract, withaferin A plays predominant modulatory activities and exhibits anti-inflammatory, pro-apoptotic, anti-invasive, and anti-angiogenic effects against various cancer conditions. Thus, it is considered as a potential drug candidate for treatment of different types of cancer. Mechanistically, withaferin A is shown to restrain TNF-induced IκB kinase and NF-κB activation and also stimulates apoptotic signals through triggering reactive oxygen species production. Withaferin A exerts multifunctional role in prevention of various cancer types through imparting signal transduction and their modulation. Thus, Ashwagandha is used in the Ayurvedic medicine for its rejuvenating power to enhance the immune system against stress, memory loss, neurodegenerative disorders, inflammation, arthritis, and high blood pressure and also used as immunomodulator in the improvement of fertility. Nowadays, this herb is extensively applied in the field of adjunctive therapy to treat the severe life-threatening disorders like cancer and other infectious diseases. Preclinical studies and clinical trials on animal models infer the importance of Ashwagandha use in the treatment of various medicinal practices to cure anxiety, cognitive and neurodegenerative disorders, and inflammation. Its chemopreventive effect on different conditions of an individual at their chemopreventive and radiotherapy level requires further more research in the near future (Fig. 12.1).

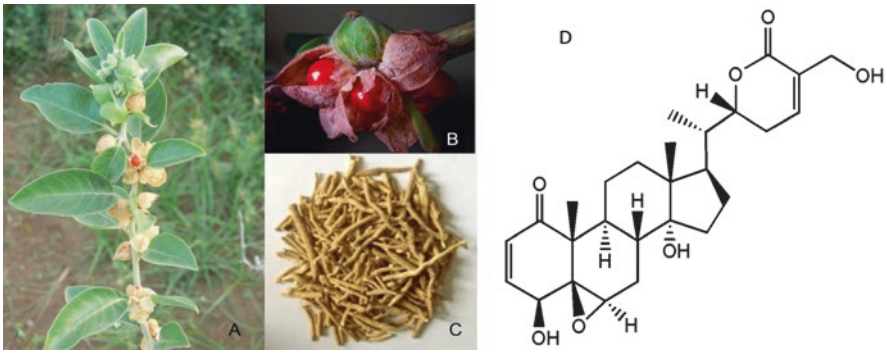

Fig. 12.1 Morphology of *Withania somnifera*; (**a**) plant, (**b**) berries, and (**c**) roots; (**d**) chemical structure of withaferin A

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Chapter 13
Anticancer Potential of Mangrove Plants: Neglected Plant Species of the Marine Ecosystem

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13.1 Introduction

Cancer is a complex form of disease which is responsible for a significant number of mortalities worldwide. It is characterized by the uncontrolled growth and spread of abnormal (unresponsive to cellular signaling) cells within or out of the tissue, resulting in accumulation, local damage, and inflammation (ACS 2016). According to WHO (2017), cancer is one of the leading causes of infirmity and fatality globally, with 8.8 million deaths in the year 2015. Common forms of cancer mortality are lung cancer (1.69 million deaths), liver cancer (788,000 deaths), colorectal cancer (774,000 deaths), stomach cancer (754,000 deaths), and breast cancer (571,000 deaths). Lung cancer (bronchogenic carcinomas) is the most common cancer among men and the ninth most common among women, whereas breast cancer remains the leading cause of cancer mortality among women (Ferlay et al. 2015; Torre et al. 2015). It has been predicted that, by the year 2030, the number of newly diagnosed cancer cases will increase globally to 21.7 million and 24 million by 2035 with about 70% of mortalities mostly occurring in low- and medium-income countries

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such as Africa, Asia, and Central as well as South America (Ferlay et al. 2015). The major causes accounting for more than one-third of deaths from cancer are due to the behavioral and dietary risks such as obesity, poor intake of fruit and vegetable, physical inactivity, tobacco, and alcohol consumption, while tobacco consumption alone is responsible for about 22% of cancer deaths worldwide. Cancer-causing infections, such as hepatitis and human papilloma virus (HPV), are also responsible for about 25% of cancer cases in low- and middle-income countries (Plummer et al. 2016).

Current treatment for different forms of cancer are carried out through surgeries, radiation therapy, and/or systemic therapies such as chemotherapy, hormonal therapy, neoadjuvant therapy, immune therapy, gene therapy, and targeted therapy. These treatments may be used alone or in combination depending on the cancer type and its stage, tumor characteristics, and the patient's age, health, and preferences. Some of the therapies are performed through supportive therapies to reduce side effects and address other patient and family quality of life concerns (ACS 2016; Yue et al. 2017). However, despite the availability of a number of treatments, many forms of cancers still remain intractable, which may be either due to delays in identification, diagnosis, sophistication, or high treatment cost. The current experimental therapy for cancer is highly sophisticated and costly and has low satisfactory outputs. Moreover, in such therapies if approved by FDA due to their serviceability, the high cost would make these treatments practically unaffordable for the use of common people in low- and middle-income countries. Henceforth current research is directed toward evaluating simpler or greener treatment of cancer, which includes the use of natural products/herbal medicines (Yin et al. 2013). Some of the conventionally used plants with anticancer properties are ginko, goldenseal, ginseng, garlic, *Echinacea*, jivanti, aloe vera, big sage, and saw palmetto (Swamy et al. 2015a; Shareef et al. 2016; Mohanty et al. 2017). Furthermore, recent researches are more focused on finding therapeutics with anticancer activity from plants of terrestrial origin, because of their easy accessibility. One such potential candidature is represented by the plants from the mangrove ecosystem, considering their rich phytochemicals, their bioactive compounds, and their unique ability to thrive in harsh environmental conditions and ethnobotanical significance (Vannucci 2000; Dissanayake and Chandrasekara 2014; Mahmud et al. 2014).

Mangrove ecosystems are marginal ecosystems inhabiting the estuarine and intertidal regions or the interface between land and sea in both tropical and subtropical latitudes confined largely to regions between 30° north and south of the equator (Vannucci 2000; Das et al. 2014). There are approx. 1,59,041.5 km^2 of mangrove forests distributed in 123 countries and territories all over the world (Saranraj and Sujitha 2015). As reported by Das et al. (2014), there are 74 true mangrove plant species within 27 genera, which belong to 20 families dispersed through the world (Kathiresan and Bingham 2001; Ward et al. 2016). There are also an innumerable numbers of bacteria, fungus actinomycetes, mollusks, etc. that together enrich the biodiversity of the mangrove ecosystem. Mangrove forests occur in rough environmental conditions such as high salinity, high temperature, muddy anaerobic soils, extreme tides, and strong winds, which fluctuate violently

and frequently (Vannucci 2000). The capability of these organisms to resist the biotic or abiotic stresses and survival are attributed to their adaptability/tolerance toward a hostile ecological condition through alterations in physiological processes resulting from several novel bioactive products like hormones, antioxidants, secondary metabolites, resistant proteins, and sterols (Bandaranayake 2002; Edreva et al. 2008; Mangamuri et al. 2016; Sofia and Teresa 2016; Prasannan et al. 2016; Basha and Rao 2017). A number of bioactive compounds from mangrove plants have substantial pharmacological properties and are being used traditionally for medication against several health disorders and ailments (Edreva et al. 2008). The therapeutic extracts of roots, barks, leaves, fruits, and cell-free extracts of the microbes have been evaluated for various ethnomedicinal uses for the complete/partial treatment of malaria, filaria, inflammation, dysentery, diarrhea, cholera, lung infection, gastrointestinal infection, metabolic disorders, etc. (Sosovele et al. 2012; Gopal et al. 2016; Thatoi et al. 2016a; Mendhulkar et al. 2017). Despite the abundant contents of bioactive compounds with diversified therapeutic activities, mangrove species had not been efficiently explored to their maximum potential till date. However, the in vitro and in vitro anticancer activities of few mangrove plants have been evaluated, despite their extensive bioactive compound profiling (Huang et al. 2016).

Various plant extract-mediated nanoparticles have been proved to possess biological significance with antimicrobial, antioxidant, and anticancer properties (Swamy et al. 2015b; Swamy et al. 2015c; Akhtar et al. 2015; Rudramurthy et al. 2016). It has been postulated that conjugation of bioactive metabolites from mangrove sources with nanoparticles can boost the molecular properties and efficiency of the compounds as a promising therapeutic agent against cancer as well as other health issues. Considering the idea as true, the present chapter focuses on the anticancer activities of mangrove species, specifically phytocompounds and their bioconjugation with nanoparticles or nanocarriers as an advanced therapeutic agent. This comprehensive information will certainly benefit cancer biology researchers and pave a way for developing a smart and efficient therapeutics against different forms of cancer.

13.2 Distribution of Mangroves in the World

Mangrove plants are distributed in 123 tropical and subtropical countries around the world comprising of 74 true mangrove species including trees, shrubs, palms, and ferns. Higher percentages of the world's mangrove ecosystem can be found in Asian and African countries followed by America (South and Central). Sundarbans of India and Bangladesh covers the largest continuous mangrove forests consisting more than 0.14% of the geographical area of the country (Kathiresan 2010; Sarker et al. 2010). Mangroves are distributed in West and Central Africa, East and South Africa, Australia and New Zealand, South Asia, North and Central America, South America, Southeast Asia, the Pacific Ocean, the Middle East, and East Asia (Spalding et al. 2010).

In South and Southeast Asia, mangroves are found in Indonesia (60%), Malaysia (11%), Myanmar (8%), Papua New Guinea (8%), and Thailand (5%). Twenty-one species out of 35 mangrove plants in Southeast Asia are rare (*Sonneratia caseolaris*, *Sueda fruticosa*, *Urochondra setulosa*, etc.). In Western and Central Africa, 17 species of mangrove plants are found including *Avicennia marina*, *Rhizophora mucronata*, *Bruguiera gymnorrhiza*, *Ceriops tagal*, *Lumnitzera racemosa*, and *Rhizophora mucronata.* Forty-five mangrove species have been recorded from Australia and New Zealand (Webber et al. 2016). The North and South America comprising Florida and the Bahamas, Mexico, Puerto Rico, Eastern Venezuela, Trinidad, Guiana, and Brazil can be found with mangrove ecosystem. Brazil comprises 15% of the mangrove forest in this region. *R. mangle*, *Laguncularia racemosa*, *Avicennia germinans*, and *Conocarpus erectus* are common in Mexico. Mangrove forests can be seen in the Arabian Peninsula, Red Sea, and Gulf regions (Bahrain, Qatar, UAE, and Oman). Four species of mangrove plants such as *A. mariana*, *R. mucranta*, *Ceriops tagal*, and *B. gymnorrhiza* are observed in the Middle East area (Giri et al. 2011; Singh et al. 2012).

13.3 Diversity of Mangrove Species

Mangroves can be broadly categorized into two groups, i.e., exclusive/ major mangrove species (also called strict/obligate/true mangroves) and non-exclusive/minor/ associate mangrove species (Tomlinson 1986). The major species are the strict or true mangroves. The minor mangrove species are less conspicuous elements of the vegetation and rarely form pure stands which mostly involve different endophytes. Mangrove-associated microbes include bacteria (*Desulfovibrio*, *Desulfotomaculum*, *Desulfosarcina*, *Desulfococcus*, *Azotobacter*, *Staphylococcus*, and *Pseudomonas* species), fungi (*Aigialus striatispora*, *Calathella mangrovei*, *Eutypa bathurstensis*, *Falciformispora lignatilis*) and fungus-like protists (*Halophytophthora vesicula* and *H. spinosa*), microalgae (*Coscinodiscus*, *Rhizosolenia*, *Chaetoceros*, *Biddulphia*, *Pleurosigma*, *Ceratium*, and *Protoperidinium*), macroalgae (*Bostrychia*, *Caloglossa*, and *Catenella*), sea grasses (*Thalassia hemprichii*, *Enhalus acoroides*, and *Halophila ovalis*), salt marsh (*Spartina*), and other floras such as different epiphytes (Kathiresan 2010). In the tropical mangrove forests, there are approximately 100 epiphytic species from the Orchidaceae, Bromeliaceae, Cactaceae, Araceae, Piperaceae, and Polypodiaceae families scattered through the canopy and on the trunks of mangrove trees.

Mangrove-associated faunal species are zooplankton (the genera *Acartia*, *Acrocalanus*, *Macrosetella*, *Euterpina*, *Oithona*), sponges (Biemna *caribbean*, *Haliclona cuaçaoensis*, *Haliclona implexiformis*), ascidians (*Ecteinascidia turbinata*), epibenthos (*Vallentinia gabriellae*), infauna (*Notomastus lobatus*, *Halmyrapseudes spaansi*), meiofauna (*Parapinnanema ritae*, *P. alii*, *P. rhipsoides*), prawns (*Alpheus euphrosyne*, *A. microrhynchus*), shrimp (*Penaeus vannamei* and *P. monodon*), crabs (*Scylla serrata*, *Clibanarius laevimanus*), insects (*Mesovelia pol-*

hemusi, *Telmapsylla*, *Nasutitermes nigriceps*, *Apis dorsata*), mollusks (*Littoraria fasciata*, *Cerithidea mazatlanica*), fish (*Rivulus marmoratus*, *Cyprinodon*, *Centropomus undecimalis*), amphibians (*Rana*, *Bufo*, *Microhyla*, *Rhacophorus*), reptiles (*Crocodylus porosus*, *Varanus bengalensis*, *V. salvator* and *V. flavescens*, *Ophiophagus hannah*, *Vipera trimeresurus*), birds (*Ajala ajala*, *Cosmorodium albus*, *Eudocimus ruber*, *Pandion haliaetus*, *Sterna hirundo*, *Dendrocygna arborea*), and mammals (*Platanista gangetica*, *Macaca mulatta*, *Lutra perspicillata*, *Pteropus conspicillatus*, *Pteropus alecto*, *Cebus paella paella*, *Rhinoceros sondaicus*, *Bubalus bubalis*, *Cervus duvauceli*, *Axis porcinus*) (Kathiresan and Bingham 2001; Giri et al., 2011).

13.4 Medicinal Potential of Various Mangrove Plants

Mangroves represent a unique ecosystem with vast biological resources and immeasurable medicinal potential waiting to be revealed (Thatoi et al. 2016a, b). Now, how far they intend to venture into the enormous possibilities of never-ending medicinal potential of these mangrove plant species is a question for those intelligent and native thinkers. In this century, the time has already taken a turn and the race has already begun. Therefore, the sooner the search is made the better is the possibility of finding something that might forever shift the paradigm to the next level of human welfare. Presently, many of the biomedical potentials from mangrove plant species are slowly and steadily uncovered by creative thinkers from different corners of the world. Some of these medicinal prospectives include antibacterial, antifungal, antiviral, antidiabetic, and anti-inflammatory activities (Boopathy and Kathiresan 2010; Kathiresan 2010; Patra et al. 2011; Chakraborty and Raola 2016). An overview of such activities has been presented in this section with a special emphasis on the anticancer prospective.

13.4.1 Anticancer Potential of Mangrove Plants

Natural products of mangrove origin have long been investigated for their potential benefits since the folk era. By consistent transformation of trails and selection, mankind has learnt the significance of these plant juices and crude extracts as therapeutics for the treatment of various human disorders and aliments. During the 1900s, most medicines were obtained through cooking, infusion, or maceration of roots, barks, leaves, or flowers (Reddy and Grace 2016a). Currently, these natural products are considered to play a substantial role in the development of new drugs and therapeutics but are confined to traditional practices and uses. A database named Traditional Chinese Medicine contains 21,334 compounds derived from 2402 plants, of which about 5278 compounds have anticancer activity against highly potent cell lines. Moreover, the anatomization manifested that about 75% of these

5278 compounds are highly similar to either preclinical, clinical, and/or approved stages of anticancer drugs (Kathiresan and Manivannan 2008; Dai et al. 2016). Despite the irreplaceable and momentous potential of these bioactive phytocompounds, they are not exploited up to their maximum potential, and there is hardly any authenticate record/list/database on anticancer compounds from mangrove origin.

The search for anticancer compounds from mangrove plants is supposed to be an extensive research of the present curio, but the eye-catching results of the classical, chemically derived therapeutic agents have somewhat blinded the renewability of present research. Despite the seemingly irreversible side effects, chemical theranostics against cancer has considerably increased during recent times in comparison to natural therapeutic agents. Among all possible natural therapeutics, the use of mangrove species is least explored, although they possesses comparatively higher contents of heterogeneous bioactive compounds which is a unique characteristic of mangrove species (Boopathy and Kathiresan 2010; Debbab et al. 2010; Valli et al. 2012). However, with the advancement of technology and growing concern of side effects from drugs of chemical origin, a search for novel metabolites from terrestrial and especially mangroves has gained a considerable attention in recent times. In specific, an apprehension to cancer bioactive compounds contained in the *plant extract of Acanthus ilicifolius was shown to be effective in* preventing DNA alterations and significantly inhibited the proliferation of ascites tumor in animals and certainly improved the survival rate (Chakraborty et al. 2007). Likewise, Khajure and Rathod (2011) evaluated the cytotoxic potential of the ethanolic acetate extract of the same plant against KB and HeLa cell lines by comet assay. They found the results very promising with an increased inhibition of cancer cells. Later, Patil et al. (2011) also studied the cytotoxic activity but of a different plant species, *Excoecaria agallocha*. They evaluated the cytotoxicity of the ethanolic extract of stems against cancerous cell lines, namely, Capan-1 and Miapaca-2, and found the IC_{50} values of 4 μg/ ml and 7 μg/ml, respectively. A study by Uddin et al. (2012) isolated seven phytocompounds, namely, tetracosane, patriscabratine, quercetin3-O-β-d-glucosyl-(6→1)-α-l-rhamnoside, quercetin-3-O-β-d-glucoside, quercetin-3-O-α-lrhamnosyl-7-O-β-d-glucoside, quercetin-3-O-α-l-rhamnopyranoside, and kaempferol from the methanolic extract of the aerial parts of *Acrostichum aureum*, a mangrove fern. Further, cytotoxicity study using FITC Annexin V apoptosis assay revealed that these biocompounds possess potential to induce toxicity through apoptosis and necrosis against AGS gastric cancer cell lines. Thus, their research highlighted the possible use of this plant as a source of biocompounds and proved its traditional usage in treating peptic ulcer. Satapathy et al.(2013) screened various bioactive compounds and evaluated the antitumor activity of different parts of few mangrove plants, namely, *Phoenix paludosa* (leaf), *Avicennia alba* (leaf), *Heritiera fomes* (stem, leaf), *E. agallocha* (stem, bark, leaf), *Sonneratia apetala* (bark), and *Suaeda maritima* (stem, leaf) from Bhitarkanika natural reserve of Odisha, India. In another study by Smitha et al. (2014), the ethyl acetate extract of a mangrove plant *Acanthus ilicifolius* leaves and roots showed a significant cytotoxicity against two cancer cell lines MCF-7 and PA-1. Both leaf and root extracts recorded the highest inhibition of

MCF-7 and PA-1 cells at the concentration of 100 μg/mL. Likewise, Neumann et al. (2015) have reported that dolabrane-type of diterpenes tagalsins isolated from a mangrove suppressed tumor growths by reactive oxygen species-mediated apoptosis and cell cycle arrest. More recently, arbutin derivatives of *Heliciopsis lobata* plant showed a moderate cytotoxic effect on MGC-803 cells (Wei-Yan et al. 2016). Some of the mangrove plants with bioactive phytocompounds possessing anticancer activity and their structures are presented in Tables 13.1 and 13.2.

Table 13.1 Bioactive compounds isolated from mangrove plants with anticancer activities

Name of plant	Compound isolated	Activity against cell lines	References
Avicennia marina	Stenocarpoquinone B	K562 and HeLa cell lines	Han et al. (2007)
Xylocarpus granatum	Catechin, epicatechin	–	Das et al. (2014)
Cucumaria frondosa	Frondoside A	Urothelial carcinoma cells	Dyshlovoy et al. (2017)
A. germinans	3-chlorodeoxylapachol	K662 and HeLa cells	Mahmud et al. (2014)
	Xylomexicanin	Human breast carcinoma and KT cells	
	Gedunin	CaCo-2 colon cancer cell line	
Xylocarpus granatum	Photogedunin		
Sonneratia ovata	Sonnercerebroside, dehydroconiferyl alcohol, methoxydehydroconiferyl alcohol	AChE inhibition and cytotoxicity against HeLa, NCI-H460, MCF-7 cell lines, and PHF cells	Nguyen et al. (2015)
Soneratiia apetala	Mitomycin C	Cancer and diabetes	Patra et al. (2014)
Bruguiera gymnorrhiza	–	HepG2 cell line	Reddy and Grace (2016a)
Aegiceras corniculatum			
Aegialitis rotundifolia			
Lumnitzera racemosa			
A. marina	–	MCF-7 cell line	Reddy and Grace (2016b)
A. officinalis			
Calophyllum inophyllum			
B. gymnorrhiza			
A. corniculatum			
Phoenix paludosa	–	High toxicity against MCF-7, MDA-MB-231, SK-BR-3, and ACHN cell lines	Samarakoon et al. (2016a)
		Less cytotoxic against normal cell lines HEK-293, MCF-10A	

(continued)

Table 13.1 (continued)

Name of plant	Compound isolated	Activity against cell lines	References
Aegiceras corniculatum	–	MCF-7, and HepG2 cell lines	Samarakoon et al. (2016b)
Avicennia officinalis			
Bruguiera gymnorrhiza			
Excoecaria indica			
Heritiera littoralis			
Lumnitzera littorea			
L. racemosa			
Nypa fruticans			
Pemphis acidula			
Phoenix paludosa			
Rhizophora apiculata			
R. mucronata			
Scyphiphora			
Hydrophyllacea			
Sonneratia alba			
S. caseolaris			
Acanthus ilicifolius	Methylapigenin 7-o-β-D-glucuronate-flavone glycosides	MCF-7 and PA-1 cell lines	Singh and Aeri (2013), Smitha et al. (2014)
Catharanthus roseus	Vincristine, vinblastine	Hodgkin's disease, choriocarcinoma cells	Sain and Sharma (2013)
Acrostichum aureum	Tetracosane	HT9 colon cancer, estrogen-dependent breast cancer (MDA-MB-231) cells, and gastric cancer cells	Uddin et al. (2011)
	Quercetin-3-*O*-βd-glucoside,quercetin-3-O-βd-glucosyl-(6 → 1)-α-l-rhamnoside, quercetin-3-*O*-α-l-rhamnoside, quercetin-3-*O*-αl-rhamnosyl-7-O-β-$_d$-glucoside (2S,3S)-sulfated Pterosin C	Moderately cytotoxic against AGS, MDA-MB-231, and MCF-7 cells	Uddin et al. (2012)
	Kaempfero, patriscabratine	Gastric cancer (AGS) cells	Uddin et al. (2013)
Heliciopsis lobata	6-[(E)-2methoxy 5cinnamoyl] arbutin1, 2-[(E)-25dihydroxy-cinnamoyl] arbutin2	Cancer and MGC-803 cells	Wei-Yan et al. (2016)

(continued)

Table 13.1 (continued)

Name of plant	Compound isolated	Activity against cell lines	References
X. granatum	Xylogranatins A, B, C, D	–	Yin et al. (2006)
	Granaxylocarpins A, B	P-388 leukemia cells	Yin et al. (2007)
Ceriops tagal	Tagalsins B, C, D, E, F, G, H, W, 9, 10	Hematologic cancer (human T-cell leukemia), HCT-8, Bel-7402, BGC-823, A549, and A2780 cell lines	Yang et al. (2015)
X. granatum	Xylogranatumine A–F	A549 tumor cell	Zhou et al. (2014)

Table 13.2 Some anticancer compounds isolated from selected mangrove plants

Plants	Isolated compounds	References
Cerbera odollam	2′-o-acetyl cerleaside A, 17b-neriifolin, cerberin	Chan et al. (2016)
Xylocarpus granatum	Photogedunin, catechin, epicatechin, procyanidins	Das et al. (2014)
Cucumaria frondosa	Frondoside A	Dyshlovoy et al. (2017)
Avicennia marina	Stenocarpoquinone B	Han et al. (2007)
Sonneratia ovata	Sonnercerebroside, (7S,8R) dehydroconiferyl alcohol	Nguyen et al. (2015)
Avicennia germinans	3-chlorodeoxylapacho, xylomexicanin, gedunin	Mahmud et al. (2014)
Acrostichum aureum	Quercetin-3-O-αl-rhamnosyl-7-O-β-d-glucoside, Tetracosane, Quercetin-3-*O*-βd-glucoside, Quercetin-3-*O*-β-d-glucosyl-(6 → 1)-α-l-rhamnoside, Quercetin-3-*O*-α-l-rhamnoside, kaempferol	Uddin et al. (2012)
Heliciopsis lobata	6′-[(E)-2″, methoxy, 5″ cinnamoyl] arbutin 1, 2′-[(E)-2″, 5″ dihydroxy-cinnamoyl] arbutin 2	Wei-Yan et al. (2016)
Ceriops tagal	Tagalsins A, B, C, D, E, F, G, and H, tagalsins W, tagalsins 9 and 10	Yang et al. (2015)
Xylocarpus granatum	Xylogranatins A, B, C, D	Yin et al. (2006)
Xylocarpus granatum	Granaxylocarpins A, B	Yin et al. (2007)
Xylocarpus granatum	Xylogranatumine A–F	Zhou et al. (2014)

13.4.1.1 Endophytes from Mangrove Plants and Their Anticancer Activity

Endophytes, mostly fungi, actinomycetes, and bacteria, are organisms that live in harmony within the intercellular spaces of plant tissue with no apparent damage to their host. In another way, it can be said that they live in a symbiotic relationship with their host plants. They epitomize a vast variety of microbial adaptations that have been developed in the special or remote environment. Plant-host interaction requires persistent and extended reactions against the defense mechanisms of the host by the endophyte (Ariole and Akinduyite 2016). The secondary metabolites produced by microorganisms in general and endophytic microorganisms in specific have been investigated and explored for various industrial purposes including pharmacological and clinical applications including anticancer therapeutics. An introduction to some of these endophytes and their anticancer activities are described below.

Streptocarbazoles A and B, two novel indolocarbazoles with an unknown feature of cyclic N-glycosidic linkages between 1,3-carbon atoms of the glycosyl moiety and two indole nitrogen atoms of the indolocarbazole core, were isolated from the marine-derived actinomycetes strain *Streptomyces* spp. which is sometimes also found in endophytic form in mangrove plants. Fu et al. (2012) found that streptocarbazole A possesses cytotoxicity against HL-60 and A-549 cell lines and could arrest the cell cycle of HeLa cells at the G2/M phase. Divergolide D, isolated from *Streptomyces* spp. HKI0576 associated with *Bruguiera gymnorrhiza*, a mangrove plant from China, was found to exhibit a significant antitumor activity against pancreatic cancer PANC-1, lung cancer LXFA 629 L, sarcoma SAOS-2, and renal cancer RXK 486 L cell lines (Xu et al. 2014). Lam et al. (2014) isolated 52 endophytic actinomycetes from 3 different species of mangrove trees, namely, *Sonneratia caseolaris*, *S. paracaseolaris*, and *Lumnitzera racemosa*, in Nam Dinh Province, Vietnam. Among them, only two strains (2E20 and 2E29) showed both antifungal and root growth inhibition activities and exhibited anticancer activity against cancer cell lines KB, SK-LU-1, HepG2, and MCF7. Wang et al. (2012) reported the cytotoxic effect of polyphenols obtained from *Penicillium expansum* 091006 associated with *Excoecaria agallocha*, a mangrove plant. Endophytic bacteria also contribute to several physiological beneficial functions of the host plants such as plant growth promotion and increased resistance against pathogens and parasites. In general, endophytic bacteria include both Gram-positive and Gram-negative bacteria that have been isolated from different plant species (Arunachalam and Gayathri 2010). There are a number of endophytic bacteria such as *Bacillus* sp., *Staphylococcus* sp., *Sporosarcina* sp., *Pseudomonas* sp., *Serratia* sp., *Stenotrophomonas*, *Micromonospora* sp., and many others that contain diverse bioactive compounds with pharmaceutical significance (Eldeen and Effendy 2013). Several sesquiterpenoids were isolated from the mangrove origin fungus, *Diaporthe* sp. (Zang et al. 2012).

SZ-685C is a natural, biologically active substance isolated from the secondary metabolites of the mangrove endophytic fungus, *Halorosellinia* spp., collected from

the South China Sea. It is an anthraquinone and has a high potency against six different cancer cell lines derived from human breast cancer (Xie et al. 2010; Hasan et al. 2015). Cultivation of *Acremonium* sp., a fungal isolate, produced two novel hydroquinone derivatives, namely, 7-isopropenyl bicyclo[4.2.0]octa-1, 3, 5-triene-2, 5-diol-5-β-d-glucopyranoside and 7-isopropenyl bicyclo[4.2.0]octa-1,3,5-triene-2, 5-diol (Abdel-Lateff et al. 2002). An endophyte (*Talaromyces flavus*) from the mangrove plant *Sonneratia apetala* contained cytotoxic norsesquiterpene peroxides (Li et al. 2011). *Penicillium chrysogenum* isolated from the mangrove plant *Acanthus ilicifolius* contained new chitin analogues A–C (1–3) and one new xanthone derivative. The penicitol A–C and penixan acid A were reported to show anticancer activity against HeLa, BEL-7402, HEK-293, HCT-116, and A549 cell lines (Wenqiang et al. 2015). Likewise, *Sonneratia ovata* Backer (Sonneratiaceae) is another widely distributed plant species in the mangrove forests of Cambodia, Vietnam, Thailand, and Indonesia. From these plant leaves, Nguyen et al. (2015) structurally elucidated the following chemical compounds such as sonnerphenolic A, sonnerphenolic B, and sonnerphenolic C, sonnercerebroside (cerebroside), lignans, steroids, triterpenoids, gallic acid derivatives, phenolic derivatives, and 1-O-benzyl-β-d-glucopyranose. Some of these isolated compounds inhibited acetylcholinesterase (AChE) activity and exhibited activity against NCI-H460 (human lung cancer), HeLa (human epithelial carcinoma), PHF (primary human fibroblast), and MCF-7 (human breast cancer) cell lines at 100 μg/mL concentration. *Pseudolagarobasidium acaciicola* isolated from *Bruguiera gymnorrhiza*, another mangrove plant, also contained 20 unknown compounds and 2 known metabolites (Merulin A and Merulin D). The compound terpene endoperoxide exhibited greater anticancer activity against the promyelocytic leukemia cell line, HL-60 (Wibowo et al. 2016). *Pestalotiopsis microspora* is another mangrove-derived endophytic fungus which contains 7 new 14-membered macrolides, pestalotioprolides C, D–H and 7-O-methylnigrosporolide, together with four known analogues, pestalotioprolide B, seiricuprolide, nigrosporolide, and 4,7-dihydroxy-13-tetradeca-2,5,8-trienolide. Some of these metabolites have shown anticancer property against murine lymphoma cell line and human ovarian cancer cell line, A2780 (Liu et al. 2016). *Streptomyces cheonanensis* VUK-A is also a mangrove-derived fungal endophyte and contains two metabolites, namely, 2-methyl butyl propyl phthalate and diethyl phthalate, the former showed cytotoxicity against MDA-MB-231, OAW-42, HeLa, and MCF-7 cell lines (Mangamuri et al. 2016). *Pestalotiopsis neglecta* (endophyte), isolated from the mangrove species *Cupressus torulosa*, was reported to possess cytotoxic activity against human embryonic kidney (HEK) cell lines (Sharma et al. 2016). Further, GC-MS analysis of the methanol extract of the species revealed the presence of different cytotoxic and antiproliferative compounds such as nonadecane and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6- methyl, which paves the way for future use as anticancer agents.

13.4.1.2 Anticancer Potential of Other Sources Found in Mangrove Ecosystem

Non-endophytic bacteria, fungi, actinomycetes, and algae found in the mangrove ecosystem produce various secondary metabolites and can be a great source of anticancer drugs. Some studies have been documented with the prevention of carcinogenesis by the potent chemicals produced from the marine flora. However, secondary metabolite of marine flora for the treatment of cancer is still not fully utilized in comparison to terrestrial habitats. Many researches have been carried out on marine flora, and their novel chemicals have shown their potentiality to be employed in finding drugs with greater efficacy for the treatment of cancer. Some of these chemical compounds are depicted in Table 13.3.

13.4.1.2.1 Bacteria

Bacterial population in the fertile mangrove water is higher than fungi. Marine bacteria produce secondary metabolites such as antibiotics (e.g., marinone), enzymes (arylsulphatase, L-glutaminase, chitinase, L-asparaginase, cellulase, protease, phosphatase), and some of the novel anticancer compounds such as bryostatins and discodermolide (Manivasagan et al. 2014).

13.4.1.2.2 Actinomycetes

Streptomyces, *Micromonospora*, *Jishengella*, and *Salinispora* are important genera of actinomycetes found in mangrove ecosystems. Indole and alkaloids including indolosesquiterpenes, indolocarbazoles, macrolides, and benzene derivatives are the main natural products of the genus *Streptomyces*. They all have been proven to be valuable sources of potentially useful bioactive metabolites for the treatment of cancer (Fu et al. 2012; Yuan et al. 2013; Dong et al. 2014; Tan et al. 2015).

13.4.1.2.3 Fungi

Mangrove consists of endophytic fungi *Halorosellinia* sp., *Guignardia* sp., and *Phomopsis* sp. and produces potent chemical derivatives which have been investigated for anticancer activity (Chen et al. 2009; Tao et al. 2010; Thatoi et al. 2013) and antifungal activities (Huang et al. 2008; Thatoi et al. 2013). Likewise, the chemicals produced by mangrove foliar fungi have been also been studied extensively for their potentiality to be utilized as anticancer drugs.

Table 13.3 Some of the marine floral derivatives and their anticancer activities

Marine flora	Chemical compounds	Biological activity	References
Actinomycetes			
Streptomyces spp.	Indolocarbazoles, streptocarbazoles A and B	Antitumor activity	Fu et al. (2012)
Streptomyces spp.	7 azlomycin F analogues, macrocyclic lactones	Antibiotic, anticancer	Yuan et al. (2013)
Streptomyces spp.	Pyrrolopyrazine, carboline and dicarboxylic acid ester	Anticancer activity	Tan et al. (2015)
Fungal flora			
Hypocrea lixii, *Irpex hydnoides*	Crude extract	Anticancer activity and cytotoxic effect	Bhimb et al. (2011)
Pestalotiopsis microspora	Crude extract	Antimicrobial and anticancer activity	Joel and Bhimba (2012)
Algal			
Stylopodium sp.	Stypoldione	Cytotoxic	Gerwick and Fenical (1981)
Cystophora sp.	Meroterpenes, usneoidone	Antitumor	Urones et al. (1992) and Boopathy and Kathiresan (2010)
Nostoc linckia, *N. spongiaeforme*	Borophycin	Cytotoxicity against human epidermoid carcinoma (LoVo) and human colorectal adenocarcinoma activity	Banker and Carmeli (1998) and Vijayakumar and Menakha (2015)
Nostoc sp.	Cyptophycin 1	Cytotoxicity against human tumor cell lines and human solid tumors	Moore (1996) and Boopathy and Kathiresan (2010)
Lyngbya boulloni	Apratoxin A	Cytotoxicity to adenocarcinoma	Luesch et al. (2001)
Stigonema sp.	Scytonemin	Antiproliferative	Stevenson et al. (2002)
Leptolyngbya sp.	Coibamide A	Cytotoxicity effect against adenocarcinoma and NCIH460 lung and mouse neuro-2a cells	Medina et al. (2008)

13.4.1.2.4 Algae Growing in Mangrove

Mangrove algae are the highest contributor among the marine flora to produce anticancer compounds. Cyanobacteria produce many bioactive compounds (toxins) which can be applied in pharmaceuticals (Jha and Zi-Rong 2004; Thajuddin 2005; Uddin et al. 2011). Scytonemin, apratoxin, cryptophycin, stypoldione, coibamide A, largazole, fucoidan, etc. are produced by mangrove algae which have been investigated for their anticancer activity.

13.4.2 Other Potential Activities of the Mangrove Species

13.4.2.1 Antibacterial Activity

Different research studies on mangrove plants have highlighted their use as an important source of antimicrobial drugs. *Suaeda maritima* is a mangrove species widely distributed on the landward margin of mangrove ecosystem across different ranges and has been reported to exhibit antimicrobial property against several pathogenic microbial strains, namely, *Shigella flexneri*, *Bacillus brevis*, *B. subtilis*, *B. licheniformis*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, and *Streptococcus aureus* (Patra et al. 2011). *Peltophorum pterocarpum* is another native mangrove species of Sri Lanka, the Andaman's, the Malay Bawong, and Malaysia whose leaves extract contains different phytochemicals and possess potent antimicrobial activity against *P. aeruginosa, S. aureus, B. cereus, Escherichia coli, Klebsiella pneumonia, Salmonella typhi, Serratia marsecens, Acinetobacter baumannii, Enterobacter* sp., *Proteus mirabilis,* and *Enterococcus* sp., (Sukumaran et al. 2011). Likewise, Saad et al. (2011) reported the antimicrobial properties of *Lumnitzera littorea* against various pathogenic microbes. *Avicennia officinalis*, a species of mangrove plants occurring at Parangipettai, Chidambaram district, also showed antimicrobial activity against the pathogenic bacterium, *B. megaterium* (Valentin et al. 2012). *Lumnitzera littorea* is another mangrove plant distributed widely in the east coast of Africa, Southeast Asia, and Australia and is known to possess antimicrobial potential against certain pathogenic bacteria like *B. cereus*, *P. aeruginosa*, and *Cryptococcus neoformans* (Saad et al. 2011). *Sonneratia apetala*, a mangrove plant extensively found in the Bhitarkanika Sanctuary along the Odisha Coastline, also possessed antibacterial activity against infectious bacteria such as *S. aureus*, *S. flexneri*, *B. licheniformi*, *B. brevis*, *Vibrio cholera*, *P. aeruginosa*, *S. epidermidis*, *B. subtilis*, and *E. coli* (Patra et al. 2014).

13.4.2.2 Antifungal Activity

Rhizophora mangle L. (*Rhizophoraceae*), a red mangrove plant widely distributed along the tropical and subtropical coasts of America from Bermuda to Florida, Occidental Africa, and the islands of Fiji, Tonga, and New Caledonia of the lower swampy zones, possesses significant antifungal properties. In traditional medicine, *Rhizophora mangle* has been used as an antifungal and antiulcer agent (Berenguer et al. 2006). The bark extract of this species had antifungal activity against a pathogenic fungus *Fusarium oxysporum* (Simlai and Roy 2013). *A. schaueriana* extensively distributed throughout the Brazilian Atlantic Forest possesses secondary metabolites such as lapachol, α-lapachone, naphtho [2,3-*b*] furan-4,9-dione, 2-isopropyl, and avicenol-C which have been evaluated and found to be active against another fungus, *Colletotrichum gloeosporioides* (Fardin et al. 2015).

13.4.2.3 Antiviral Activity

Natural herbs are one of the ancient sources for antiviral substances. The antiviral substances extracted from mangrove plants show high efficacy, low toxicity, and minor side effects. *Peltophorum pterocarpum* is a native mangrove species of Sri Lanka, the Andaman, and the Malay Bawong, Malaysia. The bark of this species contains various phytochemicals that are effective against many disease-causing viruses (Sukumaran et al. 2011). *Excoecaria agallocha* distributed across the coast regions of South China contains polyphenolic compounds, namely, excoecariphenols A–D (1–4), known to have anti-hepatitis C virus (HCV) activity (Jia et al. 2009). Similarly, the compound 2″-(methoxycarbonyl)-5″- methylpentyl 2′-methylhexyl phthalate (a novel phthalic acid ester) isolated from *A. aureum* aerial parts growing in the Bangladesh mangrove region exhibited in vitro antiviral activity against human parainfluenza virus, dengue virus, and chikungunya (Uddin et al. 2013). The mangrove plant, *Xylocarpus moluccensis*, found in the Tang forests of China contains eight new khayanolides, named as thaixylomolins G–N (1–8), and two new phragmalins (9 and 10). These compounds have a strong inhibitory potential against disease-causing virus, influenza A (Li et al. 2015). *Ceriops tagal*, another mangrove species spread along the costal belt of Africa, the South Pacific islands, and South Asia including China possessing bioactive compounds belonging to different chemical classes (alkaloids, flavonoids, and polyphenols), has been proved to be effective against white spot syndrome virus (WSSV) (Sudheer et al. 2012). Likewise, *Aegiceras corniculatum* found near Xiamen City of Fujian Province possesses four compounds and is observed to be active against HCV protease and SecA and ATPase as well as VSVG/HIV-luc pseudotyping virus (Xu et al. 2014).

13.4.2.4 Antidiabetic Activity

Several mangrove plant species have been discovered with antidiabetic properties including *Ceriops decandra* which is a traditional folk remedy for diseases like angina, diabetes, diarrhea, dysentery, hematuria, and hemorrhage. The plant extract from *C. decandra* has high potential against diabetes mellitus (Alikunhi et al. 2010). Three mangrove plants of *Rhizophora* species (*R. apiculata*, *R. mucronate*, and *R. annamalayana*) contain insulin-like antigen and exhibit antidiabetic potential against alloxan-induced diabetic in animal model (Alikunhi et al. 2011). *Nypa fruticans* Wurmb. (Arecaceae) is well-known for its traditional uses by the local practitioners against different ailments in southern regions of Bangladesh. Studies had revealed that *N. fruticans* extract possesses antihyperglycemic potential in glucose loaded in an animal model (Reza et al. 2011). *Pongamia pinnata* (L), *P. glabra* Vent., and *Millettia pinnata* (L) are widely distributed throughout the mangrove areas in the southern part of Thailand and contain pyranoflavonoids useful in the treatment of diabetes (Anusri et al. 2014).

13.4.2.5 Anti-inflammatory Activity

Certain mangrove plants have also been found to contain phytocompounds with anti-inflammatory activity. The methanolic extract of *Rhizophora apiculata*, a common mangrove plant found in Asia and Africa, when evaluated for its anti-inflammatory and antitumor activity against B16F10 melanoma cells in BALB/c, mice showed high anti-inflammatory activity (Prabhu and Guruvayoorappan 2012). Likewise *Acanthus ilicifolius* (Acanthaceae), a local inhabitant of the Sundarbans, India, is used to treat a variety of diseases. The methanolic extract of *Acanthus ilicifolius* leaves (MEAL) possesses significant anti-inflammatory properties against gastric ulcer and duck hepatitis B (Mani et al. 2012; Wei et al. 2015). The bark extracts of *Rhizophora mangle* contain various polyphenolic compounds, including tannins and other metabolites (e.g., epigallocatechin-3-gallate, procyanidins), which have anti-inflammatory effects against gastric ulcer (De-Faria et al. 2012). *Rhizophora mucronata* is a true mangrove plant extensively distributed along the coastal region of India and is rich in terpenoids with a strong potential against inflammation (Chakraborty and Raola 2016).

13.4.2.6 Antioxidant Activity

Streptomyces lincolnensis M-20 cell extract was proven to contain an antioxidant agent, protocatechualdehyde (Kim et al. 2008). This compound exhibited a potent antioxidant activity by scavenging the free radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH). In another study, Ser et al. (2015) isolated a new *Streptomyces* strain MUSC 149 T from the soil of mangrove forest located in Tanjung Lumpur, Peninsular Malaysia. *Streptomyces* MUSC 149 T-cell extract showed a dose-respondent DPPH free radical scavenging activity. The lowest activity (1.1 ± 1.4%) was found when 0.125 mg/ml of cell extract was used, while the highest activity (36.5 ± 3.0%) was observed with the use of 2.0 mg/ml. Further, chemical analysis of the extract revealed the occurrence of antioxidant agent(s) in the cell extract. The identified compounds included hexadecane, butanoic acid, 2-methyl-,benzoic acid, 3-methyl- (3R,8aS)-3-methyl-1,2,3,4,6,7,8,8a-octahydropyrrolo[1,2-a]pyrazine-1,4-dione, and Pyrrolo[1,2-a]pyrazine-1,4-dione,hexahydro.

13.5 Conclusion and Future Prospects

The past information on various uses of plants, microbes, and any other biological or chemical substances is the basis for the current practice of novel drug discovery. However, it is often the future that ignites and sets forth the path of the present advancements which leaves behind an illustrious past and a momentous future. The illusion of nanotechnology changes and revolutionizes the course of medicinal sciences, and drug discovery is based on the scientific exploration of a noble smart

seminatural therapeutic for the treatment of diseases like cancer. It is not that the current anticancer theranostic phytocompounds are incompetent in achieving the present appreciable goal, but rather the target unspecific delivery and low availability and dispersity are some of the huddles that need much attention. The remedy for these problems could be brought about by the application of nanotechnology. A wide range of nanocarriers are being used for the treatments of various types of cancer such as phytosynthesized silver and zinc nanoparticles by using *Heritiera fomes* and *Sonneratia apetala* mangrove plant aqueous extracts as a reducing agents. These biosynthesized nanoparticles also possessed strong pharmacological activities including antioxidant, antidiabetic, anti-inflammatory, and antimicrobial potentials. Thus, these nanoparticles could be very useful for various biomedical applications. The use of synthetic therapeutics with anticancer activity can alternatively replaced by the use of natural bioactive compounds conjugated to nanocarriers, which is both effective and show negligible side effects. In the coming decade advanced medication can be formulated using phytocompounds of mangrove origin as nanotherapeutics against cancer. Likewise, there are polymeric and other inorganic nanocarriers that have the capacity to carry anticancer phytocompounds of mangrove origin, but still the research is limited. Thus, various neglected species of mangroves can be a new source of natural compounds which could be successfully used for treating numerous human ailments. However, more emphasis should be given to explore these natural sources for the discovery of novel therapeutics against cancer. Despite the awareness, if still the diversifying serviceability of mangrove species is not put to their explicit use, then the incomprehensible credibility of their anticancer activity would continue to rest in its own shadow for ages.

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Chapter 14
Piper betle Linn. in Cancer: Past, Present, and Future

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14.1 Introduction

Betel vine (*Piper betle* Linn.) belonging to the family *Piperaceae* is a major remedial plant grown in Southeast Asia. The plant is a native of Malaysia and its surrounding regions and is presently cultivated on a large scale in various parts of India, Nepal, Bangladesh, Burma, and Sri Lanka (Guha 2006; Kumar et al. 2010; Annamalai et al. 2016). The leaves of this plant are the vital parts having immense therapeutic values. They are also used in a number of religious celebrations and ceremonies all across Southeast Asia. In India, guests are offered with betel leaves, commonly referred as 'tambool' as a mark of respect and used in several traditional and religious events (Warrier et al. 1995; Annamalai et al. 2016). The betel vine is a perennial creeper and bears leaves that are 4–7 in. long and 2–4 in. broad. The plant is raised by vegetative propagation method in shade areas supported on large bamboo sticks or on trees such as coconut or areca nut. The betel leaf is marketed mainly based on its size and colour. The betel vine has been referred as 'green gold of India' as over 20 million people are known to derive their livelihood by growing and marketing betel leaves in India (Janes et al. 2014). The betel leaf can be segregated into various varieties based on the leaf size, colour, taste, and fragrance. Some of the commonly cultivated varieties of betel vine in India are the Mysore, Banarasi, Salem, Magadhi, Kauri, Calcutta, Bagerhati, and Ghanagete (Satyavati et al. 1987; Warrier et al. 1995; Rai et al. 2011).

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Due to various traditional importance, betel vine has been widely investigated for its phytochemical constituents. The leaf of betel vine contains an array of biologically active compounds whose composition and concentration depend on the variety, season, and geographical area grown. The spicy odour of betel leaf is attributed to the occurrence of phenols and terpenes in its essential oils. Various phytochemicals found in betel plants include carvacrol, chavicol, chavibetol, hydroxychavicol, eugenol, eugenol methyl ether, methyl eugenol, eucalyptol, estragole, allyl catechol, 4-hydroxycatechol, cadinene, β-caryophyllene, γ-lactone, *p*-cymene, cepharadione A, dotriacontanoic acid, tritriacontane, α-terpinene,α-terpineol-2-3, dotriacontanoic acid, pentatriacontane, hentriacontane, stearic acid, triotnacontane, n-triacontanol, piperlonguminine, allylpyrocatechol diacetate, isoeugenol, α-pinene, β-pinene, 1, 8-cineol, β-sitosterol, γ-sitosterol, stigmasterol, palmitate, ursolic acid, and ursolic acid 3-acetate (Kumar et al. 2010; Gundala and Aneja 2014; Das et al. 2016). Some important phytochemicals are depicted in Fig. 14.1, and the pharmacological actions of well-studied molecules are enlisted in Table 14.1.

Ayurveda, a traditional Indian system of medicine for time immemorial, has been using betel leaf in preparation of concoction for treating indigestion, cough, cold, bronchitis, and asthma. Also various folk medicines in South Asia document use of betel vine leaf to treat ailments. Chewing betel leaf has been a traditional method for consumption and believed to prevent bad breath (halitosis), improve vocalization, strengthen the gum, conserve the teeth, and sweeten breath. The essential oils of betel leaves have been used for treating catarrhal inflammation and as a disinfectant (Satyavati et al. 1987). Several studies have demonstrated the pharmacological properties of betel vine using both in vitro and in vivo models. While most studies have validated the traditional use, however, few reports have been on exploratory lines. Preclinical experiments have demonstrated that betel leaf possesses antimicrobial, anticariogenic, antilarval, antiprotozoal, antiallergic, antidiabetic, anti-inflammatory, hepatoprotective, antiulcer, cardioprotective, antihyperlipidaemic, antiplatelet, vasorelaxation, and immunomodulatory effects (Kumar et al. 2010, Gundala and Aneja 2014). In spite of all the beneficial effects, betel leaves are infamous and are credited to be a cause of cancer. This is principally because the leaves are a component of the betel quid that also contains the tobacco and areca nut (both of which are proved to be carcinogens) (Rai et al. 2011).

Today, cancer remains to be one of the major death-causing multidiseases globally, and deaths due to cancer are projected to increase drastically to 16 million by the year 2025 (Ferlay et al. 2010; Jemal et al. 2011; Torre et al. 2015). Treatments such as surgery, chemotherapy, and radiotherapy are the current practice of cancer therapy. As cancer cells lose their regulatory mechanisms as found in normal cells, hence, they divide uncontrollably and can spread to other parts of the body. Due to this property, chemotherapeutic drugs fail to cure cancerous cells. From the past decades, several systemic drug discoveries have resulted in large collection of chemotherapeutic drugs. However, these chemical drugs own several intrinsic problems including toxicity and severe adverse effects during the chemotherapy (Desai et al. 2008; Torre et al. 2015). Currently, plant-derived compounds such as vinblastine, vincristine, vindesine, etoposide, teniposide, paclitaxel, docetaxel, and

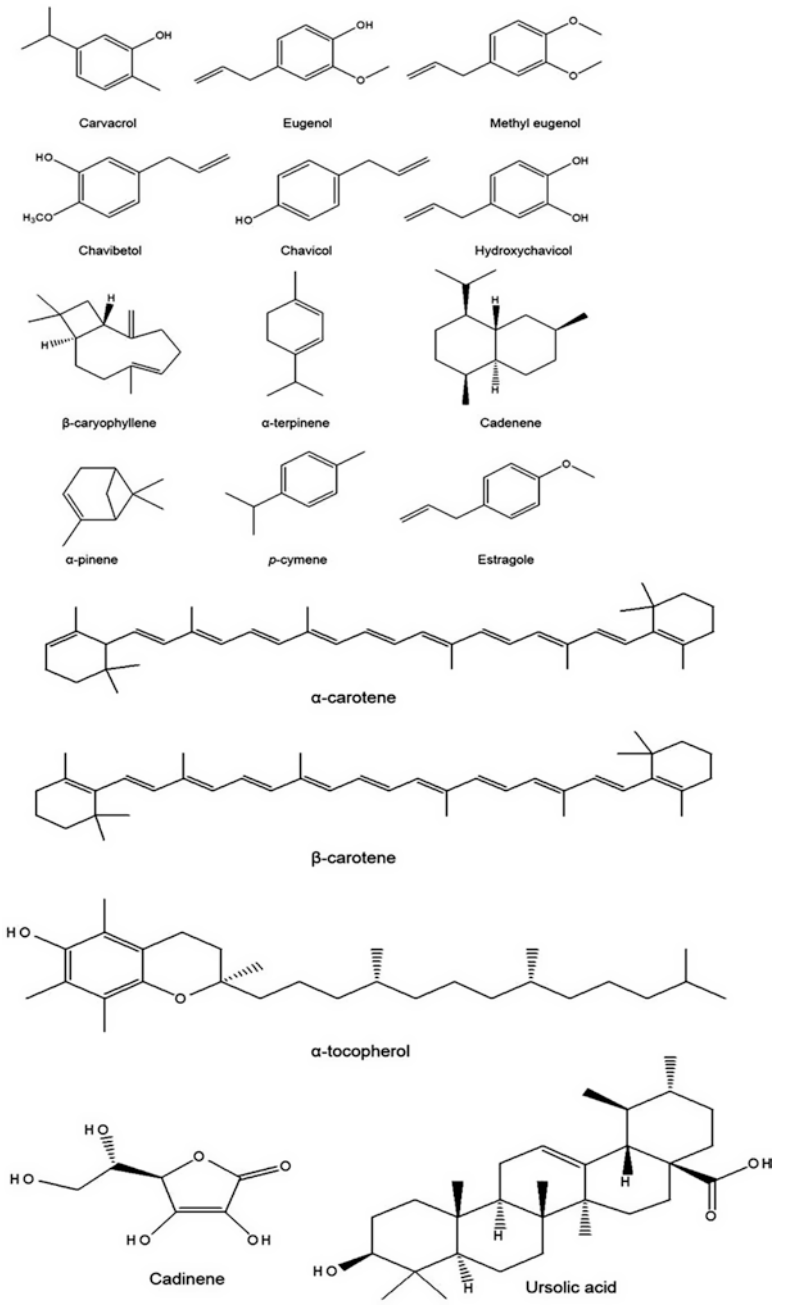

Fig. 14.1 Structures of major chemical constituents of *Piper betle*

Table 14.1 Chemical compounds of *Piper betle* and their pharmacological actions

Chemical compounds	Molecular formula	Pharmacological actions
1,8-cineol	$C_{10}H_{18}O$	Treatment of inflammatory diseases
α-cadinene	$C_{15}H_{24}$	Anticancer activity
α-humulene	$C_{15}H_{24}$	Anti-inflammatory, effective in reducing platelet-activating factor
Allylpyrocatechol diacetate	$C_{13}H_{14}O_4$	Antimicrobial activity against various obligate oral anaerobes
Aromadendrene	$C_{15}H_{24}$	Antioxidant and anti-ageing
β-elemene	$C_{15}H_{24}$	Anti-proliferative effect, used in chemotherapy for cancer treatment
β-selinene	$C_{15}H_{24}$	Antibacterial properties
Caryophyllene	$C_{15}H_{24}$	Antioxidant, anti-inflammatory, anticancer, and local anaesthetic
Eugenol	$C_{10}H_{12}O_2$	Antiseptic and local anaesthetic, in dentistry
Eugenyl acetate	$C_{12}H_{14}O_3$	Anti-virulence potential
Germacrene D	$C_{15}H_{24}$	Analgesic and anti-inflammatory properties
Globulol	$C_{15}H_{26}O$	Antimicrobial activity
Hydroxychavicol	$C_9H_{10}O_2$	Antimutagenic activity
Sabinene	$C_{10}H_{16}$	Antimicrobial activity
α-Pinene	$C_{10}H_{16}$	Anti-inflammatory and antimicrobial

camptothecin and their derivatives are the major and effective anticancer agents available in the market. Medicinal plants still remain as a natural reservoir and provide opportunity to explore new drug molecules with chemoprotective activity against cancer. Several medicinal plants with a number of bioactive compounds have been suggested for having potential anticancer activities (Taneja and Qazi 2007; Arumugam et al. 2016; Mohanty et al. 2017; Tariq et al. 2017). As natural products exhibit higher effectiveness with lower side effects, they are highly appreciated in recent times (Lachenmayer et al. 2010; Kooti et al. 2017). In addition, medicinal plants constitute multiple phytocompounds and, thus, allow to explore such compounds for the discovery of novel and key anticancer agents (Newman and Cragg 2007; Swamy and Sinniah 2016; Kooti et al. 2017). The aim of the chapter is to provide the information on betel vine's cancer preventive effects and the mechanisms involved. Moreover, it also highlighted the various phytoconstituents that promote beneficial role of betel vine leaves against different cancers.

14.2 Betel Quid and Cancer

It has been a common belief that betel causes oral cancer, if consumed regularly. This debatable accreditation is mainly because of the fact that betel leaves are generally chewed in the form of 'betel quid' that comprises areca nut (*Areca catechu*), betel

leaf, and slaked lime. Often, betel quid is consumed along with tobacco (*Nicotiana tabacum*) which is known to cause oral cancer (Brunnemann and Hoffmann 1992; IARC 2004). A number of studies have been carried out with the individual constituents of betel quid, and results have conclusively shown that both tobacco and areca nut are carcinogenic (Canniff and Harvey 1981; Sundqvist et al. 1989; Jin et al. 1996; Wang and Peng 1996; Jeng et al. 2000; Wang et al. 2003; IARC 2004; Lee et al. 2005; Boffetta et al. 2008) and slaked lime promotes carcinogenesis (Thomas and MacLennan 1992; Jeng et al. 1994). In contrast, it has been proven scientifically that betel leaf is devoid of mutagenic and carcinogenic effects. For the first time, Bhide et al. (1979) showed that aqueous extract of betel leaves fails to cause tumours in both Swiss and C17 mice, thereby proving that betel leaf was not carcinogenic. Further, various scientific findings have conclusively shown that betel vine leaves and its phytoconstituents prevent chemical-induced cancers in experimental animals (Rai et al. 2011; Fazal et al. 2014). Various chemopreventive effects of betel leaves and its phytoconstituents have been discussed in the following sections.

14.3 *Piper betle* Against Various Cancer Types

Lung, liver, colorectal, stomach, and breast are the most common types of cancer leading to major deaths around the globe. Therefore, at present, there is a high demand for finding a cure and/or methods to prevent cancer. There are numerous chemically derived drugs that have been used to treat cancer. However, toxicity and detrimental effects to nontargeted tissues are few instances that override its benefits. Therefore there is a huge demand for alternative therapies using plant-based naturally derived compounds for treating cancer. The secondary metabolites from plants include polyphenols, flavonoids, etc. that have been investigated in detail for their prospective therapeutic properties. In general, it has been shown that phytocompounds possess a diverse range of biological actions such as antioxidant activity, arresting of cell growth, apoptosis induction, and targeted cell cytotoxicity that favour anticancer activity. A few plant-based drugs are under clinical trials as a combination therapy. Betel vine leaves are widely used as condiments and in traditional medicines of Asia and Africa. The rich phytoconstituents present in betel vine leaves have been documented to possess antioxidant, immunomodulatory, anti-inflammatory, and anti-proliferative activities. Given below are the details of studies that represent the identification of its anticancer attributes of betel vine against various types of cancers.

14.3.1 Prevention of Oral Cancer

Oral cancers have been acclaimed to be one amongst most common cancers around the world with nearly 11% cases being reported in Southeast Asia (Jemal et al. 2011; Cheong et al. 2017). The use of tobacco as smoke or along with betel quid is

the maximum risk-causing habit linked with oral cancers in India, Taiwan, and other Southeast Asian countries (Warnakulasuriya 2009; Khan et al. 2014). A study conducted by Rao (1984) showed that topical application of betel vine leaf extract inhibited benzo(a)pyrene-induced oral tumorigenesis in hamsters. Subsequent studies demonstrated that the leaf extract was preventing tobacco-specific nitrosamine, namely, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and N′-nitrosonornicotine-induced carcinogenesis (Padma et al. 1989a). Likewise, the betel vine leaf extract and its two phytoconstituents, namely, β-carotene and α-tocopherol, were observed to be effective in decreasing the incidence of cancer in Syrian hamsters, by means of reducing the tumour burden, enhancing the tumour latency period, and via regressing the established tumours. Also a combined protective effect was observed when hamsters were fed with betel leaf extract along with turmeric suggesting a cooperative effect between two dietary agents (Azuine and Bhide 1992). Recently, Sarkar et al. (2015) have shown that eugenol could induce apoptosis in AGS (human gastric adenocarcinoma) cells in vitro, independent of functional p53. Thus, eugenol may be used as potential anticancer agent in cases where defective p53 confines the apoptotic response of chemotherapeutic agents.

14.3.2 Prevention of Forestomach Cancer

Gastric cancers occupy the fourth most common types of cancers amongst others in the world. In Asian countries where the consumption of betel quid and *Helicobacter pylori* infection is considerable, the incidences of gastric cancer are even higher (Jemal et al. 2011; Torre et al. 2015). A study conducted by Bhide et al. (1991a) showed that the supplementation of betel leaf extract with drinking water significantly reduced benzo[a]pyrene-induced forestomach neoplasia in mice. The anticarcinogenic activity was found to be concentration dependent. Subsequent studies with eugenol, α-carotene, β-carotene, hydroxychavicol, and α-tocopherol also showed that they are equally effective in prevention against benzo[a]pyrene-induced forestomach tumorigenesis in mice (Bhide et al. 1991a). Likewise, Manikandan et al. (2010) have demonstrated the induction of apoptosis by eugenol and its function as anti-invasive and anti-angiogenic agent in N-methyl-N′-nitro-N-nitrosoguanidine-induced gastric tumorigenesis in the rat model. Eugenol also seems to possess antimicrobial activity as demonstrated in a preclinical trial done against 30 strains of *Helicobacter pylori* indicating its usefulness (Ali et al. 2005). In addition, in vitro treatment of *H. pylori*-infected human gastric epithelial AGS cell lines with β-carotene inhibited the expression of iNOS (inducible nitric oxide synthase) and COX-2 (cyclooxygenase-2) enzymes that are known to be important in *H. pylori*-induced gastric diseases (Jang et al. 2009). Overall, these observations clearly indicate that phytochemicals present in the betel vine leaves have a protective effects against the gastric cancer.

14.3.3 Prevention of Skin Cancer

Globally, skin cancers are the leading cancer forms and prevail as major chronic diseases in a number of countries (Apalla et al. 2017). Overall, about 75% of skin cancer forms are associated with the basal cell carcinoma followed by squamous cell carcinoma (15%) and melanoma skin cancer (10%). Amongst them melanoma is highly metastatic and harmful than others (Bradford 2009). In vivo experiments have demonstrated the topical application of hydroxychavicol in Swiss albino and Swiss bare mice; however, hydroxychavicol was effective only in Swiss bare mice (Azuine et al. 1991). Other studies have shown that eugenol inhibited 7,12-dimethylbenz(α)anthracene (DMBA)-/croton oil-induced tumorigenesis in mice (Sukumaran et al. 1994; Pal et al. 2010). Also, it has been proved that eugenol-related compounds induce pro-apoptotic activities in malignant melanoma cells (Pisano et al. 2007). An elaborate study of Kaur et al. (2010) has shown that eugenol inhibits subcutaneous chemical carcinogenesis by inhibiting oxidative stress and inflammatory responses, thus promoting apoptosis in mice. Furthermore, by down-regulating H-ras and c-Myc oncogenes and activating p53-dependent apoptosis, eugenol was shown to eliminate the mutated cells (Pal et al. 2010). More recently, Shah et al. (2016) screened different solvent (petroleum ether, water, ethyl acetate, and ethanol) extracts of betel vine leaves in an in vivo model. According to their study, application of the ethyl acetate extract against mice bearing melanoma significantly reduced the size of tumour. These studies clearly indicate that betel vine leaf and its phytochemicals significantly help in preventing skin carcinogenesis.

14.3.4 Prevention of Prostate Cancer

Prostate cancer is one of the most often detected cancers amongst males and the sixth leading cause of deaths worldwide due to cancer (Haas et al. 2008; Torre et al. 2015). A recent study has shown that bioactive constituents of *Piper betle* leaf extract mainly hydroxychavicol showed significant anti-proliferative activity in both in vitro studies and in vivo models of prostate cancer (Paranjpe et al. 2013). Its anticancer activity has been mainly ascribed for its role in the regulation of cell cycle, reduction of clonogenicity, and induction of cytotoxicity, via ROS (reactive oxygen species)-induced DNA damage which involves several pro-apoptotic pathways (Gundala and Aneja 2014). Moreover, it was shown that hydroxychavicol could cause a drop in mitochondrial potential and initiate caspase-mediated apoptosis of prostate tumour xenografts, when given orally at 150 mg/kg body weight in mouse. Thus such pro-oxidant activities of phytochemicals can be credible grounds for future preclinical studies in prostate cancer management.

14.3.5 Prevention of Mammary Cancer

Worldwide breast cancer is the second most common cancer and equally leading cause of cancer-related deaths in women (Jemal et al. 2011; Torre et al. 2015). Studies conducted using the aqueous extract of betel vine leaf were shown to prevent DMBA-induced mammary cancer in rats, if administered within the initial phase of the disease. However the aqueous leaf extract had no effect, once the mammary tumours had grown, indicating that the betel vine leaf and its phytochemicals may only possess chemopreventive activity (Rao et al. 1985). Subsequent studies by Bhide et al. (1994) showed that administration of betel vine leaf extract along with drinking water decreased the tumour burden and incidence or delayed the onset of mammary tumours in the DMBA-treated mice. Also, it was found that betel vine leaf extract could effectively prevent the mammary tumorigenesis in the genetically predisposed C3H (Jax) mice, where decreased incidences of spontaneous mammary tumour were observed (Bhide et al. 1994). In another study, it has been shown that eugenol, a major phytochemical from betel vine leaf, could induce apoptosis at a lower concentration in both in vitro and in vivo cancer models. Interestingly, it was found that eugenol induces intrinsic apoptotic pathways and downregulates the expression of E2F1 and surviving genes, independent of p53 and ERα status. In addition, oncogenes (NF-κB and cyclin D1) associated with breast cancer were found to be inhibited, while cyclin-dependent kinase inhibitor p21 WAF1 protein was upregulated in the xenografted mice (Al-Sharif et al. 2013), thus reiterating the anticancer properties of eugenol against several cancer types.

14.4 Mechanisms Responsible for the Cancer Preventive Effects

Cancer is a multistage process; the main characteristic of cancer involves overall alteration in the physiology of the cells. These changes include altered growth signals, limitless cell division, avoiding programmed cell death, cell invasion, and angiogenesis. Hence, recent curative strategies have been focused on reversing the physiological deviances of cancer cells than eliminating them. This requires better understanding on mechanism of action of the drugs for aiding anticipated outcome. Thus, the potential mechanism of actions of betel leaf extracts and their possible ways in mitigating cancer is discussed below.

14.4.1 Free Radical Scavenging Effects

In the normal scenario, free radicals are constantly regenerated due to various cellular metabolisms, which mainly comprise of ROS and reactive nitrogen species (RNS) (Halliwell 2007; Fuchs-Tarlovsky 2013; Gundala and Aneja 2014). At very low concentrations, free radicals are presumed to be beneficial, while excess are deleterious and cause inflammation, cytotoxicity, and mutagenesis and initiate or aggravate carcinogenesis (Devasagayam et al. 2004). The aqueous extract of the inflorescence of betel vine was found to effectively scavenge H_2O_2, superoxide radicals, and hydroxyl radicals in an in vitro analysis. Also, the extract prevented the hydroxyl radical-induced DNA damage in the PUC18 plasmid (Lei et al. 2003). The ethanolic extract of Mysore and Bangla varieties of betel vine leaf were found to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) free radicals effectively under in vitro conditions with the highest being observed in the latter (Rathee et al. 2006). Other Indian varieties such as Kauri, Ghanagete, and Bagerhati were also found to be effective in scavenging DPPH, superoxide radicals, and hydroxyl radicals with the best observed in Kauri and the least being found in Bagerhati variety (Dasgupta and De 2004).

Previous report indicates that the hydroalcoholic leaf extract of betel vine leaves possesses the nitrogen oxide scavenging effect as evidenced by the in vitro assays (Jagetia and Baliga 2004; Gundala and Aneja 2014). Similarly, Manigauha et al. (2009) have documented that the methanolic leaf extract of betel vine leaves possesses reducing power, free radical scavenging, and deoxyribose degradation activities which corroborate with the earlier reports. On the contrary, Chowdhury et al. (2013) have shown that hydroxychavicol can exert an antileukaemic activity via the production of ROS. This study demonstrated that non-apoptotic concentrations of buthionine sulfoximine along with hydroxychavicol induced a synergistic caspase-dependent and apoptosis-inducing factor (AIF)-dependent pathways in chronic myeloid leukaemia (CML) cells that led to expression of inducible nitric oxide synthase (iNOS) resulting in apoptosis. Similarly, Chakraborty et al. (2012) have observed that the treatment of primary chronic myelogenous leukaemia (CML) and leukaemic cell lines expressing wild-type and mutated Bcr-Abl tyrosine kinase gene with hydroxychavicol induced an increase in mitochondria-derived ROS which caused increased nitric oxide generation leading to apoptosis. In a recent study, eugenol was shown to promote the release of ROS leading to abrogation of G2/M phase of the cell cycle, mitochondrial toxicity, and superoxide-mediated clastogenesis which trigger the process of apoptosis in melanoma and breast and cervical cancer cell lines (Júnior et al. 2016).

14.4.2 Increase in Antioxidant Effects

Naturally, a eukaryotic cell possesses antioxidant molecules like glutathione, vitamin E, vitamin A, vitamin C (ascorbic acid), thioredoxin, and ubiquinol and the antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase that are involved in proving protection against cell damage due to free radicals and prevent mutagenesis and the formation of cancer (Devasagayam et al. 2004; Halliwell 2007; Fuchs-Tarlovsky 2013). In vivo studies have revealed that feeding betel vine leaf extract to mice increases the hepatic levels of vitamins A and C (Padma et al. 1989a) and glutathione (GSH) and SOD enzymes too (Choudhary and Kale 2002). The phytoconstituents of leaves such as hydroxychavicol, eugenol, and α-tocopherol were also reported to elevate the levels of GSH in the mouse skin (Azuine et al. 1991) and liver (Bhide et al. 1991a). Moreover, γ-tocotrienol (an isomeric form of vitamin E) and hydroxychavicol synergistically possess anticarcinogenic properties that modulate a number of cellular signalling events (Abdul Rahman et al. 2014). Altogether, these results demonstrate that the betel vine leaf extracts and its constituents increase the cellular antioxidant molecules and enzymes that may facilitate chemopreventive effects.

14.4.3 Induction of Detoxification Enzymes

The cells induct phase II drug-metabolizing enzyme such as glutathione S-transferase (GST) in order to protect themselves against electrophilic insults induced by mutagens, carcinogens, and similar metabolites. GST conjugates the toxic metabolites and, thus, facilitates its excretion (Percival 1997). Also, the agents that trigger phase II enzymes are considered to be extremely important as chemopreventive agents. In vivo studies have revealed that intraperitoneal administration of the betel vine leaf extract and its chemical constituents such as β-carotene, α-tocopherol, and hydroxychavicol significantly increases the levels of GST in the mouse skin and liver (Azuine et al. 1991; Bhide et al. 1991a). Eugenol and other constituents in the extract were effective in increasing the GST within the skin, but not in the liver, indicating organ-specific phytochemical effects (Bhide et al. 1991a).

14.4.4 Inhibition of Lipid Peroxidation

The cell membranes are rich in polyunsaturated fatty acids, which are constantly vulnerable to peroxidative attacks by free radicals. The consequence of such attack leads to a loss of fluidity, increased permeability to ions, and eventually loss of membrane function (Devasagayam et al. 2004; Gundala and Aneja 2014). In addition, the final products of lipid peroxidation, especially malondialdehyde, are mutagenic and may contribute to carcinogenesis. It has been shown that the alcoholic

extract of betel vine leaf can inhibit radiation-induced lipid peroxidation of rat liver mitochondria (Bhattacharya et al. 2005). The aqueous extracts of betel vine leaf varieties such as Kauri, Ghanagete, and Bagerhati were also found to be effective in inhibiting $FeSO_4$-induced lipid peroxidation in egg yolk (Dasgupta and De 2004). The phytochemicals present in betel vine leaves such as chavibetol and allylpyrocatechol and their glucoside derivatives effectively prevent Fe(II)-induced lipid peroxidation of liposomes and rat brain homogenates in vitro, and the best effects were observed with the allylpyrocatechol (Rathee et al. 2006). In vivo experiments have shown that betel vine leaf extract and its phytoconstituent allylpyrocatechol inhibited or alleviated gastric ulcer induced by indomethacin and decreased the levels of lipid peroxidation (Bhattacharya et al. 2007a, b). It is proposed that similar effects may have contributed towards prevention of gastric cancer induced by using benzo(a)pyrene and similar carcinogens.

14.4.5 Anti-inflammatory Effects

Chronic inflammation has been attributed towards initiation/aggravation of many human diseases, including cancer (Halliwell 2007; Crusz and Balkwill 2015). Traditionally, betel vine leaf has been utilized as a common household medication for treating inflammation in the oral cavity (Satyavati et al. 1987; Fazal et al. 2014). Many in vivo studies using a complete Freund's adjuvant-induced model of arthritis in rats have reported the anti-inflammatory activity of the ethanolic extract of betel vine leaves. Further, a detailed investigation on its mechanisms has revealed a dose-dependent decrease of nitric oxide generation in murine peritoneal macrophages due to betel vine leaf extract treatment. These decreased levels of reactive nitrogen species were directly correlated to the downregulation of inducible nitric oxide synthase in macrophages with concurrent decrease of interleukin-12 p40. The study clearly indicated that betel vine leaf extract facilitates the downregulation of T-helper 1 pro-inflammatory responses (Ganguly et al. 2007). Eugenol, one of chief constituents of betel vine leaf, has been shown to possess anti-inflammatory effects against a wide variety of inflamagens (Lee et al. 2007; Fujisawa and Murakami 2016). In vitro analysis has shown that eugenol blocks the release of prostaglandin E2 (PGE2), IL-1β, and TNF-α (bone-resorbing mediators) from lipopolysaccharide(LPS)-stimulated human macrophages due to the suppression of LPS-induced macrophage gene expression (Lee et al. 2007). Interestingly, eugenol also suppresses the expression of COX-2 gene in LPS-stimulated mouse macrophages (Kim et al. 2003). Likewise, allylpyrocatechol exhibits anti-inflammatory effects; for instance, it induces inflammatory responses in macrophages by inhibiting COX-2, iNOS, and IL-12 p40 through downregulating the NF-κB pathway (Sarkar et al. 2008). Also, hydroxychavicol extracted from the aqueous leaf extract of betel vine is known to possess antioxidant and anti-inflammatory activities (Sharma et al. 2009). In support of this, by in vitro, Rintu et al. (2015) also suggest the antioxidant and anti-inflammatory properties of betel vine leaf extract (methanol).

14.4.6 Antimutagenic Effects

One of the fundamental steps of carcinogenesis is the involvement of inducing DNA damage that provokes mutations within the nuclear material. Previous reports have confirmed that oxidative and nitrative free radicals promote oxidative stress activities and damage the cellular DNA leading to mutations (Devasagayam et al. 2004; Waris and Ahsan 2006; Reuter et al. 2010). Alterations in the DNA sequence such as nucleotide rearrangement, base modification, miscoding, gene duplication, and activation of oncogenes play a pivotal role in the cancer initiation and progression (Halliwell 2007; Weisburger 2001).

Many studies have validated that betel vine leaf is devoid of mutagenic properties (Umezawa et al. 1981; Shirname et al. 1983; Bhattacharya et al. 2005) and instead have shown to possess antimutagenic (Shirname et al. 1983) and anticlastogenic properties (Bhattacharya et al. 2005). Moreover, betel vine leaf extract did not cause any morphological transformation in the hamster embryo cells nor induce sister chromatid exchanges in phytohaemagglutinin (PHA)-stimulated and virally transformed human lymphocytes (Umezawa et al. 1981). As tobacco is rich in nitrosamines such as N′-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, and 4-(N-nitroso methyl amino)-1-(3-pyridyl)-1-butanone that are principal carcinogens, consuming or chewing tobacco has been attributed as primary cause for initiation of carcinogenesis (Padma et al. 1989b). A pivotal study has shown that betel vine leaf possesses an effective activity against several mutagens using Ames test confirms its antimutagenic effects (Shirname et al. 1983; Nagabhushan et al. 1989). From their studies it was observed that betel vine leaf extract suppressed the mutagenicity caused by N′-nitrosonornicotine, benzo(a)pyrene and dimethylbenzanthracene, and 4-(methylnitrosamino)-1- (3-pyridyl)-1-butanone in a dose-dependent way. Also, it was observed that the acetone extract of betel vine leaf was more potent in inhibiting mutagenicity than the aqueous extract (Nagabhushan et al. 1987; Padma et al. 1989b; Bhide et al. 1991b). Furthermore, Trivedi et al. (1994) showed that the aqueous extract of betel vine leaf protected against genotoxic effects of Pan masala, a mixture of betel vine leaf with lime and areca nut (with or without tobacco) extract in the CHO (Chinese hamster ovary) cells. It was also detected that the leaf extract reduced the incidence of sister chromatid exchange and chromosome aberration induced by paan masala (Trivedi et al. 1994).

Phytochemicals present in the betel vine leaf, namely, eugenol and hydroxychavicol, were found to be non-mutagenic when evaluated against several strains of *Salmonella typhimurium* with and without metabolic activation (Amonkar et al. 1986). Both these compounds exhibited a dose-dependent inhibition of dimethylbenz(a)anthracene-induced mutagenesis in the *S. typhimurium* strain TA98 with metabolic activation. Amongst these hydroxychavicol was found to be more effective compared to eugenol and effectively abrogated mutagenicity of 4-(nitrosomethylamino)-1-(3-pyridyl)-1-butanone and N′-nitrosonornicotine in Ames test, *Salmonella*/microsome assay, and the micronucleus test in mice (Amonkar et al. 1989). In another study, both hydroxychavicol and eugenol were shown to exhibit dose-dependent suppression of nitrosation in *S. typhimurium* strain

TA100 and TA1535 (devoid of S9 mix) without affecting its survival. It was found that blocking of hydroxyl groups in the benzene ring by acetylation eliminated the anti-nitrosating activity of these molecules and, thus, abolished its antimutagenic effects. Alternatively, scavenging of nitrite ions by hydroxychavicol is also thought to be a possible mechanism for inhibition of nitrosation (Amonkar et al. 1989). Eugenol too was observed to inhibit nitrosation of methyl urea and inhibit tobacco-induced mutagenicity in dose-dependent manner (Sukumaran and Kuttan 1995).

Yokota et al. (1986) observed that direct addition of eugenol during Ames test did not prevent the mutagenic activity of benzo(a)pyrene but the S9 fraction prepared from rat liver when pretreated with eugenol-suppressed mutagenesis. This signifies that eugenol inhibits the activation of cytochrome P-450 that turns benzo(a) pyrene into a mutagen. Interestingly, it was found that 5% eugenol significantly reduced the mutagenicity of benzo(a)pyrene metabolized by microsomes isolated from ad libitum fed rats (Yokota et al. 1986). A quantitative analysis on eugenol-treated microsomes showed alleviated levels of cytochrome P-450, benzo(a)pyrene hydroxylase, and aryl hydrocarbon hydroxylase activities, which signify an inverse correlation between mutagenicity of benzo(a)pyrene and eugenol concentration fed to rats (Yokota et al. 1986). In summary, these reports emphasize the potential anti-mutagenic activity of the betel vine leaf extracts and its phytoconstituents such as eugenol and hydroxychavicol and can effectively neutralize potential carcinogens in both prokaryotes and eukaryotes.

14.4.7 Antitumour-Promoting Activities

Numerous studies have shown that agents so-called tumour promoters can improve the carcinogenic effect without being carcinogenic themselves. One amongst such is 12-O-tetradecanoylphorbol-13-acetate (TPA), which is widely used as promoter for skin cancer in laboratory animals. TPA is known to enhance the generation of ROS likely by decreasing ROS-detoxifying enzymes and also activate protein kinase C enzyme that cause a wide range of biological effects in cells and tissues (Mechali et al. 1983). A study by Murakami et al. (2000) shows that the betel vine leaf extract effectively inhibits TPA-induced Epstein-Barr virus activation in Raji cells, hence confirming its antitumour property. These studies evidently suggest that betel vine leaf extract can be a source for novel chemopreventive agents against cancer.

14.4.8 Induction of Selective Apoptosis and Cell Death of Neoplastic Cells

Programmed cell death, also known as apoptosis, is a fundamental mechanism in all tissues and organ systems that regulate the cell proliferation and deletion. Many studies have confirmed that chemopreventive agents sourced through diets can

inhibit preneoplastic and tumour cells by targeting its signalling pathways, thus inducing apoptosis (De Flora and Ferguson 2005). A study conducted by Wagh et al. (2011) demonstrated that NPB001-05, a standardized extract from betel vine (500 mg/kg once or twice a day for 2 weeks), was effective in inhibiting the development of human chronic myelocytic leukaemia in xenograft models. Interestingly, this extract is found to be more potent than imatinib (an anticancer chemical drug) without any relevant toxicity. The extract was found to inhibit tyrosine kinase activity, decrease Bcr-Abl protein levels, and induce apoptosis. Also, transcriptional profiling from microarray analysis showed that NPB001-05 induced the dysregulation of endoplasmic reticulum (ER) stress, PI3K/Akt, and MAPK signalling pathway genes (Wagh et al. 2011). Studies have evidenced that some potential phytochemicals of the betel vine leaf extract such as hydroxychavicol (Chang et al. 2002; Jeng et al. 2004), ursolic acid (Kassi et al. 2007; Yamai et al. 2009; Yu et al. 2010; Shao et al. 2011), chlorogenic acid (Rakshit et al. 2010), and eugenol (Ghosh et al. 2005; Pisano et al. 2007; Manikandan et al. 2010; Jaganathan et al. 2011) can induce apoptosis in tumour cells confirming the chemopreventive effect.

14.5 Betel Vine Leaf Extract As an Adjunct to Chemotherapy

The betel vine leaf extract has been widely used in folklore medicines. Numerous studies have shown the pharmacological benefits of betel vine leaf extracts alone. However in scenario where the patient is given a concoction of drugs during chemotherapy, it is important to gauge the interaction between the drugs and its consequences. Limited studies have shown that betel vine leaf extract can improve the cytotoxicity of drugs such as cisplatin and 5-flurouracil (5-FU) in vitro when treated on Hep G2, a hepatoblastoma-derived cell line and few colon cancer cell lines such as HCT116 and HT29 (Young et al. 2007; Ng et al. 2014). The low level of expressing multidrug resistance protein 2 (MRP2) and inhibiting the activity of total GST are some of the ways by which the betel vine leaf extract contributes to improved cytotoxicity of anticancer drugs (Young et al. 2007).

14.6 Conclusion and Future Direction

The scientific literature reviewed in this chapter evidently indicates the promising role of betel vine leaf extract and its phytocompounds against different cancers. Some of the available treatments for cancer include chemotherapy, surgery, radiotherapy, and phototherapy. However, the development of drug resistance, the absence of effective drugs, high toxicity associated with severe side effects, and a high risk of relapse are some of the major challenges in cancer therapy. Hence,

medications are shifted towards natural remedies especially using plant-based drugs in recent years as they are safe and effective. In addition, these natural products significantly enhance the efficacy of chemotherapeutic drugs in combination therapies and lower the possible side effects. In this regard, betel vine and its phytocompounds can be more beneficial against tumours. Innumerable scientific studies have highlighted the chemopreventive effects of betel vine leaf and the phytochemicals present in them. These pharmacological effects have been attributed mostly due to its antioxidant, free radical scavenging, anti-inflammatory, antimutagenic and pro-apoptotic activities against neoplastic cells. In general, betel vine leaf extract and its phytocompounds have several mechanisms of action such as inhibition of apoptosis and tyrosine kinase activity; decreasing the levels of Bcr-Abl protein; enhancing ROS generation and antioxidant effects; causing DNA damage; blocking the release of PGE2, IL-1β, and TNF-α; inhibiting lipid peroxidation; inducing detoxification enzymes; and promoting inflammatory responses by inhibiting COX-2, iNOS, and IL-12 p40 through downregulating the NF-κB pathway. Additional studies are required aiming at understanding the mechanism of action of the betel vine leaf extract plus its associated phytochemicals like hydroxychavicol, eugenol, chlorogenic acid, etc. and determining their efficacy. As betel vine leaf is safe for consumption and owing to its relative abundance in South Asian countries, it remains a potential source for novel phytochemicals with tremendous pharmacological implications and countless possibilities for further investigation. Future studies should be on testing the efficacy of beetle vine leaf and its constituents in preventing various cancers with phytochemical quantified samples. In addition, toxicity studies must be encouraged to ensure these effective phytocompounds get a considerable attention for clinical trials in the near future. The results of these experiments will pave way towards the development of beetle leaf as a source for developing novel cancer preventive agents.

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Chapter 15
Anticancer Properties of Curcumin and Its Efficacy for Treating Central Nervous System Neoplasms

Neil V. Klinger and Sandeep Mittal

15.1 Introduction

Curcuma longa is a medicinal plant that has been used in traditional Chinese and Ayurvedic medicines for thousands of years (Gupta et al. 2012). Some of its other uses include relief of swelling and sprains, hepatic and biliary disorders, rheumatism, cough, abdominal pain, skin wounds, bites, and burns (Ammon and Wahl 1991). It is also used commonly as a dye and spice. The rhizome of *C. longa* more commonly known as turmeric continues to have many of these common uses. It is a rhizomatous member of the Zingiberaceae family and is a perennial herb (Scartezzini and Speroni 2000; Biswas and Mukherjee 2003) with large leaves and flowers (Fig. 15.1a). The primary and secondary rhizomes (Fig. 15.1b) are yellow or orange when sectioned and strongly resemble the familiar pigment of the powdered turmeric (Fig. 15.1c). *C. longa* is cultivated in tropical regions around the world, with the largest being produced in India (Priyadarsini 2014). It wasn't until 1815 that the principal active compound in this plant was described and named curcumin. A pure preparation was obtained in 1870 (Jackson and Menke 1881), and the chemical structure (diferuloylmethane) was reported in 1910 (Miłobedzka et al. 1910). In 1949, a study was performed that demonstrated curcumin is a biologically active

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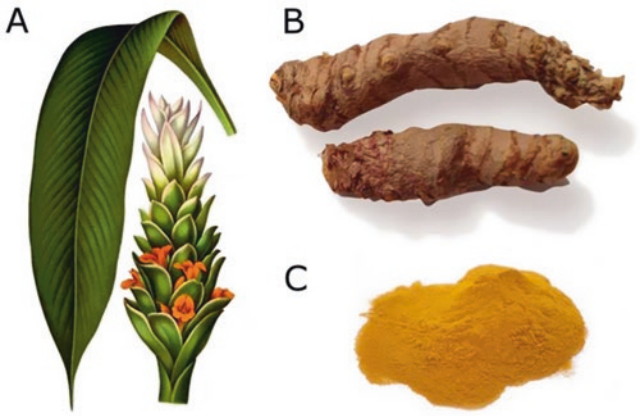

Fig. 15.1 *Curcuma longa*. (**a**) Leaf and inflorescence; (**b**) rhizomes; (**c**) powdered

compound and possessed antibacterial properties against *Staphylococcus aureus*, *Salmonella paratyphi*, *Trichophyton gypseum*, and *Mycobacterium tuberculosis* (Schraufstatter and Bernt 1949). The potential anticancer properties of curcumin were first reported in 1985, where investigators found both turmeric extract and pure curcumin were cytotoxic to human lymphocytes, leukemic cells, and Dalton's lymphoma cells. In this report, the authors concluded that the cytotoxic component found in the turmeric extract was curcumin (Kuttan et al. 1985). Since then, biomedical research interest in curcumin has experienced a near exponential growth (Fig. 15.2). Curcumin has been studied as a treatment for a multitude of disorders including inflammatory and autoimmune conditions, diabetes, skin conditions, neurological and psychiatric disorders, and cancer (Gupta et al. 2012; Kocaadam and Sanlier 2017).

15.2 Chemical and Therapeutic Properties of Curcumin

Apart from curcumin, more than 100 other distinct chemical compounds are also found in turmeric. To make matters more confusing, the term "curcuminoids" was coined to refer to several diarylheptanoid compounds of similar structure. These curcuminoids are each a distinct chemical entity and are found in turmeric with many other constituents. Though, both lay and some scientific literature have incorrectly referred to turmeric or crude turmeric extracts as curcumin, curcumin herein will refer to the single chemical entity (1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-1,6-diene-3,5-dione, and the term curcuminoids will refer to a chemical compound with structure similar to curcumin.

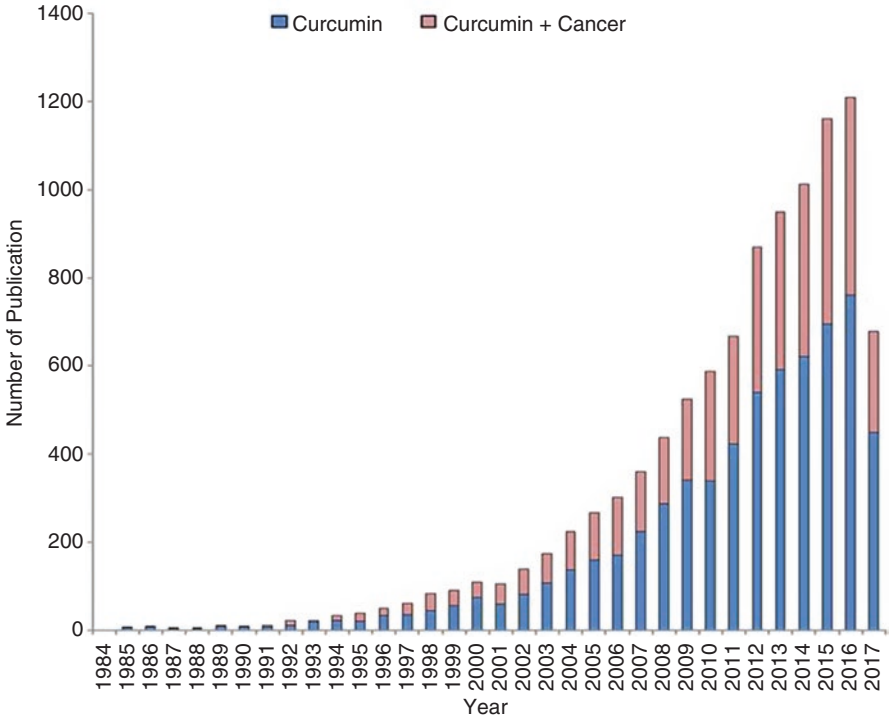

Fig. 15.2 Research interest in curcumin over the past three decades. Bar represents the number of articles published (available on https://www.ncbi.nlm.nih.gov/pubmed/) in a year. The *red* portion in bar represents the proportion of studies returned with search terms "curcumin" and "cancer," while the *blue* represents studies returned with the term "curcumin"

15.2.1 Chemical and Structural Properties

Curcumin has a molecular formula of $C_{21}H_{20}O_6$. It can undergo keto-enol tautomerization, though the enol form predominates in non- to moderately polar solvents. The crystal structure of curcumin was first reported in 1982 (Tonnesen et al. 1982). Several compounds are found in turmeric that have similar structure and are collectively called curcuminoids, comprised chiefly of curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The relative amounts of curcuminoids vary between growing regions and climates but in general account for <10% of turmeric. Extraction of curcumin was first reported in 1815 and has been improved upon substantially since then. Commonly, curcumin is extracted with the use of an organic solvent (e.g., ethanol) followed by a separation with column chromatography. Chlorinated solvents significantly improve the efficiency of extraction but are

unacceptable for use in food or medicinal applications. Detection and quantification of curcumin after extraction can be achieved with high-performance liquid chromatography (HPLC). Curcumin absorbs light both in the visible (420 nm) and UV spectra (265 nm). Synthesis of curcumin was first reported in 1918. An improved synthesis was published in 1964 and has subsequently been modified to improve yield or as a method to construct different curcuminoids (Rao and Sudheer 2011; Grynkiewicz and Slifirski 2012; Priyadarsini 2014). These production methods react boron complexed 2,4-diketones with aromatic aldehydes in polar aprotic solvents. Water is removed using alkyl borates, and primary and secondary amines help with deprotonation. The curcumin is removed by precipitation followed by column chromatography (Priyadarsini 2014).

One of the most well-known chemical properties of curcumin is that it is an exceptionally good scavenger of reactive oxygen species (ROS). It has three distinct positions that are able to undergo oxidation and has extensive resonance stabilization. Examples include scavenging of hydroxyl, peroxynitrite, superoxide, and alkoxy radicals (Priyadarsini 1997; Jovanovic et al. 2001; Sun et al. 2002; Mishra et al. 2004). In a study of 89 subjects with bronchiolitis obliterans following exposure to sulfur mustard, patients were randomized to receive treatment with curcuminoids and piperine (1500 and 15 mg/day) or placebo to see if curcuminoids affected biomarkers of oxidative stress. The authors report that the treatment group had elevated levels of glutathione (major endogenous antioxidant) and reduced malondialdehyde (indicator of lipid peroxidation) levels as well as improved lung function versus placebo (Panahi et al. 2016). A number of chemical properties make it initially difficult to consider curcumin, a suitable drug. First, it is essentially insoluble in water at pH 7.0. To complicate matters, it is unstable in aqueous solutions, especially in neutral or alkaline conditions (Price and Buescher 1997; Wang et al. 1997). For example, the $t_{1/2}$ of curcumin in 0.1 M phosphate buffer at pH 7.2 was just over 9 min and approximately 90% had decomposed within 30 min. However, when curcumin was analyzed in human blood, its $t_{1/2}$ was extended to approximately 8 h via interactions to blood lipids and proteins (Wang et al. 1997). Similar success in protecting curcumin in vitro has been found using 10% fetal bovine serum (FBS) in culture media (Priyadarsini 2014). Though not of physiologic consequence, curcumin also undergoes photodegradation (Priyadarsini 2009). This poses additional problems in production, storage, and handling of curcumin and should pose strict handling precautions when conducting experiments.

15.2.2 Pharmacokinetics

Some of the chemical properties of curcumin are unfavorable for a drug candidate. It should be noted that the pharmacokinetic properties of curcumin are also nonideal. Curcumin has very poor oral absorption. In addition, curcumin is metabolized quickly by two major pathways. This includes O-conjugation to produce curcumin glucuronide and curcumin sulfate and reduction to produce tetra-, hexa-, and

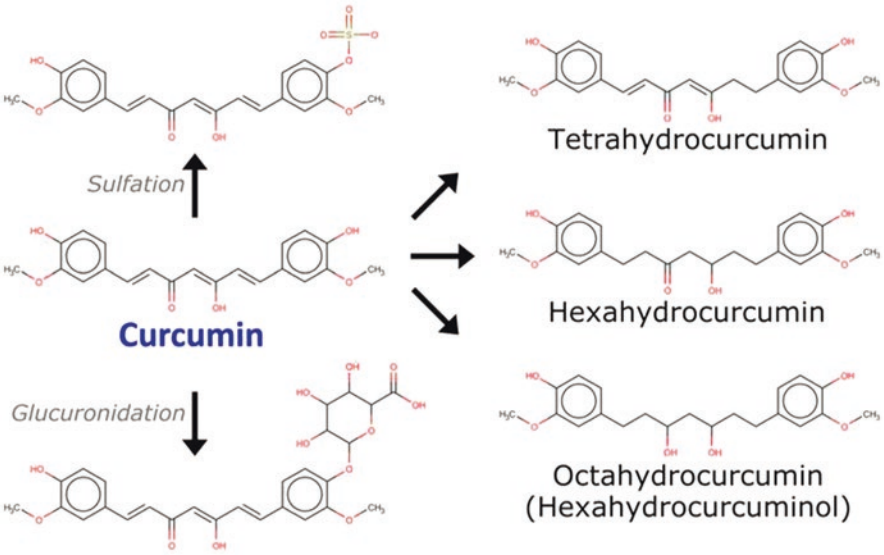

Fig. 15.3 Metabolism of curcumin by (**a**) glucuronidation, (**b**) sulfation, and (**c**) reduction to produce tetra-, hexa-, and octahydrocurcumin

octahydrocurcumin (Fig. 15.3) (Priyadarsini 2014). Though early human trials are well cited in the literature, a majority of the pharmacokinetic data obtained with curcumin and its derivatives are in rodent models. Here, we attempt to focus our discussion on the relevant human in vivo data. In a study on patients with colorectal adenocarcinoma, one, two, four, or eight capsules of curcuminoids purified from turmeric extract (each with 450 mg of curcumin, 40 mg of desmethoxycurcumin, and 10 mg of bisdesmethoxycurcumin) were administered daily. In patients receiving the highest dose, curcumin was barely detectable in the plasma with an average concentration of 11.1 nmol/l. Curcumin and its metabolites (curcumin sulfate, curcumin glucuronide) were found in the urine at levels of 0.1–1.3 μmol/l, 19–45 nmol/l, and 210–510 nmol/l, respectively (Sharma et al. 2004). In general, curcumin and its metabolites appear to be excreted in feces. Numerous other studies have been performed in humans with doses up to 12 g of curcumin per day, and plasma levels have been reported at 1.77 μM (8 g, pure synthetic curcumin) (Cheng et al. 2001), undetectable (2 g, curcuminoids extract) (Shoba et al. 1998), undetectable (180 mg curcumin in 2.2 g extract) (Sharma et al. 2001), 57.6 ng/μL (12 g, 95% curcuminoids extract) (Lao et al. 2006), and undetectable (3.6 g curcumin, 240 mg, desmethoxycurcumin, 160 mg, bisdesmethoxycurcumin from extract) (Garcea et al. 2005). The curcumin absorbed poorly and metabolized rapidly; thus, it has poor bioavailability and distribution. In spite of this, an overwhelming amount of research has been conducted in an attempt to overcome these limitations to see the therapeutic potential of curcumin come to fruition. By developing a suitable delivery vehicle, the researchers have been able to protect curcumin from degradation and increase its bioavailability.

15.2.3 Anticancer Properties

Multiple molecular mechanisms have been proposed to account for the antiproliferative properties of curcumin seen in vitro and in vivo. Here, we present the best characterized actions of curcumin which include altering cell survival signaling, inducing cell cycle arrest, inducing autophagy, and inhibition of angiogenesis.

15.2.3.1 Curcumin Alters Cell Death/Survival Signaling

Perhaps, among the best characterized molecular interactions of curcumin is with nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), a family of dimeric transcription factors that alter DNA transcription and cell survival. In unstimulated cells, NF-κB is bound to IκB proteins, preventing it from entering the nucleus. Canonical activation of NF-κB occurs when IκB proteins are phosphorylated by IκB kinase (IKK). This allows NF-κB to activate genes leading to inflammation and cell survival or proliferation among many others. In many cancers, NF-κB is constitutively active leading to uncontrolled cell proliferation and failure of normal feedback responses (Nelson et al. 2004; Vlahopoulos et al. 2015). It was first reported that curcumin was able to block NF-κB activation in 1995 (Singh and Aggarwal 1995). Subsequently, this mechanism was refined to show curcumin inhibits IKK and AKT (a serine/threonine-specific protein kinase) (Siwak et al. 2005). As a result, curcumin acts to downregulate the genes activated by NF-κB and thereby promotes cell cycle arrest and apoptosis. Specifically, activation of NF-κB can alter proliferation via cyclins D1 and E, CDK2, and MYC; promote cell survival via BFL1, BCL-2, BCL-X_L, survivin, TRAF1/2, and FLIP; promote angiogenesis via CXCL1/8, IL-1/6, VEGF, and TNF; and promote metastasis via uPA, MMPs, and adhesion molecules (Baud and Karin 2009). In addition, curcumin acts to downregulate the Akt/mTOR pathway further inhibiting cell cycle progression, cell proliferation, and increased apoptosis (Mukhopadhyay et al. 2002; Woo et al. 2003; Aggarwal et al. 2006; Beevers et al. 2006). Additionally, curcumin has been found to inhibit growth of human colon cancer by suppressing Egr-1 and thus EGFR expression (Chen et al. 2006). AP-1 is another transcription factor curcumin can downregulate, leading to normalization of c-fos/fra-1 transcription in tumorigenic HeLa cells (Prusty and Das 2005).

15.2.3.2 Curcumin Induces G2/M Cell Cycle Arrest

Many studies attempting to explain the cytotoxic effects of curcumin have noted that cells often are arrested at the G2/M stage of the cell cycle. A variety of molecular mechanisms have been proposed to explain this, though they primarily involve

the p53 tumor suppressor gene. A study using ovarian cancer cells concluded this effect was due to p53 phosphorylation and that cells entered apoptosis via caspase-3 activation (Weir et al. 2014). An in vitro study of melanoma cells reported apoptosis and cell cycle arrest after treating with curcumin via upregulation of p53, $p21^{Cip1}$, $p27^{Kip1}$, and checkpoint kinases. Curcumin also downregulates NF-κB and induces nitric oxide synthase leading to apoptosis (Zheng et al. 2004). When examining cell cycle arrest in glioma(U251) cells, Liu et al. (2007) found that low doses of curcumin appeared to induce G2/M arrest, while high doses also arrested cells in S phase. They also found the ING4 (inhibitor of growth) expression was significantly upregulated after cell cycle arrest by curcumin, as was p53 and $p21^{WAF-1/CIP-1}$.

15.2.3.3 Curcumin Induces Autophagy

Autophagy is a form of programmed cell death. It is a natural, regulated process whereby cell contents are able to recycle organelles or removed in conditions such as starvation. Autophagic vacuoles form in the cytoplasm and will eventually lead to cell death (Klionsky and Emr 2000). Some cancer cells such as glioblastoma are resistant to apoptosis but rapidly undergo autophagy when exposed to therapies such as temozolomide or radiation (Kanzawa et al. 2004; Aoki et al. 2007). Factors such as mTOR can sense amino acids and negatively regulate autophagy through p70S6K (Shigemitsu et al. 1999). Phosphatase and tensin homolog (PTEN), tumor suppressor in humans, can induce autophagy by inhibiting the PI3k/PKB pathway, while Akt activation can inhibit autophagy (Arico et al. 2001). Curcumin is able to induce autophagy by upregulation of the ERK1/2 pathway and downregulating the Akt/mTOR/p70S6K pathway (Aoki et al. 2007). Thus, curcumin would be expected to act as a chemotherapeutic against cancers susceptible to autophagic cell death. Additionally, induction of autophagy by curcumin might act synergistically with existing therapeutics.

15.2.3.4 Curcumin Inhibits Angiogenesis and Metastasis

Angiogenesis is the process of new capillary growth from existing vasculature. It is essential for growth of primary tumors and metastasis (Folkman and Shing 1992; Folkman 1995). Curcumin has been shown to inhibit differentiation of human umbilical vein endothelial cells (Thaloor et al. 1998). While matrix metalloproteinases (MMPs) play important functions in extracellular matrix remodeling in normal cells, they also allow for cancer invasion and metastasis where they are upregulated (Bachmeier et al. 2014). Curcumin is able to inhibit MMP-2 and MMP-9 (Menon et al. 1999; Hong et al. 2006) as well as VEGF and ICAM-1 (Aggarwal 2005). Curcumin can also downregulate urokinase to further decrease angiogenesis and metastasis (Yodkeeree et al. 2009).

15.2.4 Toxicity

Importantly, studies with curcumin administration to human subjects have failed to show any significant toxicity or adverse events. Even in studies with up to 12 g administered daily, the only reported reactions among these studies were occasionally rash, diarrhea, nausea, or unacceptable "bulk" of having to take so many tablets (Cheng et al. 2001; Sharma et al. 2001, 2004; Lao et al. 2006). In a pilot study with active rheumatoid arthritis patients, administration of curcumin (500 mg twice daily, as BCM-95®) for 8 weeks did not produce any attributable side effects according to the authors (Chandran and Goel 2012). Another trade preparation study using Theracurmin® with up to 400 mg of curcumin reported "no unexpected adverse events" (Kanai et al. 2013). This lack of significant side effects is reported in numerous other studies as well (Baum et al. 2008 Kanai et al. 2010; Bayet-Robert et al. 2014).

15.3 Curcumin Protection, Modification, and Delivery Systems

Modern research and preparation methods seek to overcome curcumin's pharmacokinetic barriers. Many reviews examine delivery systems in great detail (Naksuriya et al. 2014; Mehanny et al. 2016; Ahmad et al. 2016; Hussain et al. 2017). The majority of these systems are reported only using in vitro experiments, though we endeavor to focus on reviewing in vivo data when possible.

15.3.1 Natural Enhancer Piperine

Piperine is a nutraceutical compound similar to curcumin. Piperine is an alkaloid found in many species of *Piper* such as *Piper nigrum* (black pepper). Data suggests that piperine is able to inhibit hepatic and small bowel glucuronidation, thus increasing bioavailability of many drugs (Atal et al. 1985). Specifically, piperine has been shown to inhibit P-glycoprotein and CYP3A4 in humans, both of which contribute to first pass metabolism of many drugs (Bhardwaj et al. 2002). When given together with curcumin, piperine is reported to increase curcumin's bioavailability an astounding 2000%, increasing plasma concentrations to 0.18 μg/mL after a 2 g oral curcumin dose and 20 mg of piperine (Shoba et al. 1998).

15.3.2 Phospholipid Complex Preparations

Many different phospholipid complexes have been used to encapsulate curcumin. Hydrogenated soy phosphatidylcholine (HSPC) complexed with curcumin (32% w/w) increased its solubility in water over threefold, more than double the plasma concentration in orally fed (1 g/kg) rats. It also yielded a longer elimination half-life leading to a relative bioavailability of 125.8%. In addition, the authors reported superior antioxidant properties over free curcumin (Maiti et al. 2007). Curcumin formulated with phosphatidylcholine increased peak plasma concentrations from 6.5 to 33.4 nM over unformulated curcumin in rats fed 340 mg/kg by oral gavage (Marczylo et al. 2006). Solid lipid curcumin particles (SLCP) have been constructed using turmeric extract, phospholipids, fatty acids, and ascorbyl esters with a final curcumin content of 20–30%. Capsules were then formulated containing SLCP and 400–600 mg curcumin and administered orally to human patients with osteosarcoma and healthy participants. Among healthy subjects, administration of SLCP showed a peak plasma concentration of 22.4 ng/ml compared to undetectable levels from those administered unformulated curcuminoids. Among the osteosarcoma patients, administering 4000 mg versus 2000 mg SLCP led to plasma concentrations of 41 ng/ml versus 33 ng/ml and $t_{1/2}$ of 7.6 h versus 2.5 h (Gota et al. 2010). Curcumin loaded in solid lipid nanoparticles has also shown increased peak plasma concentrations in rats over free curcumin (14.3 versus 0.3 μg/ml), while the Vd decreased from 41 L/kg with free curcumin to 7.7 l/kg with similar doses of curcumin-loaded solid lipid nanoparticles (Kakkar et al. 2011).

15.3.3 Cyclodextrin

A study published in 2007 showed that cyclodextrin complexed to curcumin increases stability against hydrolytic degradation, with hydroxypropyl-β-cyclodextrin performing the best of those tested (Tomren et al. 2007). Curcumin loaded in self-assembling cyclodextrin polymers has slightly better IC_{50} values in vitro against C4-2, DU145, and PC3 prostate cancer cells compared to free curcumin (12.5, 15.9, and 16.1 versus 19.6, 19.3, and 19.4 μM). In addition, the researchers report a three- to fourfold increase in cellular uptake using cyclodextrin complexed curcumin versus free curcumin (Yallapu et al. 2010b). A study published in 2012 sought to examine the efficacy of complexing curcumin with a cyclodextrin (2-hydroxypropyl-γ-cyclodextrin) and subsequently loaded the product in liposomes. These in vitro results show that IC_{50} values were no better than conventional curcumin liposomes against the cell lines tested (KHOS, mesenchymal origin;

MCF-7, epithelial origin; and skin fibroblast) (Dhule et al. 2012). A separate study compared efficacy of free curcumin, curcumin-entrapped liposomes, β-cyclodextrin complexed curcumin, and β-cyclodextrin complexed curcumin in liposomes and found effective dose (EC_{50}) values against colon cancer cells (SW-620) of 1.9, 0.96, 2.95, and 3.25 μM, respectively. A similar pattern was obtained when these compounds were tested against lung cancer cells (A-549) (Rahman et al. 2012).

15.3.4 Polylactic-co-glycolic Acid (PLGA)

As with other delivery methods, loading curcumin in PLGA was attempted to increase its water solubility and improve its pharmacokinetics. If fact, a study published in 2011 claimed this delivery system increases curcumin's water solubility to 640-fold. The authors also report a 5.6-fold increase in bioavailability and a longer half-life after oral feedings in rats (Xie et al. 2011). In another study of curcumin-loaded PLGA particles, researchers fed rats either curcumin alone (250 mg/kg), curcumin with piperine (250 and 10 mg/kg), or the loaded PLGA particles (equivalent to 100 mg/kg curcumin). Peak plasma concentrations were found to be 90, 121, and 261 ng/ml, respectively (Shaikh et al. 2009). An in vitro study on ovarian (A2780CP) and metastatic breast (MDA-MB-231) cancer cells demonstrated that PLGA encapsulated curcumin had two- and sixfold increased cellular uptake and better IC_{50} values (13.9 and 9.1 μM) versus free curcumin (15.2 and 16.4 μM) (Yallapu et al. 2010a). Another study examining curcumin-loaded PLGA nanoparticles conjugated them to Tet-1 peptide, a targeting moiety of 12 amino acids that interacts specifically with motor neurons. By doing so, the authors postulated this may help target neuronal cells and increase efficacy for Alzheimer's disease. Their results demonstrate increased cell uptake and unaltered anti-amyloid activity with this conjugation (Fatouros et al. 2012). Another conjugation study used curcumin-loaded PLGA particles conjugated to anti-P-glycoprotein antibodies. The results showed higher uptake in cells expressing greater amounts of P-glycoprotein and greater cytotoxicity than either free curcumin or curcumin loaded in untargeted PLGA particles (Punfa et al. 2012).

15.3.5 Blood-Brain Barrier

An additional therapeutic obstacle that must be considered in the context of this chapter is the blood-brain barrier. Because the brain is "guarded," it is considerably harder to develop therapeutic agents for the central nervous system (CNS) that can be administered systemically. In particular, drugs with low molecular weights and high lipid solubility are able to cross the blood-brain barrier (Alam et al. 2010). In an in vivo study using rats, researchers infused either free curcumin or curcumin loaded in PLGA intravenously at doses of 25 mg/kg (Tsai et al. 2011). Different

organs were then analyzed. The half-life of curcumin and the curcumin loaded in PLGA was 9.2 and 14.8 min, respectively, and mean residence times were 20 and 27 min. These data show that both curcumin and curcumin loaded in PLGA particles are able to cross the blood-brain barrier into brain tissues in rats and that the PLGA-loaded particles performed slight better. Further examination found that the $t_{1/2}$ was significantly increased in the cerebral cortex (19.9 and 2.3 min) and hippocampus (16.7 and 7.6 min) using curcumin-loaded PLGA versus curcumin alone. Similar trends were noted in mean residence times within the cerebral cortex (35.1 and 17.9 min) and hippocampus (30.2 and 16.5 min) (Tsai et al. 2011). Another rodent study using Tg2576 mice (transgenic; overexpress mutant human amyloid precursor protein and Aβ) fed with 23 mg/kg curcumin, nanocurcumin, or water by gavage examined brain tissue distribution. They found mean residence time in the plasma to be undetectable in animals fed with free curcumin but 34 min in those fed with nanocurcumin. Mean residence time in brain tissue was 20 min with free curcumin and 114 min with nanocurcumin. Again, these data show that both free and modified (curcumin in (PEG-PLA) di-block polymer micelles) curcumin are able to penetrate the blood-brain barrier (Cheng et al. 2012). Interestingly, the antioxidant properties of curcumin also allow protection of the blood-brain barrier itself in addition to brain tissues. One of the study using a rat model of ischemia/reperfusion injury injected 2 mg/kg IV curcumin 30 min after ischemic insult. The authors reported significantly diminished infarct volume, improved neurologic recovery, and decreased mortality. Further in vitro experiments in this study show curcumin can prevent peroxynitrite radical-mediated damage of brain capillary endothelial cells (Jiang et al. 2007). Another group suggested curcumin is able to protect rat brain microvasculature through the heme oxygenase-1 pathway and restores damage to occludin and zonula occuldens-1 proteins of the blood-brain barrier tight junctions (Wang et al. 2013).

15.4 CNS Neoplasm Studies

The first in vivo study of curcumin's efficacy for treating brain tumors was published in 2009. Researchers injected tumor cells (B16F10 mouse melanoma) into the brain of mice and treated with either 667 μM (to an estimated plasma concentration of 35 μM) curcumin via tail vein injections or the carrier alone (DMSO). Another set of animals received direct intracerebral injections of curcumin via a cannula placed in the location of the B16F10 cell injections. In both of these experiments, the treated animals remained essentially tumor-free, whereas the carrier-treated animals were morbid by day 21. Further, in vitro studies in this experiment show dramatic decreases in cell viability by MTT assay in human oligodendroglioma and lung carcinoma (HOG and A549) as well as mouse neuroblastoma, glioma, and melanoma (N18, GL261, B16F10) cell lines after treatment with 50 μM curcumin (Purkayastha et al. 2009). An in vitro study examined NanoCurc™ (polymeric nanoparticle formulation of curcumin) for efficacy against human

medulloblastoma (DAOY, D283Med) and glioblastoma (HSR-GBM1, JHH-GBM14) cell lines. The authors report a marked reduction in growth of each of these cell lines when treated with NanoCurc™ (Lim et al. 2014). In another in vivo study, Perry et al. (2010) examined athymic mice xenografted with human U-87 malignant glioma cells being treated with curcumin. In one arm of the study, animals received intraperitoneal injections with curcumin or vehicle alone either 7 days before (chemopreventive effect) or 3 days after (therapeutic effect) subcutaneous U-87 cell inoculation. In these animals, the median tumor volume for the treated animals/control animals was 40.9% and 32.4%, respectively. These results suggest that administration of curcumin before or after tumor formation are both effective. In a separate arm of the study, 5×10^5 U-87 cells we implanted intracranially into athymic mice. They subsequently received intraperitoneal injections of curcumin or vehicle alone. Treatment with curcumin versus carrier led to a 12% increase in average survival (23.4 versus 20.9 days).

An in vitro study on human (T98G, U87MG, T67) and rat (C6) chemoradiation-resistant malignant glioma examined the effects of curcumin on sensitization to traditional chemotherapies (cisplatin, etoposide, camptothecin, and doxorubicin) and radiation. Curcumin alone was able to inhibit growth of these cell lines in a dose-dependent manner. More interestingly, in experiments where T98G and U87MG cells were pretreated with 25 μM curcumin and then exposed to five Gyradiation, a synergistic effect was noted. Similarly, pretreating T98G and U87MG cells with 25 μM curcumin led to dramatically decreased viability after administering cisplatin, etoposide, camptothecin, or doxorubicin. Cell survival was found to correlate with inhibition of NF-κB and AP-1, and sensitization by curcumin correlated with reduced DNA repair enzyme and Bcl-2 expression (Dhandapani et al. 2007). In an in vivo experiment, rats were injected intracranially with C6 glioma cells. Animals then received either curcumin (50 mg/kg/day) or vehicle intraperitoneal injections. The entire vehicle-treated animals developed significant tumor mass, whereas only 63% of the treated animals developed histochemically detectable tumors. Among the treated animals which developed tumors, 71% were significantly smaller than the vehicle-treated group. The authors also performed several intriguing in vitro experiments. First, they report IC_{50} values of different glioma cell lines (U138MG, C6, U87, U373) as well as astrocytes (29, 25, 19, 21, and 135 μM, respectively). These data suggest that curcumin spares normal glial cells at concentrations lethal to cancerous ones. In addition, the authors report U138MG and C6 cell viability as measure by MTT after treating with combinations of curcumin, cisplatin, and doxorubicin. Treatment with 25 μM curcumin, cisplatin, doxorubicin, curcumin + cisplatin, and curcumin + doxorubicin yielded 55%, 63%, 66%, 30%, and 36% viability for U138MG cells and 75%, 42%, 73%, 10%, and 46% for C6 cells (Zanotto-Filho et al. 2012). A separate in vitro study examining the coadministration of curcumin and paclitaxel for U138MG and LN18 glioblastoma cell lines revealed strong synergy between these drugs as well. When curcumin and paclitaxel were administered at 20 μM and 10 nM, a combination index (CI <1 indicates synergy) of 0.09 and 0.10 was reported for U138MG and LN18 cells, respectively (Hossain et al. 2012).

An in vivo model grew subcutaneous DAOY medulloblastoma tumors by injecting cells in SCID (the severe combined immunodeficiency) mice. These animals were fed oral curcumin by gavage and led to significant decreases in tumor burden (1442 mm^3 versus 2294 mm^3 in control animals). The authors also tested the Smo/Smo transgenic medulloblastoma model to examine the efficacy of curcumin for blood-brain barrier penetration after systemic administration. Median survival time for treated and control animals were 192 and 144 days, respectively. Additionally, the authors report biochemical analyses from the harvested tumor tissues revealing increased apoptotic markers, decreased HDAC4 levels, and elevated acetylation of α-tubulin among the treated animals (Lee et al. 2011). However, Zhuang et al. (2012) reported that curcumin may also target more stem-like precursor cells. Glioma-initiating cells (GICs) are thought to be cell populations responsible for initiation and propagation of glioblastoma. When GICs from surgically resected human tissue were grown in the presence of curcumin, they acquired markers of differentiation (Tuj1, GFAP, Olig2, βIII-tubulin) sooner than control cells and decreased expression of stem markers (CD133 and nestin). As such, curcumin is able to force these cells to differentiate. Once differentiated, the authors report these cells decrease their ability to self-renew. An in vivo xenograft arm within this study reported animals treated with curcumin had small, confined tumors with fewer microscopic abnormalities, whereas untreated animals developed large tumor burdens and microscopic features typical of glioblastoma. In addition, most of the treated mice survived the study period of 120 days, while all of the untreated animals perished. Electron microscopy of the animal tissues revealed a large number of autophagosomes in treated animals, suggesting that curcumin had induced autophagy (Zhuang et al. 2012). More recently, another study of patient-derived glioblastoma stem cells reported similar results. Administration of curcumin was able to reduce cell viability and yielded an IC_{50} of approximately 25 μM. Further, the authors report that even treatment with levels of curcumin that were not lethal to the stem cells (2.5 μM) led to significantly decreased proliferation and colony formation (Gersey et al. 2017).

15.5 Conclusions and Future Prospects

Many preclinical in vivo and in vitro studies exist to support the efficacy of curcumin for the treatment of CNS tumors. Sufficient preclinical, mechanistic, and safety studies exist to support trials of curcumin for the treatment of CNS neoplasms in humans. Thus, there is a need for improved therapeutics, especially for treating aggressive tumors such as glioblastoma. The delivery systems clearly described to overcome the pharmacokinetic barriers (poor water solubility, poor absorption, rapid metabolism) imposed on free curcumin. In addition, commercially prepared curcumin products have already been tested for increased bioavailability and safety profile. Furthermore, researches are needed to illuminate the beneficial effects of curcumins. In addition, use of curcumin in a randomized controlled trial as an adjunctive therapy for the treatment of CNS neoplasms such as glioblastoma will also be an area of future investigators.

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Chapter 16
Vitamin E: Nature's Gift to Fight Cancer

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16.1 Introduction

Vitamin E was first discovered in 1922 by Evans and Bishop and recognized as "anti-sterility factor X". Since then, vitamin E has attracted many scientists around the globe to venture into studying its properties and health benefits. The first therapeutic use of vitamin E was reported in 1938, which involved the use of wheat germ oil supplement to recover 11 of 17 premature new born babies suffering from growth failure (Bell 1987). Thereafter, further researches on vitamin E continued and highlighted its significance as a powerful lipid-soluble antioxidant (Tappel 1962; Burton and Ingold 1986; Esterbauer et al. 1991). Vitamin E contains two major substances such as tocopherols (TOCs) and tocotrienols (TCTs). These substances are present in eight different homologs, namely, α-tocopherol, β-tocopherol, γ-tocopherol, δ-tocopherol, α-tocotrienol, β-tocotrienol, γ-tocotrienol, and δ-tocotrienol (IUPAC-IUB Joint Commission on Biochemical Nomenclature 1982). Each of the TOC homologs are named according to the position and number of the methyl groups on the phenol ring, with the α-, β-, γ-, and δ-homologs containing three, two, two, and one methyl groups, respectively (Fig. 16.1). Earlier reports on the benefits of vitamin E were emphasized mainly on the effects of TOCs particularly α-TOC, which has been recognized as the most powerful lipid-soluble antioxidant (Tappel and Zalkin 1960; Tappel 1962, 1980; Dillard et al. 1978, 1980; Burton and Ingold 1981; Burton et al. 1982, 1985; Mukai et al. 1989; Pryor et al. 1988, 1993). The antioxidant activity is responsible for scavenging the propagation of reactive oxygen species (ROS) at the cellular membranes (Choe and David 2009). The massive concern on the powerful ability of α-TOC, however, had been upstaged by the emergence of the TCT derivatives following the discoveries of their potential

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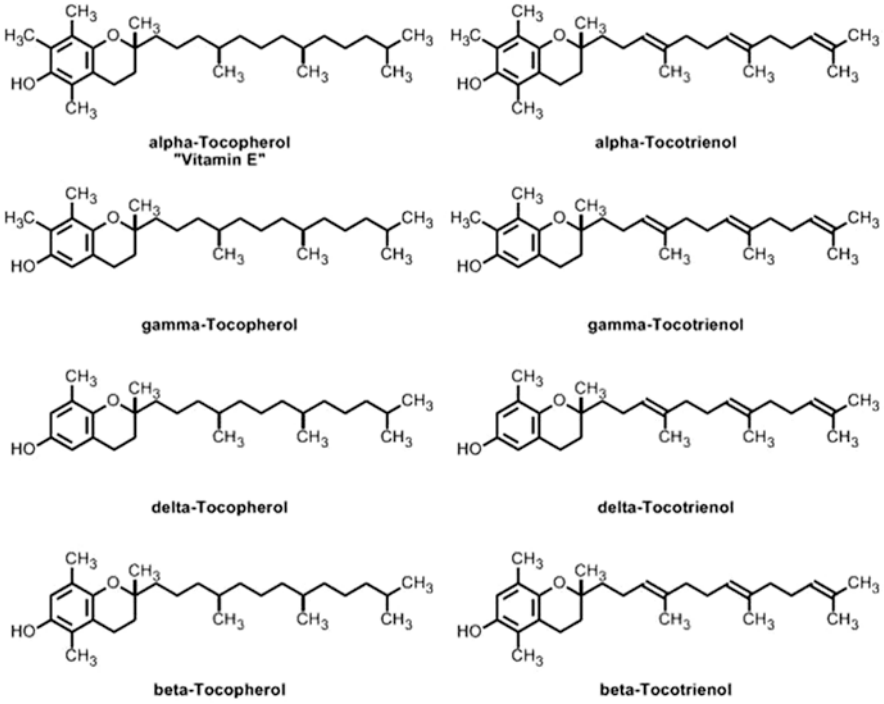

Fig. 16.1 Structural differences of TOCs and TCTs

as cholesterol-lowering (Qureshi et al. 1986) and anticancer agent (Kato et al. 1985; Sundram et al. 1989) in the late twentieth century. As a result, these findings had impacted on the redirection of vitamin E researches in the twenty-first century into wider aspects of health benefits, including antiaging, anti-angiogenesis, anticancer, cardiovascular diseases, neuroprotection, and radioprotection (Sen et al. 2007; Bharat et al. 2010).

Cancerous cells reflect the dysregulations in the signaling pathways that are supposed to control the normal proliferations. The dysregulations of the normal proliferations results in the uncontrolled cell growth, which will eventually form a mass that grows from the benign to the metastatic stage. Despite the common incidences of cancers nowadays, the establishments of reliable and comprehensive methods of treatments are still a huge disappointment for the medical community. The current uses of cancer treatments such as chemoprevention, chemotherapy, medications, and surgical methods are not completely successful in cancer prevention and treatments. The low rate of success in employing these methods were generally reported due to induction of adverse health effects, in spite of the high treatment costs the patients bear. High dependence on the chemically formulated drugs and medicines has resulted in the ineffectiveness of the current cancer treatment methods. This has raised the possibilities in moving into the use of natural products and traditional herbs. Natural plant compounds and herbs are becoming popular as one of the

proven traditional medicine that can be used in cancer prevention and treatments. The growing interest in the use of natural resources has provoked the possibility of using the plant-based medicine or food as one of the preventive and treatment methods in cancer patients. Thus, the aim of the chapter is to provide an overview of the previously published reports and also to discuss the current trends in vitamin E research and the anticipated future endeavors to maximize its uses against the cancerous cells.

16.2 Major Sources of Vitamin E

Among the various known sources of vitamin E, rice bran, palm oil, and annatto oil are the richest source. The ratio of TOCs to TCTs in rice bran is 50:50, in palm oil is 25:75, and in annatto oil is 0.1:99.9 (Tan 2014). The "tocopherol-free" aspect of annatto is highly valuable (Uchida et al. 2012). However, palm oil and rice bran contain approximately around 25–50% of α-TOC in the total vitamin E content.

16.3 Regulations and Bioavailability of Vitamin E

Vitamin E has been a well-known and studied vitamin for its antioxidant activity and potency (Tappel and Zalkin 1960; Tappel 1980; Dillard et al. 1980; Burton and Ingold 1981; Burton et al. 1985; Mukai et al. 1989; Pryor et al. 1993). The antioxidant activity of vitamin E homologs was due to the presence of relative H-atom-donating ability, which may increase the efficiency with the greater ring methyl substitution. In addition, the differences in the potency of α-TOC against the other homologs in vivo were due to the hepatic discrimination as well as the preferential metabolism. The massive concern on the powerful ability of α-TOC, however, had been upstaged by the emergence of the TCT derivatives following the discoveries of their potential as cholesterol-lowering and anticancer agent (Kato et al. 1985; Qureshi et al. 1986; Sundram et al. 1989). These discoveries were also supported by other studies which reported that although α-TCT has only one third of the biological activity of α-TOC (Bunyan et al. 1961; Weimann and Weiser 1991), it has higher or equivalent antioxidant activity compared to α-TOC (Serbinova et al. 1993; Suarna et al. 1993). In addition, a vitamin E analog [2,4,6,7-tetramethyl-2-(4′,8′,l2′-trimethyltridecyl)-5-hydroxy-3,4-dihydrobenzofuran] with equivalent biological activity to α-TOC (Ingold et al. 1990) has been reported to possess 1.5 times higher antioxidant activity (Burton et al. 1983). Thus, it has been concluded that the highest biological activity of vitamin E homologs could be due to presence of three methyl groups and a free hydroxyl on the chromanol ring, together with the isoprenoid tail. This specific structural requirement suggested the specific interactions of vitamin E homologs with proteins and other macromolecules (Traber and Packer 1995).

16.3.1 Regulation of Tocopherols (TOCs)

α-Tocopherol (α-TOC) has been mainly recognized for antioxidant activities and cell signaling aspects. Azzi et al. (2004) studied the impact of α-TOC as a signaling molecule onto the several cellular molecules and reported that it inhibits the protein kinase C (PKC) and 5-lipoxygenase. Moreover, it also activated the protein phosphatase 2A and diacylglycerol kinase (Mahoney and Azzi 1988; Boscoboinik et al. 1991a, b). Another study by Barella et al. (2004) reported that dietary vitamin E (α-tocopheryl acetate) induces changes in steroidogenesis by affecting the cholesterol homeostasis in adrenal glands and testes. In addition, the genes encoding for proteins that are involved in the uptake (LDL receptor) and de novo synthesis, for instance, the 7-dehydrocholesterol reductase, 3-hydroxy-3-methylgluteryl coenzyme A synthase, 3-hydroxy-3-methylglutaryl coenzyme A reductase, isopentenyl diphosphate δ-isomerase, and farnesyl pyrophosphate synthetase of cholesterol, which are the precursors of all steroid hormones have been identified as α-TOC-sensitive molecular targets (Barella et al. 2004).

α-TOC is also involved in the modulation of two major signaling pathways that are dependent on the involvement of PKC and phosphatidylinositol 3-kinase (PI3K). The involvement of α-TOC in PKC pathway has been reported earlier (Boscoboinik et al. 1991b). Besides, there is also a study which reported that TOCs do not only inhibit the formation of free radicals but also inhibit the tyrosine kinase activity, in a study done using the tissue plasminogen activator (TPA)-induced primary human fibroblasts or HL-60 cells (Sharma et al. 1994). Meanwhile, the involvement of α-TOC in phosphatidylinositol 3-kinase (PI3K) has been reported recently. According to Jean-Marc et al. (2015), α-TOC and the phosphorylated form of α-TOC, α-tocopheryl phosphate (α-TP), increased the vascular endothelial growth factor (VEGF)-promoter activity in a phosphatidylinositol-3-kinase gamma (PI3Kγ)-dependent manner. Other than that, there are also several genes that are being partly regulated (due to the effects of α-TOC on PKC and PI3K), and there are also genes that are being independently regulated by TOCs. These categories of genes can be divided into five different groups. The first group (Group 1) consists of the genes that are involved in the uptake and degradation of TOCs which are α-tocopherol transfer protein (α-TTP), cytochrome P450 (CYP3A), γ-glutamyl-cysteine synthetase heavy subunit, and glutathione-S-transferase. The second group (Group 2) consists of the genes that are implicated with lipid uptake and atherosclerosis such as CD36, SR-BI, and SR-AI/II. Meanwhile, the genes grouped in the third group (Group 3) consist of the genes that are involved in the modulation of extracellular proteins including tropomyosin, collagen-α-1, MMP-1, MMP-19, and connective tissue growth factor. The fourth group (Group 4) consists of the genes that are connected to adhesion and inflammation which are E-selectin, ICAM-1 integrins, glycoprotein IIb, IL-2, IL-4, IL-1b, and transforming growth factor-β (TGF-β). Finally, Group 5, consists of genes that are implicated in cell signaling and cell cycle regulation, which are PPAR-γ, cyclin D1, cyclin E, Bcl2-L1, p27, CD95 (APO-1/Fas ligand), and 5a-steroid reductase type 1 (Azzi et al. 2004).

The intracellular transportation of vitamin E is being regulated by tocopherol transfer protein (TTP). The TTP protein has been shown and described in cell cultures (Horiguchi et al. 2003), animals (Sato et al. 1991; Dutta-Roy et al. 1993; Zimmer et al. 2000), and various human tissues (Arita et al. 1995; Azzi 2007). The discovery of α-TOC specific membrane receptors (Urano et al. 1992) and cytosolic transfer proteins have strengthened the theory that vitamin E possesses other properties than just being an antioxidant agent (Traber and Packer 1995). This was supported by an earlier study which reported that α-TTP is responsible for maintaining plasma α-tocopherol concentrations (Traber et al. 1990). Following the discovery of the α-TOC specific membrane receptors, the crystal structure of α-TTP has been reported (Meier et al. 2003; Min et al. 2003). There are three important structural features of the ligand (α-TOC) needed for recognition by α-TTP. The first feature is a fully methylated chroman ring, second is a phytyl pyrophosphate-derived tail (Della Penna and Pogson 2006), and third is the R-configuration at C-2 position where the tail attaches to the chromanol ring (Panagabko et al. 2003). Besides the usage of α-TTP, vitamin E also enters the circulation from the intestine in chylomicrons. The conversion of chylomicrons to remnant particles results in the distribution of newly absorbed vitamin E to all of the circulating lipoproteins and ultimately to the tissues. This enrichment of lipoproteins with vitamin E is a key mechanism by which vitamin E is delivered to tissues (Traber et al. 2004).

16.3.2 Regulation of Tocotrienols (TCTs)

α-Tocotrienols (α-TCTs) possess better antioxidant properties than α-TOC (Serbinova et al. 1991; Serbinova and Packer 1994). This is due to the unsaturated side chain of TCTs that allows for efficient penetration through better distribution on tissue membranes with saturated fatty layer (Suzuki et al. 1993). Besides having the antioxidant activities, TCTs also exhibited the antiproliferative (Parajuli et al. 2015; Alawin et al. 2016), anti-survival (Samant et al. 2010), proapoptotic (McIntyre et al. 2000), antiangiogenic (Weng-Yew et al. 2009), and anti-inflammatory (Wu et al. 2008) activities. The effects of TCTs are regulated through a number of molecular targets. These molecular targets of TCTs can be classified into two groups, the targets that are modulated by direct binding (van Haaften et al. 2002; Zhou et al. 2004; Khanna et al. 2007; Comitato et al. 2009; Upadhyay and Misra 2009) and the targets that are modulated by indirect binding, meaning that the modulation of various targets by TCTs occurs at either the transcriptional, translational, or posttranslational levels. For instance, Src and 3-hydroxy-3-methyl-glutaryl coenzyme A (HMG-CoA) reductase are modulated through direct binding, whereas inflammatory transcription factors and the genes regulated by them and death receptors are modulated indirectly.

The antioxidant activities of TCTs are mediated through induction of antioxidant enzymes such as superoxide dismutase (Newaz and Nawal 1999; Lee et al. 2009) and glutathione peroxidase (Adam et al. 1996), which quench free radicals such as

superoxide radicals. Meanwhile, the antiproliferative activity of TCTs is mediated through modulation of growth factors such as vascular endothelial growth factor (VEGF) (Weng-Yew et al. 2009), basic fibroblast growth factor (bFGF) (Rashid et al. 2008), and transforming growth factor-β (TGF-β) (Shun et al. 2004), HER2/neu (Pierpaoli et al. 2010), and interleukin-6 (IL-6) (Wu and Ng 2010). In addition, the cyclin-dependent kinases (Cdk2, Cdk4, Cdk6) and their inhibitors, the p21, p27, and p53 (Agarwal et al. 2004; Samant et al. 2010), are also involved in the regulation of growth-suppressive effects by TCTs. Besides, the inhibition of mitogen-activated protein kinases (MAPK) such as ERK (Sun et al. 2008), p38 MAPK, and JNK (Park et al. 2010) is also a factor of the antiproliferative effects of TCTs. Furthermore, the suppression of cyclin D1 induced by TCTs also plays an important role in the anti-survival activities of this vitamin (Elangovan et al. 2008; Wali et al. 2009; Samant et al. 2010). This was also supported by an earlier study which reported that TCTs impede the survival of various tumor cells by inhibiting the expression of NF-κB-regulated gene products associated with anti-apoptosis such as XIAP, IAP-1, IAP-2, bcl-2, bcl-xl, c-FLIP, TRAF-1, survivin, and Bfl-1/A1 (Ahn et al. 2007). The growth inhibitory effect of TCTs was also reported to be through the suppression of phosphotidylinositol-3-kinase (PI3K)/AKT pathway (Samant and Sylvester 2006). In addition, the downregulation of the telomerase, c-myc, and raf-ERK signaling pathways has been also linked to TCTs' ability to inhibit cell survival (Eitsuka et al. 2006; Sun et al. 2008).

The pro-apoptotic effects are one of the many functions of TCTs, with various studies revealing that TCTs are able to induce apoptosis in a wide variety of tumor cells. These effects are mediated through activation of both extrinsic and intrinsic pathways. The extrinsic pathways involve induction of death receptors (Park et al. 2010) and activation of caspase-8, which leads to caspase-3 activation (Shah et al. 2003; Shah and Sylvester 2004). Meanwhile, the activation of intrinsic pathways by TCTs involves mitochondrial depolarization (Rickmann et al. 2007) and is mediated through the upregulation of *Bax* and *Bid* (Sakai et al. 2006), release of cytochrome *c* (Rickmann et al. 2007), and activation of caspase-9, which in turn leads to activation of caspase-3 (Sakai et al. 2006; Sun et al. 2009). Besides, the apoptosis induced by TCTs was also reported to be mediated through DNA fragmentation (Srivastava and Gupta 2006; Rickmann et al. 2007) and regulation of p53 in human colon carcinoma RKO cells (Agarwal et al. 2004). The suppression of angiogenesis by TCTs also has been reported to be mediated through the inhibition of VEGF expression (Weng-Yew et al. 2009) and VEGF receptor signaling (Miyazawa et al. 2008; Shibata et al. 2009), whereas the anti-inflammatory effect of TCTs was reported following the findings where γ-TCT suppresses the signal transducer and activator of transcription 3 (STAT3) cell signaling pathway which is involved in inflammation (Bachawal et al. 2010). Furthermore, hypoxia-induced factor-1 is another pathway that has been linked with inflammation and is modulated by TCTs (Shibata et al. 2008a). Besides all the molecular targets described above, TCTs also have been reported to inhibit various protein kinases, including protein kinase C

(Ozer et al. 1995), p60 Src (Sen et al. 2000) IκBα kinase (Sylvester et al. 2005), and glycogen synthase kinase-3β (GSK-3β) (Shah and Sylvester 2004). All of the effects induced by TCTs in the various cell signaling pathways have been linked to their (TCTs) effects against cancer, diabetes, cardiovascular problems, and neurological diseases.

16.4 Mechanistic Aspects of Vitamin E Against Cancerous Cells

The metabolic process influencing the in vitro bioactivity of different forms of vitamin E showed the concentration dependent functional regulation of different signaling pathways and groups of genes (Nesaretnam et al. 2004; Yap et al. 2008). The metabolism of TOCs and TCTs is mainly involved in an initial CYP450-dependent ω-hydroxylation followed by β-oxidation, resulting in the rapid formation of carboxyhethyl hydroxychroman acid (CEHC). CHEC is the main in vivo metabolite of vitamin E that is found in plasma and urine and considered the most useful indicator of vitamin E metabolism in humans and animals (Galli et al. 2002, 2003). The beneficial effects of vitamin E have been widely reported; however, the specificity of action and health-promoting properties of vitamin E, especially TCTs, are believed to also involve other players (Galli and Azzi 2010). These may include α-TTP-like and/or low-affinity-binding proteins and other protein-lipid and lipid-lipid interactions in cell membranes and in the cytosol (Atkinson et al. 2008), as well as the intracellular receptors such as ERβ (Comitato et al. 2009). As a consequence of a specific structure-function and in analogy with the behavior of α-TOC, TCTs may also co-localize and interact with polyunsaturated fatty acid (PUFA) and cholesterol molecules in the membrane to influence the composition and signaling function of membrane microdomains such as the "membrane rafts" (Atkinson et al. 2008). This increases their recognition as the regions of the plasmalemma that may control cell signaling and redox responses through key elements such as the enzyme NADPH-oxidase and PKC (Galli 2007a, b). In addition, the specificity of TCT signaling includes the control of the protein kinase C (Eitsuka et al. 2006) as well as of other kinases such as PI3K/Akt (Shah and Sylvester 2004; Shibata et al. 2008b; Uto-Kondo et al. 2009), ERK-MAPK (Sun et al. 2008), and cyclin-dependent kinases (Elangovan et al. 2008). Besides, the regulation of enzymes such as HMG-CoA reductase in cholesterol biosynthesis appears to be regulated by TCTs at the post-translational level (Song and De Bose-Boyd 2006). Telomerase activity, a downstream component of the PKC signaling, is also reported to possibly play a role in the TCT-dependent control of cell proliferation (Eitsuka et al. 2006). The emergence of vitamin E as an anticancer substance has been extensively studied. The various mechanisms of signaling pathways responsible for the suppression and proliferation of cancer cells are summarized in tabular form (Table 16.1).

Table 16.1 Summary of anticancer effects/mechanisms of action by vitamin E against various types of cancer cells

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
Breast	Corn oil (CO) soybean oil (SBO) crude palm oil (CPO)	CO and SBO differ greatly from the palm oils in the contents of tocopherols, tocotrienols, and carotenes, and high PO diets did not promote chemically induced mammary tumorigenesis in female rats when compared to high CO or SBO diets	Sundram et al. (1989)
	Refined, bleached, deodorized palm oil (RBD PO)		
	Metabisulfite-treated palm oil (MCPO)		
	Palm TRF	TRF inhibited the proliferation of cells by 50%	Nesaretnam et al. (1995)
	α-TOC		
	TRF	Combinations of γ-TCT or δ-TCT with tamoxifen (in ratio of 1:1) in MCF-7 cells showed a synergistic inhibitory effect on the rate of cell proliferation and growth	Guthrie et al. (1997)
	α-TOC		
	α-TCT		
	γ-TCT		
	δ-TCT		
	Palm TRF	TRF inhibited growth of MCF7 cells in both the presence and absence of estradiol with a nonlinear dose-response	Nesaretnam et al. (1998)
	α-TOC	Preneoplastic (CL-S1), neoplastic (-SA), and highly malignant (+SA) mouse mammary epithelial cells preferentially accumulate TCTs as compared to TOCs, and highly malignant (+SA) cells were the most sensitive, whereas the preneoplastic (CL-S1) cells were the least sensitive to the antiproliferative and apoptotic effects of TCTs	McIntyre et al. (2000)
	γ-TOC		
	δ-TOC		
	α-TCT		
	γ-TCT		
	δ-TCT		
	Palm TRF		
	Palm TRF	TRF exerted inhibition in early postreceptor events involved in cAMP production upstream from EGF-dependent MAPK and phosphoinositide 3-kinase/Akt mitogenic signaling in preneoplastic CL-S1 mouse mammary epithelial cells	Sylvester et al. (2002)
	Palm TRF	TCT-induced apoptosis in (+SA) mammary cancer cells is mediated through activation of the caspase-8 signaling and is independent of caspase-9 activation	Shah et al. (2003)
	γ-TCT		
	Palm TRF	TCTs are able to affect cell homeostasis in human breast cancer cells, possibly independent of the antioxidant activity	Nesaretnam et al. (2004)

(continued)

Table 16.1 (continued)

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
	δ-TCT	α -TEA- and δ-TCT-induced apoptosis involved upregulation of TGF-beta receptor II expression and TGF-beta-, Fas-, and JNK-signaling pathways	Shun et al. (2004)
	Vitamin E analog (α-tocopherol ether acetic acid analog [alpha-TEA; 2,5,7,8-tetramethyl-2R-(4R,8R,12-trimethyltridecyl) chroman-6-yloxyacetic acid]		
	γ-TCT	γ-TCT induced apoptosis through mitochondria mediated death pathway	Takahashi and Loo (2004)
	α-TCT	TCT-induced caspase-8 activation and apoptosis in malignant (+SA) mammary epithelial cells is not mediated through the suppression of the PI3K/PDK/Akt mitogenic signaling pathway and subsequent reduction in intracellular FLIP expression	Shah and Sylvester (2005a)
	γ-TCT	Antiproliferative effects of γ-TCT resulted from a reduction in Akt and NF-κB activity in neoplastic (+SA) mammary epithelial cells	Shah and Sylvester (2005b)
	γ-TCT	Antiproliferative and apoptotic effects of γ-TCT are mediated by a reduction in the Pl3K/PDK-1/Akt signaling	Sylvester et al. (2005)
	γ-TCT	Antiproliferative effects of γ-TCT in neoplastic (+SA) mouse mammary epithelial cells are mediated by a suppression in ErbB3-receptor tyrosine phosphorylation and the subsequent reduction in PI3K/PDK-1/Akt mitogenic signaling	Samant and Sylvester (2006)
	γ-TCT	Addition of γ-TCT with either EGCG or resveratrol on breast cancer cells resulted in a significant additive effect in reducing cyclin D1 and bcl-2 expression	Hsieh and Wu (2008)
	γ-TCT	ER stress apoptotic signaling is associated with γ-TCT-induced apoptosis in (+SA) mammary tumor cells	Wali et al. (2009)
	γ-TCT	Combined treatment of γ-tocotrienol with erlotinib or gefitinib prevents ErbB receptor heterodimer cooperation and inhibits EGF-dependent mitogenic signaling in (+SA) murine mammary tumor cells	Bachawal et al. (2010)

(continued)

Table 16.1 (continued)

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
	α-TCT γ-TCT δ-TCT	γ-TCT suppressed cell growth through regulation of time-dependent and dose-dependent manner, accompanied by the modulation of cell cycle regulatory proteins such as the Rb/E2F complex, cyclin D1/CDK4, and cyclin B1/CDK1, inhibition of specific Rb phosphorylation, and by the time- and dose-dependent increase in the expression of quinone reductase NQO2	Hsieh et al. (2010)
	γ-TCT	γ-T3 induced apoptosis through upregulation of DR5 and CHOP and other endoplasmic reticulum (ER) stress markers	Park et al. (2010)
	α-TCT γ-TCT δ-TCT Alpha-tocopheryl succinate (α-TOS)	γ-TCT and δ-TCT, and α-TOS induced apoptosis possibly via the mitochondrial pathway and the expression of senescent-like growth arrest markers as p53, p21, and p16	Pierpaoli et al. (2010)
	γ-TCT	Antiproliferative effects of γ-TCT on (+SA) mammary tumor cells are associated with reduction in cell cycle progression from G1 to S phase, as evidenced by increased in p27 levels, and a corresponding decrease in cyclin D1, CDK2, CDK4, CDK6 and phosphorylated Rb levels	Samant et al. (2010)
	γ-TCT α-TCT	γ-TCT-induced apoptosis is associated with suppression of Id1 and NF-κB through modulation of the upstream regulators (Src, Smad1/5/8, Fak, and LOX), as well as the induction of JNK signaling pathway	Yap et al. (2010)
	Annatto-TCTs	Antitumor effects were exerted through reduction in the HER-2/neu mRNA and p185(HER-2/neu) protein in tumors and tumor cell lines	Pierpaoli et al. (2013)

(continued)

Table 16.1 (continued)

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
Cervix	α-TOC α-TOC acetate γ-TCT	α-TOC and γ-TCT induced apoptosis through enhanced expressions of p53, Bax and Caspase-3, and the activity of Caspase-3 in cervical carcinoma CaSki cell	Hasani et al. (2008)
	α-TOC α-TCT γ-TCT δ-TCT	TCTs demonstrated a dose-dependent and time-dependent induction of cell death through cell cycle arrest at G2/M phase (downregulation of cyclin D3, p16, and CDK6 expression) and inhibition of HeLa cell proliferation through the upregulation of IL-6	Wu and Ng (2010)
	α-TOC Palm TRF	Palm TRF exerted the antiproliferative effects in CaSki cells through downregulation of the MEK-2 and ERK-2 protein expression	Hasani et al. (2011)
	γ-TCT	γ-TCT inhibits the spherical cell growth of cervical cancer cells	Gu et al. (2015)
Colon	Palm TRF	TRF-induced apoptosis in colon carcinoma cells mediated by p53 signaling which appears to be independent of cell cycle association	Agarwal et al. (2004)
	δ-TCT	The inhibitory mechanism of δ-TCT is by reducing HIF-1α protein expression or increasing HIF-1α degradation. δ-TCT also tend to suppress the hypoxia-induced COX-2 protein expression (implying a possible posttranscriptional mechanism by δ-TCT)	Shibata et al. (2008a)
	γ-TCT	γ-TCT inhibits cell proliferation and induces apoptosis in a time- and dose-dependent manner, accompanied by cell-cycle arrest at G0/G1 phase, increase in Bax/Bcl-2 ratio, and activation of caspase-3. Nuclear factor-κB p65 protein also may be involved	Xu et al. (2009)
	Atorvastatin (ATST) with γ-TCT and celecoxib (CXIB)	The synergistic action of the three compounds (ATST, γ-TCT, and CXIB) was showed by their induction of G0/G1 phase cell cycle arrest and apoptosis	Yang et al. (2010)
	γ-TCT	γ-TCT sensitizes tumor cells to tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) by upregulating DRs through the ROS/ERK/p53 pathway and by downregulating cell survival proteins	Kannappan et al. (2010)

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Table 16.1 (continued)

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
Liver	TOC	TCT induces apoptosis involving the activation of caspase-8 activity	Sakai et al. (2004)
	TCT		
	α-TOC	TCT reduced the cell viability possibly through apoptosis	Har and Keong (2005)
	TCT		
	α-TCT	Antiproliferative effect of δ-TCT through induction of apoptosis, S-phase arrest, and increase in CYP1A1 gene expression in hepatocellular carcinoma HepG2 cells	Wada et al. (2005)
	β-TCT		
	γ-TCT		
	δ-TCT		
	γ-TCT	γ-TCT induced apoptosis in Hep3B cells through caspase-8 and caspase-9 activation, suggesting Bax and Bid genes were involved	Sakai et al. (2006)
	γ-TCT	Antiproliferative effect of γ-TCT in HepG2 cells is probably through the downregulation of HMGCoA reductase activity	Aida et al. (2007)
	γ-TCT	γ-TCT possibly has direct influence on the expression dynamics of peroxiredoxin-4 (Prx4) to control proliferation in HepG2 cells	Sazli et al. (2015)
Lung	Synthetic redox-silent analog of alpha-tocotrienol (T3) (6-O-carboxypropyl-alpha-tocotrienol (T3E))	T3E induced cytotoxicity on accumulation of cells in the G1-phase and subsequently induced apoptosis following the inhibition of Ras farnesylation and a marked decrease in the levels of cyclin D and Bcl-xL	Yano et al. (2005)
	Synthetic redox-silent analog of alpha-tocotrienol (T3) (6-O-carboxypropyl-alpha-tocotrienol (T3E))	T3E suppresses hypoxia adaptation of lung cancer cells by the inhibition in hypoxia-induced activation of Src signaling	Kashiwagi et al. (2008)
	Synthetic redox-silent analog of alpha-tocotrienol (T3) (6-O-carboxypropyl-alpha-tocotrienol (T3E))	T3E inhibited the chemoresistant mesothelioma cell growth through G2/M arrest in cell cycle and induction of apoptosis, inhibition of Src activation and Src-independent Stat3 activation	Kashiwagi et al. (2009)
	δ-TCT	δ-TCT inhibited cell growth and induced apoptosis by downregulation of Notch-1 via inhibition of NF-κβ signaling pathway	Ji et al. (2011)

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Table 16.1 (continued)

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
	δ-TCT Cisplatin	Combined treatment of δ-TCT and cisplatin inhibited cell growth and induced apoptosis by downregulation of Notch-1, inhibition of NF-κβ signaling pathway, and increased caspase-3 and PARP expressions	Ji et al. (2012)
Ovary	D-alpha tocopheryl acetate	D-alpha tocopheryl acetate suppressed endogenous telomerase activity in ovarian cancer cells	Bermudez et al. (2007)
	Palm TRF Cyclophosphamide (CPA)	Coadministration of TRF with CPA confers protection against apoptosis in ovaries from chemotherapy-associated damage	Saleh et al. (2014)
	Palm TRF Cyclophosphamide (CPA)	TRF administration reversed the abnormal folliculogenesis with accompanied reduced ovulation rate, follicular edema, increased vascularity, and inflammatory cell infiltration induced by CPA	Saleh et al. (2015)
Pancreas	α-TOC α-TCT β-TCT γ-TCT δ-TCT Palm TRF	TCTs selectively triggered apoptosis by targeting the mitochondrial permeability transition pore	Rickmann et al. (2007)
	d-δ-TCT	D-delta-tocotrienol induced cell cycle arrest at the G1 phase and apoptosis	Hussein and Mo (2009)
	γ-T3	γ-T3 inhibited the growth and sensitize the pancreatic tumor cells to gemcitabine by suppressing NF-κB-mediated inflammatory pathway	Kunnumakkara et al. (2010)
	γ-TCT δ-TCT	TCTs can induce apoptosis through the suppression of proliferative signaling pathways such as those mediated by the PI3-kinase/AKT and ERK/MAP kinases via downregulation of Her2/ErbB2 expression	Shin-Kang et al. (2011)
	Meta-analysis	A high level of vitamin E was significantly linked to a decreased risk of pancreatic cancer – a meta-analysis suggesting vitamin E might effectively prevent pancreatic cancer	Lujian et al. (2015)

(continued)

Table 16.1 (continued)

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
Prostate	γ-TOC	γ-TOC and γ-CEHC showed an inhibitory effect on prostate cancer cell growth	Galli et al. (2004)
	γ-CEHC (γ-carboxyethyl-hydroxychro-man)		
	γ-TCT	The size of the tumors were reduced by almost 40% in γ-TCT-treated and irradiated mice	Kumar et al. (2006)
	γ-TCT	γ-TCT induced apoptosis and necrosis in LNCaP and PC-3 cells. Treatment with γ-TCT results in cytochrome *c* release and PARP cleavage in both cells	Jiang et al. (2006)
	Palm TRF	Treatment with palm TRF decreases cell viability and colony formation through a dose-dependent G0/G1 phase arrest and sub G1 accumulation in prostate cancer cell lines	Srivastava and Gupta (2006)
	γ-TCT	γ-TCT showed selective growth inhibition activity	Campbell et al. (2007)
	Palm TRF	TCTs induced apoptosis and cell cycle arrest in PC-3 cells through increased expression of Fas receptor, Fas ligand, caspase 8, caspase 3, and bax gene	Nesaretnam et al. (2008)
	α-TOC		
	α-TCT		
	γ-TCT		
	δ-TCT		
	γ-TCT	γ-TCT induced apoptosis by activation of procaspases and the presence of sub G1 cell population. Apoptosis was associated with suppression of NF-κB, EGF-R, and Id family proteins (Id1 and Id3), induction of JNK-signaling pathway, suppression of mesenchymal markers, and the restoration of E-cadherin and gamma-catenin expression	Yap et al. (2008)
	TCT (\#947;-T3)	\#947;-T3 may exert anticancer effect by the downregulation of the prostate cancer stem cell markers (CD133/CD44) expression	Luk et al. (2009)
	Vitamin E natural homologs (α-TOC, β-TOC, γ-TOC, δ-TOC, α-TCT, β-TCT, γ-TCT and δ-TCT)	Vitamin E homologs and synthetic derivatives may induce cell death in prostate tumors that are resistant to caspase-activating therapeutic agents	Constantinou et al. (2009)
	Succinate synthetic derivatives		
	α-TS, β-TS, γ-TS, δ-TS (TS=tocopheryl succinates)		

(continued)

Table 16.1 (continued)

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
	γ-TCT Docetaxel (DTX)	γ-TCT (alone or in combination with DTX) may provide improve therapeutic efficacy against prostate cancer cells	Yap et al. (2010)
	Mixed TCTs (D-α-TCT (12–14%), D-β-TCT (1%), D-γ-TCT (18–20%), D-δ-TCT (4–6%), and D-α-TOC (12–14%)	Mixed TCTs suppress prostate tumorigenesis in the transgenic adenocarcinoma mouse prostate (TRAMP) mice by modulating cell cycle regulatory proteins and increasing expression of proapoptotic proteins	Barve et al. (2010)
	γ-TCT	γ-TCT inhibits the growth of cancer cells through the downregulation of the latent precursor and the mature forms of TGFβ2 and the concomitant disruptions in TGFβ receptor I, SMAD-2, p38, and NF-κB signaling	Campbell et al. (2011)
	γ-TCT	γ-TCT downregulated the expression of cancer cell markers (CD133/CD44)	Luk et al. (2011)
	γ-TCT	γ-TCT treatment induced apoptosis, necrosis, and autophagy in human prostate cancer cells	Jiang et al. (2012)
	α-TOC γ-TOC γ-TCT	γ-TCT exerted cytotoxic effects and induces cellular apoptosis through the modulation of phospho-c-Jun and MAPK pathways	Mahboob et al. (2013)
	Annatto-TCT (mainly contains δ-TCT)	Annatto-TCT suppresses cell growth in human prostate cancer cells through inhibition of Src and STAT3 genes	Sugahara et al. (2015)
	d-δ-TCT Geranylgeraniol	Combinations of d-δ-TCT and geranylgeraniol cumulatively suppressed the level of membrane HMG-CoA reductase and putatively reduced membrane Ras protein	Yeganehjoo et al. (2015)
	d-δ-TCT Geranylgeraniol	d-δ-TCT and geranylgeraniol synergistically induced cell cycle arrest at G1 phase, accompanied by downregulation of HMG-CoA reductase expression and reduce in membrane K-RAS protein	Yeganehjoo et al. (2017)
Skin	γ-TCT	γ-TCT induced apoptosis by activation of procaspases and the accumulation of sub-G1 cell population and was associated with suppression of NF-κB, EGF-R, and Id family proteins, induction of JNK signaling pathway. Also, γ-TCT suppressed the mesenchymal markers and the restoration of E-cadherin and γ-catenin expression	Chang et al. (2009)

(continued)

Table 16.1 (continued)

Cancer cell types	Types of vitamin E	Effects/mechanism of action	References
Stomach	γ-TCT	γ-TCT induced apoptosis through downregulation of the extracellular signal-regulated kinase (ERK) signaling pathway, and it was accompanied by downregulation of Bcl-2, upregulation of Bax, activation of caspase-3, and subsequent poly (ADP-ribose) polymerase cleavage	Sun et al. (2008)
	γ-TCT	γ-TCT induced apoptosis in gastric cancer cells through mitochondria-dependent apoptosis pathway	Sun et al. (2009)
	γ-TCT	γ-TCT mediates the antitumor metastasis activity in gastric cancer cells	Liu et al. (2010)

α-TOC, alpha-tocopherol; *β-TOC*, beta-tocopherol; *γ-TOC*, gamma-tocopherol; *δ-TOC*, delta-tocopherol; *α-TCT*, alpha-tocotrienol; *β-TCT*, beta-tocotrienol; *γ-TCT*, gamma-tocotrienol; *δ-TCT*, delta-tocotrienol; *α-TOS*, alpha-tocopheryl succinate; *γ-CEHC*, gamma-carboxyethyl-hydroxy-chro-man); *TS*, tocopheryl succinates; *TRF*, Tocotrienol-rich fraction

16.5 Clinical Studies on Vitamin E

Vitamin E has been reported as an effective anticancer agent, and its uses in treatments of cancers have been the subject of significant clinical trials. The earlier clinical use of vitamin E has been documented way back in the 1980s. According to Machlin (1985), early supplementation of vitamin E to infants with low birth weight (less than 1500 g) resulted in the decrease of the symptoms of retinopathy of prematurity and a reduced occurrence of intraventricular hemorrhage. In addition, neurological symptoms such as areflexia, ataxia, and sensory neuropathy were prevented or reversed when vitamin E was given to children with cholestatic liver disease before 3 years of age. The author also reported on the epidemiological studies indicating that subjects with higher levels of blood vitamin E have lower risk of death from ischemic heart disease and cancer, a lower risk of breast cancer, and a lower incidence of infections (Machlin 1985).

Following the report by Machlin (1985), together with the reports on its anticancer effects by Kato et al. (1985) and Sundram et al. (1989), vitamin E has received much attention due to the claim on its ability in reducing the risk of cancer. This has resulted in the extensive research on the benefits of vitamin E on the clinical aspects. Surprisingly, the obtained reports from the clinical studies on cancer patients are contradictory to the reports from the laboratory experimentations. This had led to a continuous controversial debate on the effectiveness of the use of vitamin E in cancer prevention and treatments to date. For instance, a clinical study has reported on the adverse effects of α-TOC and β-carotene on the incidences of lung cancer (Albanes et al. 1996). The study involved a total of 29,133 men aged 50–69 years who smoked five or more cigarettes daily. They were randomly assigned to receive

50 mg α-TOC, 20 mg β-carotene, α-TOC and β-carotene, or a placebo daily for 5–8 years. Findings of this study indicated that no overall effect was observed for lung cancer from α-TOC supplementation; however β-carotene supplementation was associated with increased lung cancer risk. The authors concluded that supplementation with α-TOC or β-carotene does not prevent lung cancer in older men who smoke. It was also assumed that β-carotene supplementation at pharmacologic levels may modestly increase lung cancer incidence in cigarette smokers (Albanes et al. 1996).

Another major hallmark study in the clinical use of vitamin E in cancer treatment also has been reported (Eric et al. 2011). The study was named as Selenium and Vitamin E Cancer Prevention Trial, which was simply known as SELECT. The aim of this study was to determine the long-term effect of vitamin E and selenium on the risk of prostate cancer in relatively healthy men. It involved a total of 35,533 men from 427 study sites in the United States, Canada, and Puerto Rico. The study subjects were assigned to receive oral selenium (200 μg/day) from *L*-selenomethionine with matched vitamin E placebo, vitamin E (400 IU/day of all *rac*-α-tocopheryl acetate) with matched selenium placebo, both agents, or both matched placebos. It was revealed from the results the emergence of increased risk of prostate cancer with the supplementation of vitamin E in healthy men. In contrast to the SELECT study, interestingly, Chung et al. (2012) conducted an animal study and revealed that γ- and δ-TOC as well as the naturally occurring mixture of TOCs are exerting the preventive activity compared to α-TOC alone. The authors attempted to explain on the failures to show the cancer preventive effect of α-TOC in the SELECT study, suggesting that administration with vitamin E diet or supplements that are rich in γ- and δ-TOC are cancer preventive, whereas supplementation with high doses of α-TOC alone is not having any effect. Nevertheless, a lot of studies are required in order to gain a comprehensive understanding on the effects of vitamin E homologs in clinical cancer prevention and treatments.

16.6 Current Trends in Vitamin E Research

During the earlier period of vitamin E researches, TCTs have not been studied as extensively as TOCs. The benefits of TCTs came into the attention only during the late 1980s, when their cholesterol-lowering potential (Qureshi et al. 1986) and anticancer effects were published (Kato et al. 1985; Sundram et al. 1989). Since then, the benefits of TCTs have begun to be extensively studied including their effects on hypocholesterolemia (Qureshi et al. 2002), skin protection (Traber et al. 1997, 1998; Weber et al. 1997), antiaging (Schaffer et al. 2005), cardiovascular diseases (Sylvester and Theriault 2003), nervous system disease (Sen et al. 2004), and bone health (Norazlina et al. 2002; Ima-Nirwana and Suhaniza 2004; Nizar et al. 2012). In addition, the effects of vitamin E in reproductive-related problems including fertility and preimplantation embryonic development have also been studied (Mokhtar et al. 2008; Rajikin et al. 2009, 2015; Nasibah et al. 2012a, b; Kamsani et al. 2013;

Lee et al. 2014; Saidatul et al. 2014; Syairah et al. 2014, 2015, 2016). In continuation to these studies, current trends in vitamin E researches are focused toward gaining an in-depth knowledge on the molecular structures of vitamin E and its molecular interactions, the mode of interactions between vitamin E homologs and the regulating molecules, and the basis of the various inter-signaling regulations of the pro-survival and pro-apoptotic pathways. These are really important as they will serve as the fundamental evidence in the future development of vitamin E-based cancer therapeutics.

16.7 Conclusions and Future Prospects

The anticancer potential of vitamin E only came into attention in the last few decades. This may increase the interest of researchers in understanding the ability of vitamin E as a promising natural anticancer substance. Despite the numerous numbers of published reports, a lot of works on human and animal studies are still warranted in order to gain comprehensive understandings on the anticancer effects of vitamin E. Furthermore, the anticancer effects of vitamin E should take into account some of the important impelling factors including the optimum dose intakes, synergistic effects of vitamin E with other compounds/drugs, types of cancer, severity of the patients, and other related factors. It is also important to note that nutritional inadequacy of vitamin E is found only in patients with various genetic or acquired diseases. Also, in the cases where vitamin E has a claimed efficacy, an unknown pharmacologic effect unrelated to the normal functioning of the vitamin could probably be involved. It is also highly desired to develop biomarkers of vitamin E for clinical trials. Thus, this area of research still needs more efforts to validate vitamin E for the development of anticancer drugs against the various types cancerous cells.

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Chapter 17
Use of Plant Secondary Metabolites as Nutraceuticals for Treatment and Management of Cancer: Approaches and Challenges

Zahid H. Siddiqui, B. Hareramdas, Zahid K. Abbas, Talat Parween, and Mohammad Nasir Khan

17.1 Introduction

The incidence of cancer is rising at the global level, and it has become one of the major burdens to the public health. Earlier, cancer was regarded as the disease of a developed countries; now it is rapidly increasing irrespective of developed and less developed countries (Newman and Cragg 2007; Torre et al. 2015). As per the American Cancer Society (ACS) (2015), when nations are classified according to income, cancer is the second leading cause of death in high-income countries and third leading cause of death in low- and middle-income countries. Besides that globally, one in seven deaths is due to cancer, and cancer causes more deaths than AIDS (Acquired immune deficiency syndrome), tuberculosis, and malaria combined (ACS 2015). Due to the growth and aging of the population, the occurrence of cancer is expected to grow worldwide, and by 2050, 27 million new cancer cases and 17.5 million cancer deaths are projected to occur in the world (Ferlay et al. 2015).

Cancer is an irregular growth of cells which tend to proliferate in an uncontrolled way and, in some cases, to metastasize. If the spread is not controlled, it can result in death. There are 200 types of cancer which affect humans worldwide and are caused by external and internal factors. The external factors comprise tobacco, infectious organisms, and an unhealthy diet, whereas internal factors are inherited genetic mutations, hormones, and immune conditions. These factors may act together or in sequence to cause cancer (ACS 2015). Chemotherapy, radiotherapy,

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and surgery are the major treatment lines; these treatments are effective when the tumor is small in size and localized. These therapies might cause certain types of side effects and are very costly for the patients. Therefore, it is the need of the hour for a cost-effective, high-efficacy anticancer drugs with a reduced side effects.

In order to control cancer, anticancer potential of medicinal plants and their phytochemicals are regularly updated (Shukla and Mehta 2015; Fernando and Rupasinghe 2013; Rao et al. 2016). The fight to combat diseases including cancer is an ancient art, and plants were used in diverse form for the treatment of cancer in the form of crude extracts, herbal teas, juices, tinctures, fluid extracts, powders, tablets, and capsules (Farnsworth et al. 1985; Mann 2002; Morrison 2010; Mondal et al. 2012). Morrison (2010) published a detailed review on cancer chemotherapy covering its history of the last 5000 years. In short, one of the most famous medical textbooks in history is *The Canon of Medicine*, written by the Persian scientist and physician Ibn Sina (Papac 2002). He described how cancers progressively increase in size and invade and destroy neighboring tissues. Dioscorides lists genus *Vinca* (later the genus, *Catharanthus*) as likely to have antitumor activity, and as we know today, drugs derived from them, VCR and VLB, are effectively used in different cancer chemotherapy protocols (Gidding et al. 1999). However, *Catharanthus* is used as an antitumor agent since the first century AD; its importance is still not diminished because of its potency. Despite *Catharanthus* and *Colchicum* alkaloids, over the years several drugs from plants have been isolated such as the paclitaxel and its derivatives from *Taxus* sp., the camptothecin and its analog (toptotecan, irinotecan) from *Camptotheca acuminata*, and the epipodophyllotoxin lignans (etoposide, teniposide, etoposide phosphate) from *Podophyllum* sp. (DeVita et al. 2008; Kinghorn et al. 2011; Cragg and Newman 2013). DeBono et al. (2015) reported a new alkaloid noscapine from *Papaver somniferum*, which shows antimitotic activity; a lot of research is going on with noscapine and its semisynthetic derivatives against various kinds of cancers. Recently, induction of apoptosis in human leukemia cells through an intrinsic pathway by cathachunine, a unique alkaloid isolated from *C. roseus*, was reported by Wang et al. (2016). Further, Wang et al. (2012, 2014) and Zhang et al. (2013a, b) identify various forms of new dimeric indole alkaloids from *C. roseus* with significant cytotoxicity to human breast carcinoma (MCF-7), human hepatocellular carcinoma (HepG2), and human colorectal carcinoma (Lovo) cell lines. Siddiqui et al. (2011, 2013) published report for increasing rate of somatic embryogenesis and alkaloid contents in *C. roseus*. However further research is necessary for the isolation of these compounds.

Nowadays, a new trend is emerging due to accessibility of information on the Internet; a lot of cancer patients claimed to be "cancer survivor" by use of dietary supplements (Wargovich et al. 2010). The cancer patients are unsatisfied with the costly drugs that induced toxicity and other side effects and are looking for alternative or complementary useful products particularly nutraceuticals (Rao et al. 2008). Around 2500 years ago, Hippocrates quoted "Let food be thy medicine and medicine be thy food," which is very apt in today's context. Nutraceuticals diminish the line between food and drugs and emerged as an exciting opportunity to treat cancer

and source of revenue for food industry (Dillard and German 2000). In the last couple of years, the supply of food products containing bioactive compounds as well as non-food products has increased (Ranzato et al. 2014). The use of nutraceuticals has many folds and may be used to prevent chronic diseases, delay the aging process, increase life expectancy, improve overall health, or support the structure or function of the body. Nutraceuticals are also of considerable interest due to their potential nutritional, safety, and therapeutic effects. Recent studies have shown promising results for these compounds in various complications (Nasri et al. 2014). As per a market estimate, the worldwide nutraceuticals demand would reach US $250 billion by 2018 (Hardy 2000). These products are enriched with phytochemical extracts; therefore it cannot be put in the category of "food" and defined as "nutraceuticals." The nutraceuticals are available for almost all of the therapeutics areas such as cold and cough, digestion, osteoporosis, anti-arthritic, cholesterol control, pain killers, sleeping disorders, depression, blood pressure, diabetes, and prevention of certain cancers (Pandey et al. 2010). A lot of research and development is going on in this emerging field due to its therapeutic importance and revenue generation ability. However, standardization of the nutraceutical compounds or products is the need of the hour, and execution of clinical studies is required to provide scientific evidence for the claims made by the patients as well as nutraceutical companies (Das et al. 2012). The aim of this chapter is to focus on the sources of nutraceutical compounds and their importance in the management of cancer. Moreover, the mechanism of action is also highlighted.

17.2 Nutraceuticals

Internationally, there is no precise definition of nutraceuticals or functional foods or of similar terms, such as "health foods," or terms related to herbal products (Arnonson 2017). However, nutraceuticals are pure extracted phytomolecule, whereas semi-purified plant products, which are not taken as regular food, are named as functional food (Roudebush et al. 2004). On the other hand, Health Canada defines nutraceutical as "a product prepared from foods, but sold in the form of pills, or powder or in other medicinal forms, not usually associated with foods" (Bull 2000; Wildman 2001). Food supplements are those products which can be taken regularly as foods to maintain the general health. Thus, the nutraceutical as a food or part of a food, such as a dietary supplement, has a medical or health benefit, including the prevention and treatment of disease. There are widespread inconsistencies and contradictions in the many published definitions of "nutraceuticals" and "functional foods," demonstrating wholesale uncertainty about what they actually are. Arnonson (2017) explicitly dealt with these questions. However, as stated in the introduction, in this chapter, we will consider nutraceutical as a hybrid word derived from "nutrition" and "pharmaceuticals." These are natural bioactive products with food value and promising therapeutic properties in several diseases. Nutraceutical

availability ranges in the products of pharmaceutical industry, food industry, herbal and dietary supplement market, and other agribusiness-based units (Das et al. 2012). The range of nutraceuticals is not limited to isolated nutrients, engineered food, and dietary supplements but also included food and processed products such as beverages, soups, and cereals (Dureja et al. 2003).

17.2.1 Role of Nutraceuticals in Cancer

Nowadays, the dietary phytomolecules have garnered a lot of attention in the field of cancer research. The use of nutraceuticals in many cancer patients increases their sense of well-being while undergoing cancer therapy. These patterns frequently follow into the period of cancer survivorship (Wargovich et al. 2010). There is data as per ACS (2009) which suggest that one third of all cancers can be prevented by change in diet, maintenance of proper body weight, and performing routine physical exercises. There are a lot of nutraceuticals such as green tea, curcumin, isoflavones, polyphenols, lycopenes, resveratrol, etc. which have evidence to reverse, prevent, or delay the carcinogenic process (Li and Sarkar 2002; Surh 2003; Khan et al. 2007). As per Wargovich et al. (2010), the use of dietary supplements and nutraceuticals in the cancer patient is increasing at global level. Tuomisto et al. (2004) reported that foods with relatively low simple carbohydrates, moderate amounts of high-quality protein, fiber, and omega-3 fatty acid series are very beneficial for cancer patients. There are also reports where nutraceuticals found helpful in reducing the side effects of cancer due to radiation and chemotherapy, and it also helps cancer patients to lead a better life condition (Grimble 2003). These nutraceuticals are rich source of antioxidants and show different mechanisms of action. Most of them, being antioxidants, target signaling pathways related to redox-mediated transcription factors and moderate the endocrine system, immunological cascade, and enzymes related to inflammation. Some of them also have direct effects on DNA repair and cleavage process (Ranzato et al. 2014). Recently, Zheng et al. (2016) reviewed in details about different nutraceuticals and their role in combating different types of cancer.

17.3 Some Important Nutraceuticals Studied for Cancer Treatments

17.3.1 Camellia sinensis *(Green Tea)*

Green tea has pluripotent effects on a large number of molecular processes; green tea constituents inhibit angiogenesis and neovascularization. The active constituent of green tea which is thoroughly researched is epigallocatechin-3-gallate (EGCG)

(Sagar et al. 2006; Butt and Sultan 2009). EGCG inhibits proliferation of different types of tumor cell notably colon, breast, and head and neck cancer cell lines (Sagar et al. 2006; Hazgui et al. 2008; Kato et al. 2008; Shankar et al. 2008; Larsen and Dashwood 2010). EGCG inhibits VEGF as well as interferes with irregular cell signaling pathways Akt and ERK 1 and 2 signaling systems (Issa et al. 2007; Volate et al. 2009). Further research is yet to elucidate the effectiveness of pharmacological doses of EGCG on angiogenesis and proliferation of metastatic lesions in humans (Wargovich et al. 2010). However, the anticancer properties of EGCG have also been recognized on less common cancer cells such as anaplastic thyroid carcinoma (De Amicis et al. 2013) and malignant mesothelioma (Ranzato et al. 2012). Besides that the biotransformation of green tea polyphenols is different in human from rat and mouse, which may explain the interspecies difference in antitumor properties (Clifford et al. 2013). The variable role of EGCG suggests that this compound has a huge potential of anticancer activity.

17.3.2 Capsicum *sp. (Chili Pepper)*

Capsicum sp. is a global spice commonly used for its pungent taste; the red chilies are pungent due to the presence of capsaicin, the active constituents (Oyagbemi et al. 2010). There are reports (Yang et al. 2009; Lee et al. 2010) which show that capsaicin delays the multiplication of cancer cells, hinders inflammatory response, and regulates apoptosis through influence on the GSKβ3 component of the beta-catenin pathway. Zhang et al. (2008) reported controlling effects of oral capsaicin on human pancreatic cancer, grown as xenografts in nude mice. In case of human colorectal cancer, Lee et al. (2012) reported the capsaicin preventive role by suppressing β-catenin-/TCF-dependent pathways by the following mechanisms, suppression of β-catenin transcription, activation of proteosomal degradation of β-catenin, and disruption of β-catenin/TCF-4 interactions. As per Lau et al. (2014), capsaicin promotes apoptosis of human small lung cancer cells through the TRPV6 and downstream calpain pathway. This result suggested the possible role of TRPV6 as a molecular target for the treatment of human small cell lung cancer. Venier (2015) reported that capsaicin inhibits the proliferation of prostate cancer both under in vitro and in vivo conditions. Further Cao et al. (2015) reviewed the anticancer effects and mechanisms of capsaicin in chili peppers, whereas Clark and Lee (2016) reviewed the multiple molecular targets responsible for the anticancer mechanism of capsaicin. Further the author reported the benefits of combinational use of capsaicin with other nutritional or chemotherapeutic compounds, concentrating on synergistic antitumor activities. These reports are suggesting the promising role of capsaicin in cancer management.

17.3.3 Crocus sativus *(Saffron)*

Saffron is a world-famous condiment used for coloring and flavoring food; it is one of the most expensive spices in the world which is also used in folk medicines. In the past few years, reviews are published elaborating the anticarcinogenic effect of saffron and its ingredients (Zhang et al. 2013c; Samarghandian and Borji 2014; Bhandari 2015; Ruba et al. 2017). The active constituents of saffron are crocin and crocetin, known for their antineoplastic activity by inhibiting cell proliferation and induction of apoptosis (Zheng et al. 2016). There are also reports which suggest selective effects of saffron against cancer cell at relatively low concentration (Abdullaev 2003; Schmidt et al. 2007). Saffron extract and crocetin can bind easily at the PCP binding site of the NMDA receptor and at the sigma-1 receptor, whereas picrocrocin and crocins had no effective binding effect (Lechtenberg et al. 2008). In human pancreatic cancer cell line (BxPC-3), cell viability was performed using MTT, and it was recorded that crocin led to apoptosis of assayed by BxPC-3 cells (Bakshi et al. 2010). Crocetin inhibits invasiveness of MDA-MB-231 breast cancer cells via downregulation of matrix metalloproteinases (Chryssanthi et al. 2007). The ethanolic extract of saffron inhibits the cell viability of in vitro human alveolar basal epithelial carcinoma cells (Samarghandian et al. 2011); in another study they further reported the cytotoxic effects of aqueous extract of saffron in A549 cells by inducing apoptosis and inhibiting cell proliferation by caspase-dependent pathways (Samarghandian et al. 2013). In case of cancer of the digestive system, reports suggested that crocetin induced apoptosis, suppressed Bcl-2, and upregulated Bax expression in gastric adenocarcinoma cells (Bathaie et al. 2013). Further, Amin et al. (2015) reported that crocin can induce autophagy-independent classical programmed cell death in colon cancer cells.

17.3.4 *C*urcuma longa *(Turmeric)*

In India, use of turmeric is an age-old practice (Aggarwal et al. 2008); the active constituent of turmeric is curcumin, a diferuloylmethane (Goel et al. 2008). Curcumin is known for its anti-inflammatory activity and has a potential to curb cancer along with diabetes, obesity, metabolic syndrome, and atherosclerosis (Bachmeier et al. 2010). Among the nutraceuticals, curcumin is most extensively investigated in the papers, which might be useful in prevention and treatment of a broad spectrum of cancers. Wargovich et al. (2010) suggested its regulatory role in controlling the effects of TNF-α and for its ability to reduce a group of molecular signals mediated by the nuclear transcription factor, NF-κβ. The inhibition of NF-κβ can have strong consequences for a tumor cell. The important proteins controlled by curcumin are COX-2, VEGF, the chemokines MCP-1 and MCP-4, the interleukins IL-1 and IL-6, and IGF. In case of nasopharyngeal carcinoma cells (NPC), curcumin induced G2/M phase arrest and apoptosis by mediating apoptosis-inducing

factor and caspase-3-dependent pathways (Kuo et al. 2011). Yang et al. (2012) reported apoptosis in small cell lung cancer NCI-H446 via ROS-mediated mitochondrial pathway and not cell death receptor pathway, whereas Starok et al. (2015) reported the dual mode of action of curcumin in lung cancer cells by inhibiting the enzymatic activity of the epidermal growth factor receptor (EGFR) intracellular domain and by influencing the cell membrane environment of EGFR. In case of breast cancer, the role of curcumin was highlighted by Strofer et al. (2011), Kim et al. (2012), and Mukherjee et al. (2014). Recently, Zheng et al. (2016) reviewed the role of curcumin in different types of cancer and their treatments. However, for cancer treatments, the ingestion of turmeric for curcumin and its effect on cancer cell are yet to go a long way. However, along with green tea, curcuminoids may help in cancer therapy by chemosensitization of cancer cells or in co-influencing molecular processes that make cancer cells more docile to therapy.

17.3.5 Glycine max *(Soy) Refe Done*

In terms of nutrition, soy is a rich source of protein, whereas nutraceutically it is a varied source of anticancer agents; however, research has been focused on soy phytoestrogens, a type of flavonoids found in soybeans and in edible soyfoods. Soy phytoestrogens including genistein, daidzein, and glycitein are isoflavonoids with structural similarity to human 17β-estradiol (Hooper et al. 2009). Soy phytoestrogens show a wide range of biological actions (Kwon and Song 2011; Valeri et al. 2012) including chemopreventive activities in breast and prostate cancer (Horie 2012; Wada et al. 2013). The isoflavones have the ability to control the estrogen receptor (ER) which is one of the key metrics, diagnosed with breast cancer (Rakha et al. 2010). Besides that there are soy isoflavones which can bind and transactivate ERs. Therefore, there is a potential that if soy were consumed in large amounts, compounds such as genistein could have an ill effect on breast cancer (Franke et al. 2009). Soy isoflavones gives mixed response to metastatic process; in prostate cancer metastasis, it has both beneficial and harmful effects (Lakshman et al. 2008) in xenografted human breast cancer; in animals, genistein has been shown to increase the susceptibility for metastatic spread (Vantyghem et al. 2005), and it has no effect on advanced pancreas cancers (El-Rayes et al. 2011). The therapeutic potential of genistein in mouse melanoma models and in bladder cancer was also reported (Farina et al. 2006; Singh et al. 2006). There are several potential mechanisms which have been reported for the antineoplastic activity of soy isoflavones such as inhibition of different signaling pathways (PTK, Akt, NF-κB, MAPK, COX-2, AP-1), adhesion and metastasis (FAK, MMPs), angiogenesis (HIF-1α, VEGF), and inhibition of topoisomerases I and II, 5α-reductase, and protein histidine kinase (Sarkar and Li 2002; Wang et al. 2013). Recently a detailed chapter summarizing the role of soy isoflavones in the breast cancer has been published by Kubatka et al. (2016) from preclinical findings to clinical strategy.

17.3.6 Piper nigrum *(Black Pepper)*

Piper nigrum is an herb generally used in folk medicine and broadly consumed as spice. The most common studied active constituent of black pepper is piperine (Srinivasan 2007), and it shows antineoplastic activities in variety of cancer (Zheng et al. 2016). It is known to enhance the uptake and bioavailability of curcumin and tea polyphenols by inflection of drug metabolism isoforms in the liver (Aggarwal 2010). Bezerra et al. (2008) recorded the in vitro and in vivo antitumor effect of 5-fluorouracil combined with piplartine and piperine and found that the therapeutic potential of piplartine is greater than piperine. However, in different tumor models including lung metastasis piperine, it has been shown to have anticancer activity (Pradeep and Kuttan 2002), whereas Kakarala et al. (2010) reported inhibiting effects of piperine in mammosphere formation. Yaffe et al. (2012) reported the inhibiting effects of piperine in the metabolic activity of HRT-18 human rectal adenocarcinoma cells by preventing cell cycle progression and induce apoptosis. Later, Yaffe et al. (2015) reported that piperine inhibits the growth of HT-29 human colon cancer cells via g1 arrest and recorded that apoptosis is activated by endoplasmic reticulum stress and loss of mitochondrial membrane integrity. In case of breast cancer, Do et al. (2013) reported that piperine inhibits proliferation and induces apoptosis by activation of caspase-3 and PARP cleavage. Piperine is also responsible for the enhanced effects of paclitaxel by sensitizing human epidermal growth factor receptor. In another study piperine was found to be the most potent adjuvant for enhancing the efficacy of TNF-related apoptosis (Abdelhamed et al. 2014). Besides, piperine is reported to induce apoptosis in triple-negative breast cancer xenografts in immune-deficient mice by mitochondrial pathway (Greenshields et al. 2015).

17.3.7 Trigonella foenum-graecum *(Fenugreek)*

Fenugreek is a very famous spice known for increasing the sensory quality of food. It is widely known for its medicinal value such as antioxidant, antidiabetic, immunological, hypocholesterolemic, and anticarcinogenic activities. Recently there are reviews and chapters on nutraceutical properties and utilization of fenugreek in various food products (Garg 2016; Wani and Kumar 2018). The active constituent of fenugreek seeds is diosgenin a saponin steroid and found to be safe at very high doses in rats (Taylor et al. 2000). It has been reported as a suppressor of human colon cancer cell lines, myelogenous leukemia cells, and breast cancer cell lines (Shishodia and Aggarwal 2006; Raju and Bird 2007; Srinivasan et al. 2009). The whole crude extract of fenugreek has been shown more potential than the individual active compounds used in isolation as reported by Shabbeer et al. (2009). In estrogen receptor-positive breast cancer cell line, Sebastian and Thampan (2007) checked the growth with ethanolic extracts of fenugreek and reported decreased cell viability

and mitochondrial membrane potential. Diosgenin acts as an inhibitor for COX enzymes and NF-κβ activity and has possible effects on 5-LOX activity (Raju and Mehta 2009), whereas Li et al. (2010) reported the mediating role of diosgenin in STAT3 signaling pathway in hepatocellular carcinoma by suppressing the activation of c-Src, JAK1, and JAK2. Khoja et al. (2011) suggested that the extract of fenugreek has the ability to increase apoptosis by enhancing the expression of pro-apoptotic genes. Ethanolic extract of dry fenugreek acts as an inducer of cellular death by autophagy in human T-lymphoma Jurkat cells. The Jurkat cell death was preceded by the appearance of morphological changes, formation of multiple large vacuoles, and membrane disintegration which coincided with increased transcriptional upregulation of LC3 (Al-Daghri et al. 2012). In a panel of cancer cell lines, including T-cell lymphoma, cytotoxic effects of fenugreek extract were recorded that show the usefulness of fenugreek for antineoplastic activity (Alsemari et al. 2014). In another report, crude methanolic extract of fenugreek seeds was prepared to elucidate its antineoplastic activity using HepG2 cell line. It was found that the crude extract exhibits cytotoxic effect and apoptosis induction, facilitated by upregulation of p53, Bax, PCNA, and caspase-3 activation (Khalil et al. 2015).

17.3.8 Syzygium aromaticum *(Cloves)*

Clove is the sun-dried unopened flower bud used as a spice and in traditional herbal preparation. It exerts antiseptic, antibacterial, antifungal, and anticancer properties. The active constituent of clove is eugenol (4-allyl-1hydroxy-2-methoxybenzene) which is also present in several other plants such as basil, cinnamon, and bay leaves. However, the anticancer effects of eugenol were recorded on studies of human cancer cell lines and in the MNNG rat model of gastric cancer (Slamenova et al. 2009; Manikandan et al. 2011). Further, Manikandan et al. (2010) reported that eugenol suppresses cell growth by inhibiting nuclear factor kappa beta (NF-κβ). Dwivedi et al. (2011) reported comparative antitumor potential of clove against cancer cell lines of various anatomical origins. The author uses water, ethanol, and oil extracts of clove; the oil extract exhibited maximum cytotoxic activity in TE-13 esophageal cancer cell lines. The antiproliferative and molecular mechanism of eugenol-induced apoptosis in cancer cells was reported by Jaganathan and Supriyanto (2012), whereas Shamaladevi et al. (2013) reported proapoptosis, antiproliferative, and antiandrogen receptor transcription activities, which suggested the potential use of aqueous allspice extract and ericifolin eugenol fraction against prostate cancer. In oral squamous cell carcinoma cell line, eugenol reduced ATP utilization and oxidative stress by changing metabolic profiles (Koh et al. 2013). The role of clove as a therapeutic herb for the treatment of colorectal cancer was elucidated by Liu et al. (2014); oleanolic acid was one of the constituents in ethyl acetate extracts of cloves responsible for anticancer activities; similarly Iwano et al. (2014) reported the contribution of eugenol for treatments against liver cancer. Recently Yi et al. (2015) reported the combined effects of myricetin, and methyl eugenol has enhanced

anticancer activity, cell cycle arrest, and apoptosis induction in HeLa cervical cancer cell lines.

17.3.9 Vitis vinifera *(Grape)*

Grape is one of the most commonly consumed fruits in the world both as fresh fruit and processed fruit (grape juice, raisins, wine, and molasses) (Schamel 2006; Percival 2009). It is a rich source of antioxidants and phenolics and reported to have antineoplastic activity. Under in vitro conditions, seed extracts and grape skin exhibit strong free radical scavenging and chelating activities and inhibit lipid oxidation (Zhou and Raffoul 2012). Proanthocyanadins are the active constituents of common grape and muscadine (wild grape); it showed promising results on antitumor activity (Yi et al. 2005; Wargovich et al. 2010). Wild grapes are relatively new to nutraceutical market, and cranberries are also a rich source of proanthocyanidins (Chatelain et al. 2008). American cranberry proanthocyandin-rich extracts suppress MMP-2, MMP-9, and TIMP-2 activities in the DU145 human prostate cancer cell line (Déziel et al. 2010). Among proanthocyanadins, the most studied is resveratrol both in cell culture and animal models (Hsieh and Wu 2010). The resveratrol has been reported to inhibit both forms of COX, COX-1, and COX-2 (Yance and Sagar 2006). In another late-stage cancer experiment, resveratrol showed its antiangiogenic potential (Liu et al. 2010; Uchiyama et al. 2010). Further, resveratrol treatment significantly inhibits human glioma (Gagliano et al. 2010). Grape seed proanthocyanidins have been shown to induce apoptosis of non-small-cell lung cancer A549 and H1299 cell (Singh et al. 2011). In another report grape seed extract was reported to cause cell cycle arrest and selective inhibition in growth and apoptotic death in both Detroit 562 and FaDu HNSCC cells by activating DNA damage checkpoint cascade, including ataxia and caspases (Shrotriya et al. 2012). Del Follo-Martinez et al. (2013) reported the combined effects of resveratrol and quercetin (1:1) on colon cancer cells and found their repressing role on oncogenic microRNA. Procyanidin, the most active constituent of grape seed extract, is found to be very effective against prostate cancer. Procyanidin reportedly inhibits cell growth, decreases clonogenicity, and induces cell cycle arrest and apoptic cell death by targeting NF-κB, Stat3, and AP1 transcription factors (Tyagi et al. 2014). In a latest report, Mohansrinivasan et al. (2015) elucidate the anticancer activity of grape seed extract on skin cancer cell lines A431.

17.3.10 Zingiber officinale *(Ginger)*

Ginger is a common spice in foods and beverages used across worldwide. It is rich in different bioactive phenolic compounds, including nonvolatile pungent compounds such as gingerols (6-gingerol), paradols, and shogaols (Karna et al. 2012).

There are reports suggesting the active role of gingerol as a promising anti-inflammatory agent with ability to suppress motility, invasion, and adhesion in human hepatocarcinoma cells, human breast cancer cell lines, and other cell lines (Rhode et al. 2007; Kundu et al. 2009; Dugasani et al. 2010). Encapsulated ginger, used as a treatment for chemotherapy-induced nausea and vomiting, showed promising results (Zick et al. 2009); other trials are ongoing to further define the use of ginger in cancer management. The crude ethanolic extract of ginger was used to investigate the cytotoxicity and anticancer activity of ginger against cholangiocarcinoma, an adenocarcinoma which arises from the epithelial cells of the bile ducts (Plengsuriyakaran et al. 2012). Park et al. (2014) performed in vitro studies to evaluate the anticancer properties of ginger leaf under in vitro condition and reported reduction in cell viability and apoptosis. The mechanism involved may be a result of ATF3 promoter activation and subsequent increase of ATF3 expression through ERK1/2 activation in human colorectal cancer cells. Recently, Akimoto et al. (2015) by using ethanol-extracted materials of ginger reported an inhibition in cell cycle progression and eventually induced the death of human pancreatic cancer cell lines. The underlying mechanism involved was autosis, a recently categorized form of cell death. Prasad and Tyagi (2015) reviewed the role of ginger and its constituents in the prevention and treatments of gastrointestinal cancer.

17.4 Conclusions and Future Prospects

Nutraceuticals are products, which provide nutrition as well as can be used as medicine. It has properties to provide physiological benefits or protection against chronic diseases. During the last few decades, nutraceuticals garnered a significant interest due to their potential nutritional, safety, and therapeutic effects especially in the management of lung, liver, breast, stomach, colorectum, cervix, and prostate cancers. The research has suggested that almost one third of all cancers can be prevented easily by doing regular physical activity, maintenance of optimum body weight, and modification of diet. The nutraceuticals contain several phytochemicals, such as eugenol, gingerol, capsaicin, epigallocatechin-3-gallate, crocin, crocetin, curcumin, diosgenin, proanthocyanidins resveratrol, piperine, and soy isoflavones. These nutraceuticals are useful for the inhibition of tumor advancement and/or could also be useful for the treatment of human carcinomas in combination with conventional therapeutics. As per the published reports, there are several mechanisms by which nutraceuticals control cancer management such as induction of apoptosis, DNA damage, causing G2/M arrest, inhibition of proliferation, migration and invasion of cancer cells, and sensitizing cancer cells to chemotherapy and radiotherapy. However, nutraceuticals proved to be very effective in various kinds of cancer; still a lot of research is required to fully appreciate nutraceuticals because the active constituents of plants vary depending upon their geographical environments. This can be achieved by in vitro mechanistic studies and in vivo animal studies together with clinical trials to eradicate the menace of cancer in a cost-effective

manner. Moreover, research involving electrostatic spray and nanoscale delivery of the active components present in these nutraceuticals extracts can pave a faster way for cancer management.

Conflict of Interest The author declares that there is no conflict of interest.

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Chapter 18
Usefulness of *Ocimum sanctum* Linn. in Cancer Prevention: An Update

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18.1 Introduction

Cancer is a group of ailment and is as old as mankind (Baliga et al. 2013). It is characterized by an uninhibited cell division leading to malignancy with the capability of invading neighboring tissues through the bloodstream or lymphatic system (Croce 2008). Cancer destroys organ systems through a localized invasive growth and by metastasizing to other distant tissues and organs. At the molecular level, all these events are initiated due to failures in the regulatory mechanisms of oncogenes and tumor suppressor genes. The tumor cells differentiate rapidly and progress with metastatic growth (Croce 2008). Cancer is today's leading death causing diseases along with cardiovascular diseases. According to a survey report by the American Cancer Society, nearly 1,665,540 tumor cases were detected in 2014 alone (Torre et al. 2015; Sridevi et al. 2016). The incidence of cancer is expected to increase by 75% by the year 2030, and the faulty lifestyle behaviors and sedentary lifestyle are proposed as the major contributing factors (Torre et al. 2015).

From a therapeutic perspective, cancer is treated by using chemotherapy, radiotherapy, and surgery either alone or in conjunction with each other, and the modalities are decided by the patient's general health status and the stage of the disease (Baliga et al. 2013). However, the side effects associated with the use of chemotherapy and radiotherapy are immense and negate the therapeutic benefit (Baliga et al. 2013). In addition to the standard therapeutic modalities, the prevention of

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cancer termed as “chemoprevention” is also being emphasized and is being appreciated as one of the promising methods to delay or prevent cancer formation (Baliga et al. 2013). From a definition perspective, chemotherapy involves the use of chemical, natural, or biological agents to prevent or suppress the progression of carcinogenic to become invasive metastatic cancer (Tsao et al. 2004).

Medicinal plants have largely contributed to the modern-day therapeutics and have been used for thousands of years in the folk medicines by Asian and African populations, and many plants are consumed for their health benefits in several developed nations (Martins 2014). To substantiate this, epidemiological investigations constantly report that a regular intake of vegetables and fruits strongly lowers the risk of cancer. In addition, some of the phytochemicals were reported to prevent tumor initiation and progression by inducing antioxidative stress effects and anti-inflammatory activities and by modulating signaling pathways (nuclear factor (NF-κB), Keap1-NRF2-ARE, activator protein (AP-1), etc.) (Liu 2004; Guarino et al. 2007). Additionally, the greatest benefit of plant-based compounds is that they are orally administrable and have a high safety index (Baliga et al. 2013; Ozkan et al. 2016). In this chapter, detailed information on the anticancer and chemopreventive potential of tulsi plant and its phytoconstituents are discussed giving more emphasis on their mechanistic aspects against different cancer types. This summarized data will allow opportunities for pharmaceutical exploration of tulsi plant in bringing its phytoconstituents into the drug market against several cancers.

18.2 Botany and Morphology of Tulsi

India has a vast history of use of medicinal plants, and *Ocimum sanctum* L. (synonym *O. tenuiflorum* L.), commonly known as tulsi or holy basil, is one of the most revered and widely used plants in various folk systems of medicines (Baliga et al. 2013). Tulsi, belonging to the family Lamiaceae, is a tropical annual herb which is a branched and erect subshrub with scented simple opposite green or purple leaves and hairy stems (30–60 cm tall). The leaves measure about 5 cm in length with an ovate and to some extent toothed structure. They bear purplish flowers in an elongated raceme in the closed whorls (Prakash and Gupta 2005). Morphologically and on the basis of leaf color, tulsi can be classified into two types, the *Shyama* or *Krishna* Tulsi, having purple- or dark-colored leaves, and the *Rama* or *Shri* or *Lakshmi* Tulsi, with green or light leaf variety. These varieties have been proposed to have different potency in terms of their medicinal effects with dark variety being more potent than the green variety (Prakash and Gupta 2005).

Although, the geographical distribution of this plant is throughout the world tropics including tropical Asia, eastern and northern parts of Africa, parts of China, Hainan island province of China, and Taiwan, it has been believed to have originated in India dating back to thousands of years. It is widely used in Ayurveda system of medicine in India (Prakash and Gupta 2005). In ancient Indian texts, tulsi

meaning the "incomparable one" has been called as "queen of herbs" owing to its widespread healing properties (Pattanayak et al. 2010).

18.3 Nutritional and Phytochemical Composition of Tulsi

Diverse factors such as different strains, geographical location, the procedures of growing and harvesting, and storage conditions determine the chemical composition of tulsi (Anandjiwala et al. 2006; Zheljazkov et al. 2008). This high degree of variability makes it a highly complex plant containing a diverse milieu of nutrients and other biologically active compounds. The major phytonutrients in tulsi are vitamin A, vitamin C, minerals such as zinc, calcium, iron, and several other nutritional phytoconstituents. The nutritional composition of tulsi per 100 g is as follows: protein, 4.2 g; carbohydrate, 2.3 g; fat, 0.5 g; phosphorus, 287 mg; calcium, 25 mg; iron, 15.1 mg; and vitamin C, 25 g (Anbarasu and Vijayalakshmi 2007; Pattanayak et al. 2010).

The various parts of tulsi are rich in biologically active constituents including flavonoids, saponins, tannins, triterpenoids, and other phenolic compounds. Tulsi leaves are the richest in eugenol and methyl eugenol in addition to the presence of vicenin, orientin, ursolic acid, molludistin, luteolin, luteolin-7-O-glucuronide, apigenin, and apigenin-7-O-glucuronide (Kelm et al. 2000; Baliga et al. 2013). The essential oils of tulsi also constitute carvacrol, linalool, limatrol, and a sesquiterpene hydrocarbon, caryophyllene. Additionally, some of the phenolic compounds present in fresh leaves and stems include cirsimaritin, cirsiliol, apigenin, isothymusin, and rosmarinic acid (Baliga et al. 2013). Other beneficial compounds of tulsi include a number of monoterpenes and sesquiterpenes such as α-elemene, bornyl acetate, myrtenal, neral, α-pinene, β-pinene, campesterol, camphene, β-sitosterol, and stigmasterol (Singh et al. 1996, 2007, 2012; Kelm et al. 2000; Gupta et al. 2002; Shishodia et al. 2003; Prakash and Gupta 2005; Anandjiwala et al. 2006; Zheljazkov et al. 2008; Baliga et al. 2013). Some of the important phytochemicals of tulsi are depicted in Fig. 18.1.

18.4 Pharmacological Significance of Tulsi

Tulsi is one of the well examined plants with many pharmacological effects. The broad range of biological activities include immunomodulation, anti-ulcer, anti-inflammation, antimicrobial, antifertility, antihypertensive, cardioprotective, hepatoprotective, antidiabetic, radioprotective and as a chemopreventive agent. Additionally as an adaptogen, tulsi is also helpful and supports in adapting to stress (Godhwani et al. 1987; Pandey and Madhuri 2010). Different solvent extracts from tulsi leaves show antibacterial activity when analyzed against *Staphylococcus aureus* and *Salmonella typhimurium* pathogenic bacteria which cause diarrhea

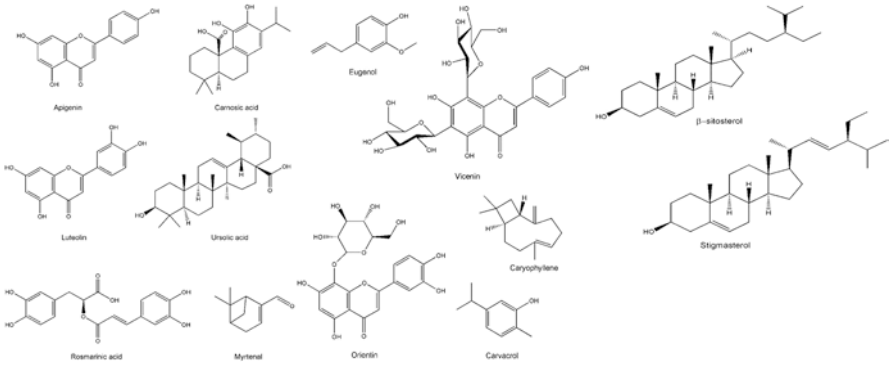

Fig. 18.1 Some of the important phytochemicals of *Ocimum sanctum*

(Eswaret al. 2016). Tulsi has been reported to possess ameliorative properties and to possess antidiabetic properties (Mahaprabhu et al. 2011). Wound healing effects of tulsi were studied using incisional wound model in rats, and it was found that it has a wound healing potential (Eyo et al. 2014). Aqueous extract of tulsi reduced the level of LDL cholesterol, total cholesterol, and triglycerides levels in acute hyperlipidemia induced rats by Triton WR-1339 in rats. Administration of the ethanolic extract of tulsi in alloxan diabetic rats was shown to reduce hyperglycemia (Vats et al. 2002). It was found that 60% and 80% of the benzene and petroleum ether extracts of tulsi leaves produces antifertility activity in female rats. In an experimental study, tulsi extract was found to be highly effective as a hepatoprotective agent. It reduced the liver damage induced by paracetamol in albino rats (Lahon and Das 2011). Studies have also shown that tulsi is beneficial in cancer, and this review summarizes the anticancer effects of tulsi against different cancer types (Baliga et al. 2013), and the subsequent paragraphs address these aspects in detail.

18.4.1 Role of Tulsi in Breast Cancer

Breast cancer ranks the second most common cancer types worldwide and is one of the major reasons of mortality due to cancer (Wiseman 2008; Torre et al. 2015). Using the Matrigel plug assay method, Nangia et al. (2004) have reported that the breast cancer cells, MDA-MB-435, treated with tulsi leaf extract effectively inhibit the migration and capillary tube formation and reduce the number of blood vessels. In another study, the breast cancer cells, MDA-MB-231, induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) also showed the inhibitory effect of tulsi leaf extract and increased the levels of cyclooxygenase-2 (COX-2) enzyme (Nangia et al. 2004). These results successfully establish the anti-tumorigenic and anti-angiogenic properties of tulsi leaf extracts.

The phytochemicals such as eugenol have been shown to possess anticarcinogenic activity against the breast cancer cells (MCF-7) owing to their redox modulatory activities (Vidhya and Devaraj 2011). Along the similar lines, flavonoids (luteolin and apigenin) found in tulsi were demonstrated to induce apoptosis in various human breast cancer cells such as MDA-MB-453, SK-BR-3, and MCF-7 by overexpressing HER2 oncogene. Further, these phytochemicals are known to regulate key signaling molecules involved in the cell cycle pathways (CCNA2, PCNA, CDKN1A, CCND1, PLK1), estrogen signaling pathways (GTF2H2, NCOR1, TAF9, NRAS, NRIP1, POLR2A, DDX5, NCOA3), histone deacetylase activity, and cell survival, invasion, and cancer growth (Markaverich et al. 2011; Attoub et al. 2011; Kim et al. 2012). Additionally, carnosic acid and rosmarinic acid are shown to possess growth inhibitory effects in in vitro studies (Yesil-Celiktas 2010).

This anti-tumorigenic potential of these compounds has been further validated experimentally using tumor-bearing nude mice and mammary cancer induced by treating dimethylbenz[a]anthracene (DMBA) and medroxyprogesterone acetate (MPA) in experimental Sprague-Dawley rats. The study results revealed that MPA increases vascular endothelial growth factors (VEGF) and suppresses vascular endothelial growth factor receptor-2 (VEGFR-2) in the hyperplastic regions. The suggested possible mechanism of action could be useful for such therapeutic applications (Chen et al. 2007; Mafuvadze et al. 2011; Mafuvadze et al. 2012).

18.4.2 Role of Tulsi in Skin Cancer

A long-term exposure or chronic contact of the body to sunlight's ultraviolet (UV) radiation is the major etiologic agent causing the skin cancers such as squamous-cell skin cancer (SCC), basal-cell skin cancer (BCC), and melanoma. Majority of these tumors are BCC involving non-melanocytic skin cells, followed by cutaneous malignancy, i.e., SCC. A plethora of studies has authenticated that UV-B radiation (290–320 nm) initiates tumor formation and also acts as a tumor promoter. Acute exposure of UV-B radiation to human keratinocytes increases the secretion of diacylglycerols, prostaglandins, and free arachidonic acid and upregulates COX-2 protein expression (Buckman et al. 1998). Another cause of skin cancer is the exposure to toxic xenobiotic compounds. Their constant exposure alters the structure of the skin and promotes skin cancer. Medicinal plants possessing phytocompounds with free radical scavenging and antioxidant activities are well documented to have radioprotective properties.

Leaves of tulsi have been demonstrated to possess selective radioprotective effects at a nontoxic concentration (Baliga et al. 2016). Earliest studies have observed that the topical applications of ethanolic extract of tulsi leaves decrease DMBA-induced skin papillomagenesis. It has been reported that pretreatment of the skin with tulsi extract before the application of carcinogens decreases the tumor formation incidences (Baliga et al. 2013). The essential oil extracted from tulsi seeds has reduced MCA-induced skin carcinogenesis and increased the survival of

tumor-bearing mice (Baliga et al. 2013). Additional studies have shown that tulsi increases the levels of GSH (glutathione), GST (glutathione S-transferase), and other enzymes with antioxidant activities, by decreasing the activity of ornithine decarboxylase (ODC) and cytochrome P450 enzymes and reducing the expression of GST-P (glutathione S-transferase placental form) and GGT (gamma-glutamyl transpeptidase). Also, the levels of lipid peroxidation were found to decrease, suggesting its protective role. Tulsi leaves contain various biologically active phytocomponents mainly eugenol, apigenin, and luteolin which are beneficial in curing the dermal toxic effects of xenobiotics and UV-B radiation (Baliga et al. 2013).

Eugenols hinder the formation of superoxides and lipid peroxidation; decrease the oxidative stress, inflammation, and cell multiplication; and induce apoptosis (Manikandan et al. 2010; Baliga et al. 2013; Sarkar et al. 2015). The pretreatment with eugenol effectively inhibits nuclear factor kappa-light-chain (NF-kB), an enhancer of activated B cells, ornithine decarboxylase (ODC) activity, and expression of nitric oxide synthase (iNOS) and COX-2 and decreases the levels of proinflammatory cytokines (Baliga et al. 2013). Primary melanoma cells established from patient tissues have shown that eugenol causes a concentration-based suppression of the cell growth. Apigenin also inhibits the DMBA-initiated tumorigenesis and reduces UV-B-induced increase of COX-2 levels in both mouse and human keratinocyte cell lines and prevents UV-B-induced skin cancer in mice. Likewise, luteolin present in tulsi has the potential to increase the survival of normal human keratinocytes (NHKs) after UV-B irradiation. However, malignant cells were not affected and remained unchanged. Luteolin was reported to attenuate UV-B-induced cell death by delaying or inhibiting intrinsic pathways of apoptotic signals and enhancing antioxidant activities (Verschooten et al. 2010; Baliga et al. 2013).

18.4.3 Role of Tulsi in Lung Cancer

Lung cancer is another leading death causing cancers which is estimated to a total of 1.5 million deaths worldwide annually. The lung cancer is only diagnosed at a later stage due to local invasion or distal metastases (Perlikos et al. 2013). Carcinogens with the ability to cause mutagenesis induce the activation of oncogenes and lead to lung carcinogenesis. Lung cancer metastasis is due to transition of epithelial cells to mesenchymal cell type which occur though the activation of numerous cell signaling pathways including Akt/GSK3β, MEK-ERK, and Fas (Perlikos et al. 2013).

The extract of tulsi induces apoptosis in A549 (lung cancer) cells and suppresses lung cancer development in C57BL/6 mice (Magesh et al. 2009). The molecular mechanism by which tulsi prevent lung carcinoma is through the phosphorylation of survival genes (Akt and ERK), increasing the levels of cytochrome C, and reducing the expression of Bcl-2, an anti-apoptotic protein (Bhattacharyya and Bishayee 2013). Treatment of tulsi plant extracts to animals with carcinoma was shown to inhibit the tumor growth in a dose-dependent manner (Bhattacharyya and Bishayee 2013).

It is also reported that the leaf extract of tulsi inhibits cell adhesion and decreases matrix metallopeptidase 9 (MMP-9) and increases antioxidant enzyme levels (Nangia-Makker et al. 2004). The administration of the extract to mice relatively reduced the formation of tumor lumps and significantly reduced the weight of lung. Luteolin, one of the major component of tulsi induces the arrest of G2 phase of the cell cycle, induces apoptosis and suppresses the growth and migration of adenocarcinomic A549 (human alveolar basal epithelial) cells and helps in preventing the lung cancer (Magesh et al. 2009; Baliga et al. 2013; Nana-Sinkam and Powell 2013).

18.4.4 Role of Tulsi in Liver Cancer

According to the recent report of the American Cancer Society, liver cancer is the fifth and eighth most common cancer in men and women, respectively, worldwide (Torre et al. 2015; Siegel et al. 2016). The major risk factors associated with liver cancer are chronic infections of HBV (hepatitis B virus) and HCV (hepatitis C virus) and high alcohol consumption (Baliga et al. 2013). There are numerous phytomedicines which are being used for preventing and treating different liver disorders or hepatotoxicity (Ashfaq and Idrees 2014). Tulsi is also one of them. Scientific studies have revealed that the extracts of tulsi and its oil significantly elevated the function of cytochrome b5, cytochrome P450, and glutathione S-transferase in the liver; all of these play an important role in detoxifying carcinogens and mutagens (Siegel et al. 2016).

It has been reported that tulsi-treated lung cancer cell lines, NCI-H460, exhibit anticancer activity by increasing intracellular ROS, by decreasing cell proliferation, and by altering the mitochondrial membrane potential (Das et al. 2006). Apigenin, a phytochemical from tulsi, has been shown to inhibit the phenobarbital (PB)-promoted and N-nitrosodiethylamine (NDEA)-induced experimental hepatocellular cancer initiation (Sridevi et al. 2016). Similarly, the ethanolic extract of tulsi leaves at a dose of 400 and 800 mg/kg body weights was shown to modulate aryl hydrocarbon hydroxylase and cytochrome P450 enzymes that are known to metabolize carcinogens. In vivo studies have shown that the ethanolic leaf extract of tulsi can alleviate damages in the liver due to antituberculosis drugs in rats (Ubaid et al. 2003). In their study, it has been revealed that tulsi exhibits the protective effects by reducing the levels of protein and lipid oxidation, by decreasing phase I enzymes, and by enhancing phase II and antioxidant enzymes.

In vitro studies with the primary hepatocytes in rats have revealed that feeding the leaf extract (ethanolic) of tulsi (20–500 μg/ml) before DMBA treatment (10 or 50 μg) cause the decrease the levels of DMBA-DNA adducts significantly. It suggests the capability of tulsi extract in preventing the DMBA-induced tumorigenesis in the early stages (Prashar et al. 1998). Further, they have suggested that leaf extract of tulsi blocks the events that are linked to chemical tumorigenesis by suppressing the metabolic activation of carcinogenic substances (Aggarwal and Mali 2015).

Experiments have shown that the ethanolic extract of tulsi leaves administered orally at a dose of 200 mg/kg in male Wistar albino rats gave protection against the liver injury induced by paracetamol (Chattopadhyay et al. 1992). Likewise, the cold water extract (3 g/100 g, administered orally for 6 days) of tulsi was found to be highly effective against carbon tetrachloride (0.2 ml/100 g, subcutaneously)-induced liver damage in experimental rats (Seethalakshmi et al. 1982).

Various investigations have shown that phytochemicals of tulsi prevent hepatocarcinoma in rats and the protective effects are achieved through mitigating the oxidative stress. A pentacyclic triterpene acid, ursolic acid isolated from tulsi, has been stated to inhibit the activities of NF-κB triggered by carcinogenic substances including tumor necrosis factor, phorbol ester, hydrogen peroxide, okadaic acid, and tobacco smoke (Srinivas et al. 2016). According to them, ursolic acid suppresses the degradation and phosphorylation of IκBα, activation of IκB kinase, phosphorylation of NF-kB-p65 subunit, nuclear translocation of p65 subunits, and NF-κB-dependent reporter gene expression. In addition, ursolic acid suppresses the rate of cell proliferation and promotes apoptotic events and arrests the cell cycle phases (Srinivas et al. 2016). Moreover, the in vitro cell culture assays have revealed that treating ursolic acid induces cytotoxicity and apoptosis in various cell lines including HA22T (Gayathri et al. 2009), HepG2 (Tang et al. 2009), Huh (Yang et al. 2010), and H22 (Wang et al. 2011). It also has some antiangiogenic effects and decreases the expression of VEGF and maintains GSH levels and reduces the cell invasion and migration in HA22T and Hua7 cells (Lin et al. 2011).

The phytoconstituent, apigenin (a flavone), present in tulsi has been documented with antioxidant, anti-inflammatory, and anticancer potential (Shukla and Gupta 2010). Cell culture studies have shown that it causes apoptosis mediated through the generation of ROS (Choi et al. 2007). Furthermore, apigenin has been shown to inhibit human hepatoma cancer cell (Huh7) growth by inducing apoptosis mediated via altered expression of several regulatory genes and inhibiting PI3K/Akt/mTOR signaling pathways (Cai et al. 2011; Tong and Pelling 2013). Likewise, luteolin is a type of flavonoid most often found in tulsi leaves that induces apoptosis in HepG2 cells mediated by activating the release of cytochrome C enzymes and mitochondrial translocation of Bak and Bax apoptogenic proteins (Lee et al. 2010). Myrtenal, which is a monoterpene present in tulsi, has been found very effective in preventing diethylnitrosamine (DEN)-induced hepatocellular carcinoma in rats (Babu et al. 2012). Its administration corrected the modified levels of enzymes, involved in metabolism of carbohydrate, as compared to the carcinogen alone treated groups (Lingaiah et al. 2012). It also reduced the levels and activities of phase I enzymes (cyto b5, cyto P450 CPR, CBR) and concomitantly increased the phase II enzymes (GST, UGT). It modulated the levels of p53, TNF-α, and caspase-3.

18.4.5 Role of Tulsi in Gastric Cancer

Gastric cancer is another more frequently diagnosed cancer types in worldwide population. According to the epidemiological survey report on cancers worldwide, nearly 22,220 cases of gastric cancer patients are detected annually in the United States of America alone. Among them, about 10,990 patients are expected to die (Siegel et al. 2016), and based on the 2009–2013 data, the number of new cases of gastric cancer was 7.4 per 100,000 patients per year which accounts for 8% of the total cases (Jemal et al. 2011). Nowadays, many herbs have been investigated for bioactive compounds, and modern research studies are mainly aimed to understand their antitumor activity against various cancers, and the results are found positive to a certain extent (Ovadje et al. 2015). Tulsi is a rich source of phytochemical compounds and thus exhibits innumerable pharmacological effects including anticancer activity. A dose of 400 and 800 mg/kg of tulsi leaf extract has been found to modulate the carcinogen-metabolizing enzymes such as GST, hydrocarbon hydroxylase, and cytochrome P450 that play a role in detoxifying mutagens and carcinogens (Banerjee et al. 1996).

Tulsi plant leaves administered orally to benzo[a]pyrene-induced gastric cancer in mice and N-methyl-N^1-nitro-N-nitrosoguanidine (MNNG)-induced gastric cancer in rats effectively prevented the tumorigenesis (Aruna and Sivaramakrishnan 1992). In their preclinical experiments, tulsi leaves were incorporated into diet (200 mg/g) before and after tumor induction to prevent forestomach tumorigenesis. The animals fed with the leaves of tulsi showed very low incidences (29%) of tumorigenesis suggesting the chemopreventive role of tulsi (Aruna and Sivaramakrishnan 1992). Likewise, 70% ethanolic leaf extract when administered to MNNG-induced gastric cancer rats significantly reduced the incidences of carcinogenesis (Manikandan et al. 2007a).

Mechanistic investigations of tulsi revealed that its extract selectively induces apoptosis in MNNG-induced gastric tumors. However, it does not affect the normal tissues of the stomach. Also, tulsi extract influences the molecular cascades and mechanisms that are involved in cancer progression (Manikandan et al. 2007b). Tulsi extract when administered in animals has shown that it decreases the levels of cytokeratin, proliferating cell nuclear antigen (PCNA), VEGF (angiogenesis), proteins involved in proliferation (GST-pi), and anti-apoptotic protein (Bcl-2) with a synchronized rise in the levels of proapoptotic proteins such as cytochrome C, Bax, and caspase-3 (Manikandan et al. 2007b, 2010, 2011). Administration of tulsi methanolic leaf extract (100 mg/kg) showed a significant ulcer protection against ethanol and pyloric ligation-induced gastric ulcers in animal models (Goel et al. 2005). Besides that, the leaf extract significantly inhibited the secretion of offensive acid-pepsin and increased the mucin secretion, gastric defensive factors, and cellular

mucus. Eugenol is one of the most abundant essential oils present in the leaves of tulsi that has an anticancer capability (Udupa et al. 2006). Preclinical studies have revealed that it also has an effective activity against MNNG-induced gastric carcinogenesis in rats and benzo[a]pyrene-induced forestomach tumors in mice (Bhide et al. 1991; Manikandan et al. 2010).

Manikandan et al. (2011) has investigated and invented that eugenol exerts its protective effects by the suppression of NF-κB activation and altered expression of NF-kB target genes that inhibit or promote the survival and proliferation of cells. Likewise, eugenol treatment was shown to increase apoptosis by the modulation of Apaf-1, caspases, Bcl-2 family proteins, and cytochrome C (Bhide et al. 1991). In a study, eugenol was shown to promote apoptosis of AGS (human gastric cancer) cells, mediated by both intrinsic and extrinsic cell signaling apoptotic pathways. During the course of gastric carcinoma development, alterations in p53 occur. Eugenol inhibited the cell proliferation rate as evidenced from the decreased population of cells at the S-phase which corresponds to a decreased level of proliferating cell nuclear antigen (PCNA) expression with its treatment. Thus, the antiproliferative potential of eugenol was found to be dependent on p53 (Sarkar et al. 2015). Also, in vitro studies have shown that luteolin, another chemical component of tulsi, initiates cell death and apoptosis in AGS tumor cells (Wu et al. 2008). Furthermore, apigenin (Zhang et al. 2006) and β-sitosterol (Zhao et al. 2009) are also reported to induce apoptosis and cause cell death. Additionally, ursolic acid treatments of BGC-803 cells have also shown to arrest cell cycle at G0/G1 stage and enhance apoptosis by enhancing the expression of caspase-8 and caspase-3 genes (Wang et al. 2011). Likewise, ursolic acid from tulsi has been shown to inhibit the activities of NF-κB activation induced by several carcinogens that include phorbol ester, hydrogen peroxide, tumor necrosis factor, and cigarette smoke. Finally, it inhibits multiplication of cells, prompts apoptosis, and arrests the cell cycle (Aggarwal et al. 2011).

18.4.6 Role of Tulsi in Oral Cancer

Oral cancer is common in the world with approximately 90% of them being reported from the regions of Southeast Asia, where the people are more inclined toward smoking and tobacco chewing. Mouth ulcers and other infections related to the mouth are very effectively reduced by leaves of tulsi, and it has been found that eugenol extracted from tulsi prevents early events of DMBA-induced buccal pouch tumorigenesis (Karthikeyan et al. 1999). The oral administration of the ethanolic and aqueous tulsi leaf extracts and tropical application of its leaf paste decreased the occurrence of squamous cell carcinomas (Shivpuje et al. 2015).

Phytochemical studies have shown that the flavonoid, apigenin, exhibits chemopreventive effects against the DMBA-induced oral carcinogenesis in buccal pouches of golden Syrian hamsters. It has been also found that a natural benzenediol abietane diterpene, carnosic acid, and a phenolic compound, rosmarinic acid, are equally efficient in inhibiting DMBA-induced oral carcinogenesis in hamsters. Both

compounds mediated the protective effects by enhancing antioxidant and detoxification enzymes and decreasing the levels of lipid peroxidation (Manoharan et al. 2010; Anusuya and Manoharan 2011).

18.5 Conclusions and Future Prospects

Information accrued from preclinical studies suggests that tulsi is useful in cancer prevention. Tulsi plant possesses several bioactive compounds belonging to different classes of phytocompounds including polyphenols, alkaloids, terpenes, steroids, etc. Some of the major active principles of tulsi include eugenol, apigenin, ursolic acid, luteolin, carvacrol, linalool, rosmarinic acid, β-sitosterol, and stigmasterol. Both plant extracts and individual compounds of tulsi were proved to be effective in preventing several types of cancers as evidenced from the in vitro and in vivo study models. Some of the molecular events that are involved in the prevention of tumorigenesis include the induction of apoptosis mediated by activating the release of cytochrome C enzymes and mitochondrial translocation of Bak and Bax apoptogenic proteins, p53, TNF-α and caspases, Apaf-1, and Bcl-2 family proteins, decreasing the expression of VEGF, maintaining GSH levels, reducing the cell invasion, phosphorylation of IκBα, activation of IκB kinase, phosphorylation of NF-kB-p65 subunit, nuclear translocation of p65 subunits, and NF-κB-dependent reporter gene expression. Additionally, studies have also confirmed that tulsi has a high margin of drug safety and does not possess toxic effects. However, additional studies are required to aim for a better understanding of the mechanisms of action of the tulsi leaf phytoconstituents, and also to determine their efficacy in animal models. Only a few isolated compounds, such as eugenol, luteolin, and apigenin from tulsi have been proved to have antiproliferative activity. Therefore, future research should focus on isolating more anticancer compounds from tulsi plant and extensively evaluate to understand their role in the cytotoxicity events. Moreover, combination therapy involving tulsi plant compounds should be considered for a safe and effective cancer treatment. With wide distribution, easy availability, and safety in the consumption, tulsi plant has a tremendous therapeutic potential. However, its myriad prospects require additional investigations to understand its cancer preventive effects in humans.

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Chapter 19
Phytochemicals with Anticancer Potential: Methods of Extraction, Basic Structure, and Chemotherapeutic Action

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19.1 Introduction

Phytochemicals or secondary metabolites are chemical compounds formed during the normal plant metabolic processes and useful in the protection of plants (Watson et al. 2001; Ning et al. 2009). Most of these phytochemicals possess an important medicinal properties and have been found to have many applications in pharmaceutical industries. Free radical-scavenging molecules such as flavonoids, tannins, alkaloids, quinones, amines, vitamins, and other metabolites possess anti-inflammatory, anticarcinogenic, antibacterial, and antiviral activities (Sala et al. 2002). People have been relying on the natural source (plants) of treatments for various diseases even today that is still the case especially in rural areas, where traditional healers outnumber the western doctors. Most medicines used by the western doctors are also derived from natural plants. Most phytochemicals have antioxidant activity and protect human cells against oxidative damages. Plants with antioxidant properties are used for minimizing the severity of the inflammation-related diseases, and a health-promoting effect of antioxidants from plants is thought to arise from their protective effects by counteracting reactive oxygen species (ROS) (Wong et al. 2006). Studies also have looked at the intake of specific phytochemicals and found a link to reduce cancer risk. One study found that only specific flavonoid subgroups were associated in decreasing the risk of breast cancer. They have found that the reduced risk of cancer was not as strong by individual phytochemicals when compared with that of the foods rich in several phytochemicals. The consumption of cruciferous vegetables such as broccoli, cabbage, and cauliflower has been associated with a decreased risk

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of prostate, lung, breast, and colon cancers. Isothiocyanate found in cruciferous vegetables, especially sulforaphane in broccoli, has been studied extensively and is believed to offer some degrees of cancer prevention. Foods containing phytochemicals are already a part of our daily diet (Daffre et al. 2008). In fact, most foods contain phytochemicals except for some refined foods, such as sugar or alcohol. Some foods, such as whole grains, vegetables, beans, fruits, and herbs, contain many phytochemicals. The easiest way to get more phytochemicals is to eat more fruit (blueberries, cranberries, cherries, and apple) and vegetables (cauliflower, cabbage, carrots, and broccoli). It is recommended to take daily at least five to nine servings of fruits or vegetable. Fruits and vegetables are also rich in minerals, vitamins, and fiber and low in saturated fat. Phytochemicals are naturally present in many foods, but it is expected that through bioengineering, new plants can be developed, which will contain higher levels. This would make it easier to incorporate enough phytochemicals into our daily food.

The present chapter describes the importance of some plants of the genera *Philenoptera* (family Fabaceae), *Xanthocercis* (family Fabaceae), and *Euphorbia* (family Euphorbiaceae) and crucifer (family Cruciferae) and their significance in the treatment of cancer and other diseases with respect to their chemical structure and methods of extraction. Moreover, anticancer properties of some important phytochemicals like crocetin, cyanidins, diindolylmethane (DIM) or indole-3-carbinol (I3C), epigallocatechin-3-gallate, fisetin, genistein, gingerol, kaempferol, broccoli, and lycopene have also been discussed.

19.1.1 Plant Species Containing Anticancer Phytochemicals

Many plant species are known to have phytochemicals which are active against cancer and other life-threatening diseases. Some species that are effective against cancer are discussed below:

19.1.1.1 *Xanthocercis* and *Philenoptera*

Both *Xanthocercis* and *Philenoptera* genera belong to the Fabaceae family (Rahman and Choudhary 2001). Trees, herbs, vines, and shrubs of this plant family are native to all regions of the world and are commonly cultivated (Kinghorn et al. 2003). *Xanthocercis zambesiaca* is found in Africa and is known as Muchetuchetu/Musharo in Shona and as Nyala berry in English. *X. zambesiaca* is traditionally used to treat diabetes mellitus and has been scientifically proven to have antihyperglycemic effects (Kaskiw et al. 2009). *P. violacea* is also found in Africa, known as Mohata in Shona, Mphata in Sotho, and apple leaf in English. It has been used in traditional remedies to treat gastrointestinal problems, powdered root bark for colds and snakebite treatment and root infusions as hookworm remedy, and most part of the plant has been used to treat diarrhea (Yan et al. 2009). The extracts were found to be

active in the five-cell line panel consisting of MCF7 (breast cancer), HCT116 (colon cancer), TK10 (renal), UACC62 (melanoma), and PC3 (prostate cancer) by sulforhodamine B (SRB) assay at the CSIR. Qualitative phytochemical analysis of these plant extracts confirmed the presence of tannins, flavonoids, steroids, terpenoids, alkaloids, and cardiac glycosides from *P. violacea* extract, while *X. zambesiaca* extract showed the presence of flavonoids, saponins, terpenoids, and glycosides.

19.1.1.2 *Euphorbia tirucalli*

The historical use of *E. tirucalli* (family: Euphorbiaceae) in traditional medicine in the Middle East, India, Africa, and South America was to treat a range of ailments, including syphilis, asthma, cancer, colic, intestinal parasites, skin diseases, and leprosy (Greer et al. 2005). Consequently, this has prompted scientific interest in its pharmacological properties. Chromatographic and spectroscopic analyses of extracts from the photosynthetic stems have identified a range of phenolics and terpenes, the most prominent of which are the triterpenes, euphol, and tirucallol (Greer et al. 2005). Leaf/stem extracts have been shown to possess potent antioxidant properties as key factor in combating cellular oxidative stress. Methanol extracts of *E. tirucalli* whole plant has positive antioxidant activity, potentially due to their high phenolic content, and have been deemed an excellent and accessible source of natural antioxidant activity. The use of *E. tirucalli* latex in traditional medicine as a treatment for cancer has attracted the recent interest of the West. However, this must be treated with caution, as whole plant aqueous extracts have been shown to interact with antioxidant enzyme systems in human leukocytes via upregulation of key antioxidant enzyme genes. This leads to increased cytotoxicity, confirming the need for precise investigations into dose and administration of *E. tirucalli* extracts for medicinal purposes (Harborne and Williams 1992). A further study assessed the anticancer properties of euphol extracted from *E. tirucalli* latex, (Stray and Storchova 1991) finding it to exhibit dose- and time-dependent cytotoxic effects against a significant number of cell lines, with most prominent effects against esophageal squamous cell and pancreatic cell carcinomas.

19.1.1.3 Cruciferae Family

Cruciferae family which is one of the largest families in the plant kingdom is rich in medicinal plants. It includes 338 genera and 3350 species that are distributed worldwide (Okwu 2005; Peter 2013). Various studies indicate that consumption of large number of cruciferous vegetables like broccoli, cabbage, kale, and brussels sprouts are associated with a reduced incidence of cancer (Peter 2013). These contain various primary and secondary metabolites. The breakdown products of glucosinolates are indole-3-carbinol (I3C) and diindolylmethane (DIM). These degradation products have properties like antibacterial, anticancer, and antifungal properties

(Emam and Abd El-Moaty 2009). The present study deals with phytochemical profiling of cabbage for the presence of various phytochemicals. The extract was found to contain various secondary metabolites like tannins, flavonoids, sugars, alkaloids, phenols, and anthocyanidin. The secondary metabolites, glucosinolates, are the characteristic compounds of the crucifer family (Shapiro et al. 2001). These are group of compounds that are hydrolyzed either enzymatically with myrosinase or nonenzymatically to form primarily isothiocyanates and/or nitriles. Isothiocyanates were attributed to chemopreventive activity and induce phase II detoxification enzymes, boost antioxidant status, and protect animals against chemically induced cancer. For further identification, of its degradation products, thin-layer chromatography (TLC) was performed (Patil and Shettigar 2010).

19.1.1.4 Saffron

Saffron is a spice from the flower of the *Saffron crocus* and a food colorant present in the dry stigmas of the plant *Crocus sativus* L. In a recent review article, saffron is listed as a potential agent for a novel anticancer drug against hepatocellular carcinoma (Amin et al. 2011; Abdullaev and Espinosa-Aguirre 2004). Saffron and its ethanolic extracts are also reported for the studies on human lung cancer (Samarghandian et al. 2010, 2011), pancreatic cancer cell line (Bakshi et al. 2010), skin carcinoma (Das et al. 2010), colorectal cancer cells (Aung et al. 2007), and breast cancer (Chryssanthi et al. 2011). Its applications and mechanism of actions are reviewed by Bathaie and Mousavi (2010), but till now the exact mechanism of action is not clear. In general, crocetin affects the growth of cancer cells by inhibiting nucleic acid synthesis, enhancing anti-oxidative system, inducing apoptosis, and hindering growth factor signaling pathways. Nam's study has shown that crocetin is effective for the inhibition of LPS-induced nitric oxide release; for the reduction of the produced TNF-α, IL-1β, and intracellular reactive oxygen species; for the activation of NF-κB; and for blockage of the effect of LPS on hippocampal cell death (Nam et al. 2010). Although some studies beyond those mentioned above are successfully conducted, more thorough understanding of the mechanism on crocetin and its effects are needed.

19.1.1.5 Cyanidin

Cyanidin is a extract of pigment from red berries such as grapes, blackberry, cranberry, and raspberry, apples and plums, and red cabbage and red onion. It possesses antioxidant and radical-scavenging effects which may reduce the risk of cancer. It is reported to inhibit cell proliferation and iNOS and COX-2 gene expression in colon cancer cells (Kim et al. 2008). Another study shows that cyanidin-3-glucoside (C3G) attenuated the benzo[a]pyrene-7,8-diol-9,10-epoxide-induced activation of AP-1 and NF-κB and phosphorylation of MEK, MKK4, Akt, and MAPKs and blocked the activation of the Fyn kinase signaling pathway, which may contribute to

its chemopreventive potential (Lim et al. 2011). C3G blocks ethanol-induced activation of the ErbB2/cSrc/FAK pathway in breast cancer cells and may prevent/reduce ethanol-induced breast cancer metastasis. (Xu et al. 2011) Cyanidin-3-O-glucoside, cyanidin-3-O-rutinoside, and the ethanol extract of their source of freeze-dried black raspberries selectively caused significant growth inhibition and induction of apoptosis in a highly tumorigenic rat esophagus cell line (RE-149 DHD) but not in a weakly tumorigenic line (RE-149) (Zikri et al. 2009). Cyanidin markedly inhibited UVB-induced COX-2 expression and PGE2 secretion in the epidermal skin cell line by suppressing NF-κB and AP-1 which are regulated by MAPK. In that study, MKK-4, MEK1, and Raf-1 are targets of cyanidin for the suppression of UVB-induced COX-2 expression (Kim et al. 2010). Indole-3-carbinol (I3C) is found in *Brassica* vegetables, such as broccoli, cauliflower, collard greens. Diindolylmethane (DIM) is a digestion derivative of indole-3-carbinol via condensation formed in the acidic environment of the stomach. Both are studied for their anticarcinogenic effects. I3C has been studied for cancer prevention and therapy for years (Kim and Milner 2005) for tobacco smoke carcinogen-induced lung adenocarcinoma in A/J mice, and it was found that the lung cancer preventive effects are mediated via modulation of the receptor tyrosine kinase/PI3K/Akt signaling pathway, at least partially. I3C and DIM demonstrated exceptional anticancer effects against hormone-responsive cancers like breast, prostate, and ovarian cancers (Acharya et al. 2010). In a recent study, it is concluded that DIM rather than I3C is the active agent in cell culture studies (Bradlow and Zeligs 2010).

19.1.1.6 Fisetin

Fisetin is a flavone found in various plants such as *Acacia greggii*, *Acacia berlandieri*, Eurasian smoke tree, parrot tree, strawberries, apple, persimmon, grape, onion, and cucumber (Maher et al. 2011; Arai et al. 2000). Fisetin has been found to alleviate aging effects in the yeast or fruit fly (Howitz et al. 2003; Wood et al. 2004) and exert anti-inflammatory effect in LPS-induced acute pulmonary inflammation and anticarcinogenic effects in HCT-116 human colon cancer cells (Geraets et al. 2009; Lim and Park 2009). Fisetin is also a potent antioxidant and modulates protein kinase and lipid kinase pathways. Fisetin, along with other flavonoids such as luteolin, quercetin, galangin, and EGCG, induced the expression of Nrf2 and the phase II gene product HO-1 in human retinal pigment epithelial (RPE) cells which could protect RPE cells from oxidative stress-induced death with a high degree of potency and low toxicity and reduced H_2O_2-induced cell death. However, Khan et al. (2012) found dual inhibition of PI3K/Akt and mTOR signaling in human non-small cell lung cancer cells by fisetin.

19.1.1.7 Genistein

Genistein is an isoflavone that originates from a number of plants such as lupine, fava beans, soybeans, kudzu, *Psoralea*, *Flemingia vestita*, and coffee. Functioning as antioxidant and anthelmintic, genistein has been found to have antiangiogenic effects (blocking formation of new blood vessels) and may block the uncontrolled cell growth associated with cancer, most likely by inhibiting the enzymes that regulate cell division and cell survival (growth factors). Genistein's activity was chiefly functioned as a tyrosine kinase inhibitor by inhibiting DNA topoisomerase II (Lopez-Lazaro et al. 2007). In vitro and in vivo studies show that genistein has been found to be useful in treating leukemia (Wang et al. 2008; Sanchez et al. 2009; Raynal et al. 2008; Yamasaki et al. 2007). Estrogen receptors are overexpressed in around 70% of breast cancer cases (ER-positive). Binding of estrogen to the ER stimulates proliferation of mammary cells, with the resulting increase in cell division and DNA replication. Estrogen metabolism produces genotoxic waste, which may cause disruption of cell cycle, apoptosis, and DNA repair, and forms tumor.

19.1.1.8 Gingerol

Gingerol is the active component of fresh ginger with distinctive spiciness. Gingerol is known for its anticancer effects for tumors in the colon (Jeong et al. 2009), breast, ovary (Lee et al. 2008; Rhode et al. 2007), and pancreas (Park et al. 2006). A recent review by Oyagbemi et al. (2010) summarized the mechanisms in the therapeutic effects of gingerol. In short, gingerol has demonstrated antioxidant, anti-inflammatory, and antitumor promoting properties and decreases iNOS and TNF-alpha expression via suppression of IκBα phosphorylation and NF-κB nuclear translocation (Oyagbemi et al. 2010). Treating K562 cells and MOLT4 cells with gingerol, the ROS levels were significantly higher than control groups, inducing apoptosis of leukemia cells by mitochondrial pathway. On human hepatocarcinoma cells, gingerol, along with 6-shogaol, was found to exert anti-invasive activity against hepatoma cells through regulation of MMP-9 and TIMP-1, and 6-shogaol further regulated urokinase-type plasminogen activity.

19.1.1.9 Kaempferol

Kaempferol is a natural flavonol isolated from tea, broccoli, witch hazel, grapefruit, brussels sprouts, apples, etc. Kaempferol has been studied for pancreatic cancer (Nothlings et al. 2007) and lung cancer (Cui et al. 2008). It has been investigated for its antiangiogenic, anticancer, and radical-scavenging effects (Gacche et al. 2011). Kaempferol displayed moderate cytostatic activity of 24.8–64.7 μM in the cell lines of PC3, HeLa, and K562 human cancer cells. Kaempferol has been studied as aryl hydrocarbon receptor (AhR) antagonist showing inhibition of ABCG2

upregulation, thereby reversing the ABCG2-mediated multidrug resistance, which may be useful for esophageal cancer treatment. Lycopene is a bright red pigment and phytochemical from tomatoes, red carrots, watermelons, and red papayas. It demonstrates antioxidant activity and chemopreventive effects in many studies, especially for prostate cancer. Poorly soluble in water, lycopene has high solubility in organic solvents. Its anticancer property is attributed to activating cancer preventive enzymes such as phase II detoxification enzymes (Giovannucci et al. 1995). Lycopene was found to inhibit human cancer cell proliferation and to suppress insulin-like growth factor-I-stimulated growth. This may open new avenues for lycopene study on the role of the prevention or treatment of endometrial cancer and other tumors. Lycopene also possesses inhibitory effects on breast and endometrial cancer cells (Nahum et al. 2001), prostate cancer cells (Giovannucci et al. 1995), and colon cancer cells. However, in a study conducted by Erdman and group using xenocraft prostate tumors into rats, it was found that the tumors grew more slowly in those given whole dried tomato powder but not in those given lycopene, which may indicate that lycopene may be an important component in tomato but not the only component in tomato that actively suppressing the growth of the prostate cancer (Canene-Adams et al. 2007).

19.2 Extraction Processes of Phytochemicals

General methods of extraction of phytochemicals are discussed below.

19.2.1 Solvent Extraction

Various solvents have been used to extract different phytoconstituents. The plant parts are dried immediately either in an artificial environment at low temperature (50–60 °C) or dried preferably in shade so as to bring down the initial large moisture content to enable its prolonged storage life. The dried berries are pulverized by mechanical grinders and the oil is removed by solvent extraction. The defatted material is then extracted in a Soxhlet apparatus or by soaking in water or alcohol (95% v/v). The resulting alcoholic extract is filtered, concentrated in vacuum or by evaporation, treated with HCl (12N), and refluxed for at least 6 h. This can then be concentrated and used to determine the presence of phytoconstituents. Generally, the saponins do have high molecular weight, and hence their isolation in the purest form poses some practical difficulties. The plant parts (tubers, roots, stems, leaves, etc.) are washed, sliced, and extracted with hot water or ethanol (95% v/v) for several hours. The resulting extract is filtered and concentrated *in vacuum*, and the desired constituent is precipitated with ether. Exhaustive extraction (EE) is usually carried out with different solvents of increasing polarity in order to extract as much as possible the most active components with highest biological activity.

19.2.2 Supercritical Fluid Extraction (SFE)

This is the most technologically advanced extraction system. Supercritical fluid extraction (SFE) involves use of gases, usually CO_2, by compressing them into a dense liquid. This liquid is then pumped through a cylinder containing the material to be extracted. From there, the extract-laden liquid is pumped into a separation chamber where the extract is separated from the gas, and the gas is recovered for reuse. Solvent properties of CO_2 can be manipulated and adjusted by varying the pressure and temperature that one works at. The advantages of SFE are the versatility it offers in pinpointing the constituents you want to extract from a given material and the fact that your end product has virtually no solvent residues left in it (CO_2 evaporates completely). The downside is that this technology is quite expensive. There are many other gases and liquids that are highly efficient as extraction solvents when put under pressure.

19.2.2.1 Coupled SFE-SFC

In this system, a sample is extracted with a supercritical fluid and then placed in the chromatographic system, and the extract is directly chromatographed using supercritical fluid.

19.2.2.2 Coupled SFE-GC and SFE-LC

In this system, a sample is extracted using a supercritical fluid which is then depressurized to deposit the extracted material in the inlet part or a column of gas or liquid chromatographic system, respectively. SFE has characteritic features such as robustness of sample preparation, reliability, high yield, less time consuming, and also has potential for coupling with a number of chromatographic methods.

19.2.3 Microwave-Assisted Extraction

Applications of innovative, microwave-assisted solvent extraction technology known as microwave-assisted processing (MAP) include the extraction of high-value compounds from natural sources including phytonutrients, nutraceutical and functional food ingredients, and pharmaceutical actives from biomass. Compared to conventional solvent extraction methods, MAP technology offers some combination of the following advantages: (1) improved products, increased purity of crude extracts, improved stability of marker compounds, and possibility to use less toxic solvents and (2) reduced processing costs, increased recovery and purity of marker compounds, very fast extraction rates, and reduced energy and solvent usage. With microwave-derived extraction as opposed to diffusion, very fast extraction rates and

greater solvent flexibility can be achieved. Many variables, including the microwave power and energy density, can be tuned to deliver desired product attributes and optimize process economics. The process can be customized to optimize for commercial/cost reasons, and excellent extracts are produced from widely varying substrates. Examples include, but are not limited to, antioxidants from dried herbs, carotenoids from single cells and plant sources, taxanes from taxus biomass, essential fatty acids from microalgae and oilseeds, phytosterols from medicinal plants, polyphenols from green tea, flavor constituents from vanilla and black pepper, essential oils from various sources, and many more.

19.2.4 Solid-Phase Extraction

This involves sorption of solutes from a liquid medium onto a solid adsorbent by the same mechanisms by which molecules are retained on chromatographic stationary phases. These adsorbents, like chromatographic media, come in the form of beads or resins that can be used in column or in batch form. They are often used in the commercially available form of syringes packed with medium (typically a few hundred milligrams to a few grams) through which the sample can be gently forced with the plunger or by vacuum. Solid-phase extraction media include reverse phase, normal phase, and ion exchange media. This is a method for sample purification that separates and concentrates the analyte from solution of crude extracts by adsorption onto a disposable solid-phase cartridge. The analyte is normally retained on the stationary phase, washed and then evaluated with different mobile phases. If an aqueous extract is passed down a column containing reverse-phase packing material, everything that is fairly non-polar will bind, whereas everything polar will pass through (Greer et al. 2005).

19.2.5 Chromatographic Fingerprinting and Marker Compound Analysis

Chromatographic fingerprint of a herbal medicine (HM) is a chromatographic pattern of the extract of some common chemical components of pharmacologically active and/or chemical characteristics. This chromatographic profile should be featured by the fundamental attributions of “integrity” and “fuzziness” or “sameness” and “differences” so as to chemically represent the HM investigated. It is suggested that with the help of chromatographic fingerprints obtained, the authentication and identification of herbal medicines can be accurately conducted (integrity) even if the amount and/or concentrations of the chemically characteristic constituents are not exactly the same for different samples of this HM (hence, “fuzziness”) or the chromatographic fingerprints could demonstrate both the “sameness” and “differences” between various samples successfully. Thus, we should globally consider multiple

constituents in the HM extracts and not individually consider only one and/or two marker components for evaluating the quality of the HM products. However, in any HM and its extract, there are hundreds of unknown components, and many of them are in low amount. Moreover, there usually exists variability within the same herbal materials. Hence it is very important to obtain reliable chromatographic fingerprints that represent pharmacologically active and chemically characteristic components of the HM. In the phytochemical evaluation of herbal drugs, TLC is being employed extensively for the following reasons: (1) it enables rapid analysis of herbal extracts with minimum sample cleanup requirement, (2) it provides qualitative and semi-quantitative information of the resolved compounds, and (3) it enables the quantification of chemical constituents. Fingerprinting using HPLC and GLC is also carried out in specific cases. In TLC fingerprinting, the data that can be recorded using a high-performance TLC (HPTLC) scanner includes the chromatogram, retardation factor (Rf) values, the color of the separated bands, their absorption spectra, and shoulder inflection(s) of all the resolved bands. All of these, together with the profiles on derivatization with different reagents, represent the TLC fingerprint profile of the sample. The information so generated has a potential application in the identification of an authentic drug, in excluding the adulterants and in maintaining the quality and consistency of the drug. HPLC fingerprinting includes recording of the chromatograms, retention time of individual peaks, and the absorption spectra (recorded with a photodiode array detector) with different mobile phases. Similarly, GLC is used for generating the fingerprint profiles of volatile oils and fixed oils of herbal drugs (Xie et al. 2006). Furthermore, the recent approaches of applying hyphenated chromatography and spectrometry such as high-performance liquid chromatography-diode array detection (HPLC-DAD), gas chromatography-mass spectroscopy (GC-MS), capillary electrophoresis-diode array detection (CE-DAD), high-performance liquid chromatography-mass spectroscopy (HPLC-MS), and high-performance liquid chromatography-nuclear magnetic resonance spectroscopy (HPLC-NMR) could provide the additional spectral information, which will be very helpful for the qualitative analysis and even for the online structural elucidation.

19.2.6 Advances in Chromatographic Techniques

19.2.6.1 Liquid Chromatography

19.2.6.1.1 Preparative High-Performance Liquid Chromatography

There are basically two types of preparative HPLC. One is low-pressure (typically under 5 bars) traditional PLC, based on the use of glass or plastic columns filled with low-efficiency packing materials of large particles and large size distribution. A more recent form of PLC, preparative high-performance liquid chromatography (preparative HPLC), has been gaining popularity in pharmaceutical industry.

The aim is to isolate or purify compounds, whereas in analytical work the goal is to get information about the sample. Preparative HPLC is closer to analytical HPLC than traditional PLC, because its higher column efficiencies and faster solvent velocities permit more difficult separation to be conducted more quickly. In analytical HPLC, the important parameters are resolution, sensitivity, and fast analysis time, whereas in preparative HPLC, both the degree of solute purity and the amount of compound that can be produced per unit time, i.e., throughput or recovery, are important. This is very important in pharmaceutical industry of today because new products (natural, synthetic) have to be introduced to the market as quickly as possible. Having available such a powerful purification technique makes it possible to spend less time on the synthesis conditions (Dass 2007).

19.2.6.1.2 Liquid Chromatography-Mass Spectroscopy (LC-MS)

In pharmaceutical industry LC-MS has become the method of choice in many stages of drug development. Recent advances include electrospray, thermospray, and ion spray ionization techniques which offer unique advantages of high detection sensitivity and specificity; liquid secondary ion mass spectroscopy, later laser mass spectroscopy with 600 MHz, offers accurate determination of molecular weight proteins and peptides. Isotopes pattern can be detected by this technique (Narod et al. 1998).

19.2.6.1.3 Liquid Chromatography-Nuclear Magnetic Resonance (LC-NMR)

The combination of chromatographic separation technique with NMR spectroscopy is one of the most powerful and time-saving methods for the separation and structural elucidation of unknown compound and mixtures, especially for the structure elucidation of light- and oxygen-sensitive substances. The online LC-NMR technique allows the continuous registration of time changes as they appear in the chromatographic run automated data acquisition, and processing in LC-NMR improves speed and sensitivity of detection. The recent introduction of pulsed field gradient technique in high resolution NMR as well as three-dimensional technique improves application in structure elucidation and molecular weight information. These new hyphenated techniques are useful in the areas of pharmacokinetics, toxicity studies, drug metabolism, and drug discovery process (Christophoridou et al. 2005).

19.2.6.2 Gas Chromatography

19.2.6.2.1 Gas Chromatography Fourier Transform Infrared Spectrometry

Coupling capillary column gas chromatographs with Fourier transform infrared spectrometer provides a potent means for separating and identifying the components of different mixtures (Chaimbault 2014).

19.2.6.2.2 Gas Chromatography-Mass Spectroscopy

Gas chromatography equipment can be directly interfaced with rapid scan mass spectrometer of various types. The flow rate from capillary column is generally low enough that the column output can be fed directly into ionization chamber of MS. The simplest mass detector in GC is the ion trap detector (ITD). In this instrument, ions are created from the eluted sample by electron impact or chemical ionization and stored in a radio frequency field; the trapped ions are then ejected from the storage area to an electron multiplier detector. The ejection is controlled so that scanning on the basis of mass-to-charge ratio is possible. The ion trap detector is remarkably compact and less expensive than quadrupole instruments. GC-MS instruments have been used for identification of hundreds of components that are present in natural and biological system (Narod et al. 1998).

19.2.6.3 Supercritical Fluid Chromatography (SFC)

Supercritical fluid chromatography is a hybrid of gas and liquid chromatography that combines some of the best features of each. This technique is an important third kind of column chromatography that is beginning to find use in many industrial, regulatory, and academic laboratories. SFC is important because it permits the separation and determination of a group of compounds that are not conveniently handled by either gas or liquid chromatography. These compounds are either nonvolatile or thermally labile so that GC procedures are inapplicable or contain no functional group that makes possible the detection by spectroscopic or electrochemical technique employed in LC. SFC has been applied to a wide variety of materials including natural products, drugs, foods, and pesticides (Smith et al. 1988).

19.2.6.4 Other Chromato-Spectrometric Studies

The NMR techniques are employed for establishing connectivity between neighboring protons and establishing C-H bonds. INEPT is also being used for long-range heteronuclear correlations over multiple bonding. The application of thin-layer chromatography (TLC), high-performance chromatography (HPLC) and HPLC coupled with ultraviolet (UV) photodiode array detection, liquid chromatography-ultraviolet (LC-UV), liquid chromatography-mass spectrophotometry (LC-MS), electrospray (ES), and Liquid chromatography-nuclear magnetic resonance (LC-NMR) techniques for the separation and structure determination of antifungal and antibacterial plant compounds is on the increase frequently (Narod et al. 1998). Various chromatographic and spectroscopic techniques in new drug discovery from natural products are available. Computer modeling has also been introduced in spectrum interpretation and the generation of chemical structures meeting the

spectral properties of bioactive compounds obtained from plants. The computer systems utilize 1H, 13C, 2D-NMR, IR, and MS spectral properties. Libraries of spectra can be searched for comparison with complete or partial chemical structures. Hyphenated chromatographic and spectroscopic techniques are powerful analytical tools that are combined with high-throughput biological screening in order to avoid re-isolation of known compounds as well as for structure determination of novel compounds. Hyphenated chromatographic and spectroscopic techniques include LC-UV-MS, LC-UV-NMR, LC-UV-ES-MS, and GC-MS (Narod et al. 1998).

19.3 Chemical Structures of Anticancer Phytochemicals

There are more than thousand known phytochemicals. Phytochemicals may have biological significance, for example, carotenoids or flavonoids, but are not established as essential nutrients. There may be as many as 4000 different phytochemicals. Some are responsible for color and other organoleptic properties, such as the deep purple of blueberries and the smell of garlic. Some of the well-known phytochemicals are lycopene in tomatoes, isoflavones in soy, and flavonoids in fruits. Some important class of phytochemicals (Table 19.1) is discussed below.

Table 19.1 Summary of plants, anticancer phytochemicals, and plant source

Plant species	Phytochemicals	Chemical compounds	Effects
Tomato	Lycopene	Flavones	Effective against prostate cancer
Tea, broccoli, witch hazel, grapefruit, brussels sprouts, apples	Kaempferol	Flavones	Reduced the pancreatic cancer
Ginger	Gingerol	Flavonoids	Checked the colon cancer, breast, and ovarian tumors
Fava beans, soybeans, kudzu	Genistein	Isoflavone	Anthelmintic and antiangiogenic effects
Smoke tree, parrot tree, strawberries, apple, persimmon, grape, onion, cucumber	Fisetin	Flavones	Reduced the lung cancer
Grapes, blackberry, cranberry, raspberry, or apples and plums, red cabbage and red onion	Cyanidin	Glucoside	Antioxidant, anticancer properties
Saffron crocus, the plant *Crocus sativus* L.	Crocetin	Alkaloid	Active against hepatocellular carcinoma, lung cancer

Table 19.2 Structure of some pharmacologically important anticancer phytochemicals

Phytochemicals type	Name of phytochemicals
Alkaloids	Caffeine, morphine, codeine
Glycosides	A-Terpineol, cinnamyl acetate, eugenol taxifolin-7-o-β glucoside
Flavonoids	Flavan, flavone, dihydroflavone
Phenolics	Caffeic acid, chlorogenic acid
Terpenes	Cubebene
Anthraquinone	Luteolin, methyl luteolin
Tannins	Gallic acid, genistein, glycitein, daidzein
	Glycetein R1 = H, R2 = OCH3, R3 = OH Daidzein R1 = R2 = H, R3 = OH

19.3.1 Alkaloids

These are the largest group of secondary chemical constituents made largely of ammonia compounds comprising basically of nitrogen bases synthesized from amino acid building blocks with various radicals replacing one or more of the hydrogen atoms in the peptide ring, most containing oxygen (Table 19.2). The compounds have basic properties and are alkaline in reaction, turning red litmus paper blue. In fact, one or more nitrogen atoms that are present in an alkaloid, typically as 1°, 2°, or 3° amines, contribute to the basicity of the alkaloid. Degree of basicity varies considerably, depending on the structure of the molecule and the presence and location of the functional groups that react with acids to form crystalline salts without the production of water. Solutions of alkaloids are intensely bitter. In nature, the alkaloids exist in large proportions in the seeds. Basic structures of some pharmacologically important plant derived alkaloids and roots of plants and often in combination with vegetable acids. Alkaloids are having pharmacological applications as anesthetics and CNS stimulants; more than 12,000 alkaloids are known to exist in about 20% of plant species and only few have been exploited for medicinal purposes (Wrobleski et al. 2004). The name alkaloid ends with the suffix -ine, and plant-derived alkaloids in clinical use include the analgesics morphine and codeine, the muscle relaxant (+)-tubocurarine, the antibiotics sanguinafine and berberine, the anticancer agent vinblastine, the antiarrhythmic ajmaline, the pupil dilator atropine, and the sedative scopolamine (Table 19.2).

19.3.2 Glycosides

Glycosides in general, are defined as the condensation products of sugars (including polysaccharides) with a host of different varieties of organic hydroxy (occasionally thiol) compounds (invariably monohydrate in character), in such a manner that the hemiacetal entity of the carbohydrate must essentially take part in the condensation. Glycosides are colorless and crystalline substances containing carbon, hydrogen, and oxygen (some contain nitrogen and sulfur), water-soluble phytoconstituents,

and found in the cell sap. Chemically, glycosides contain a carbohydrate (glucose) and a non-carbohydrate part (aglycone or genin); alcohol, glycerol, or phenol represents aglycones. Glycosides are neutral in reaction and can be readily hydrolyzed into its components with ferments or mineral acids. Glycosides are classified on the basis of type of sugar component, chemical nature of aglycone, or pharmacological action. Glycosides are purely bitter principles that are commonly found in plants of the Genitiaceae family and though they are chemically unrelated but possess the common property of an intensely bitter taste. The bitters act on gustatory nerves, which results in increased flow of saliva and gastric juices (Table 19.2) (Londono et al. 2010).

19.3.3 Flavonoids

Flavonoids are important group of polyphenols widely distributed among the plant flora. Structurally, they are made of more than one benzene ring in its structure (a range of C15 aromatic compounds), and numerous reports support their use as antioxidants or free radical scavengers (Angelini et al. 2010). The compounds are derived from parent compounds known as flavans. Over 4000 flavonoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol, and quercitrin are common flavonoids present in nearly 70% of plants. Other groups of flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins (Table 19.2), calchones and catechin, and leucoanthocyanidins (Londono et al. 2010).

19.3.4 Phenolics

Phenolics, phenols, or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously as natural color pigments responsible for the color of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defense against pathogens and herbivore predators and thus is applied in the control of human pathogenic infections. They are classified into (1) phenolic acids, (2) flavonoid polyphenolics (flavonones, flavones, xanthones, and catechins), and (3) non-flavonoid polyphenols. Caffeic acid is regarded as the most common of phenolic compounds distributed in the plant flora followed by chlorogenic acid known to cause allergic dermatitis among humans. Phenolics essentially represent a host of natural antioxidants, used as nutraceuticals and found in apples, green tea, and red wine for their enormous ability to combat cancer, and are also thought to prevent heart ailments to an appreciable degree and sometimes are anti-inflammatory agents. Other examples include flavones, rutin, naringin, hesperidin, and chlorogenic (Table 19.2) (Dai and Mumper 2010).

19.3.5 Terpenes

Terpenes are among the most widespread and chemically diverse groups of natural products. They are flammable unsaturated hydrocarbons, existing in liquid form commonly found in essential oils, resins, or oleoresins. Terpenoids include hydrocarbons of plant origin of general formula (C5H8)n and are classified as mono-, di-, tri-, and sesquiterpenoids depending on the number of carbon atoms. Examples of commonly important monoterpenes include terpinen-4-ol, thujone, camphor, eugenol, and menthol. Diterpenes (C20) are classically considered to be resins, and Taxol, the anticancer agent, is the common example. The triterpenes (C30) include steroids, sterols, and cardiac glycosides with anti-inflammatory, sedative, insecticidal, or cytotoxic activity. Common triterpenes, such as amyrins, ursolic acid, and oleanolic acid, and sesquiterpene (C15), like monoterpenes, are major components of many essential oils. The sesquiterpene acts as irritants when applied externally, and when consumed internally, their action resembles that of gastrointestinal tract irritant. A number of sesquiterpene lactones have been isolated, and broadly they have antimicrobial (particularly antiprotozoal) and neurotoxic action. The sesquiterpene lactone, palasonin, isolated from *Butea monosperma* has anthelmintic activity, inhibits glucose uptake, and depletes the glycogen content in *Ascaridia galli* (Jiang et al. 2016) (Table 19.2).

19.3.6 Anthraquinones

These are derivatives of phenolic and glycosidic compounds. They are solely derived from anthracene giving variable oxidized derivatives such as anthrones and anthranols. Other derivatives such as chrysophanol, aloe-emodin, rhein, salinosporamide, luteolin, and emodin have in common a double hydroxylation at positions C1 and C8. To test for free anthraquinones, powdered plant material is mixed with organic solvent and filtered, and an aqueous base, e.g., NaOH or NH4OH solution, is added to it. A pink or violet color in the base layer indicates the presence of anthraquinones in the plan (Table 19.2) (Shami 2015).

19.3.7 Tannins

These are widely distributed in plant flora. They are phenolic compounds of high molecular weight. Tannins are soluble in water and alcohol and are found in the root, bark, stem, and outer layers of plant tissue. Tannins have a characteristic feature to tan, i.e., to convert things into leather. They are acidic in reaction, and the acidic reaction is attributed to the presence of phenolics or carboxylic group. They form complexes with proteins, carbohydrates, gelatin, and alkaloids. Tannins are divided into hydrolyzable tannins and condensed tannins. Hydrolyzable tannins,

upon hydrolysis, produce gallic acid and ellagic acid, and depending on the type of acid produced, the hydrolyzable tannins are called gallotannins or ellagitannins. On heating, they form pyrogallic acid. Tannins are used as antiseptic and this activity is due to the presence of the phenolic group. Common examples of hydrolyzable tannins include theaflavins (from tea), daidzein, genistein, and glycitein (Table 19.2) (Rhazi et al. 2015).

19.4 Action of Phytochemicals

19.4.1 Antioxidant Agents

In normal conditions, the human body possesses many defense mechanisms against oxidative stress, including antioxidant enzymes and nonenzymatic compounds (Kahkonen et al. 1999). The natural antioxidant mammalian mechanism sometimes become insufficient, and then the excess of free radicals can damage both the structure and function of a cell membrane in a chain reaction leading to many degenerative diseases (Wong et al. 2006). Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases, and inflammatory diseases (Gacche et al. 2011). Natural plants are a cheap source for the extraction of antioxidant compounds, thus providing important economic advantage. The DPPH radical is a stable organic free radical with an absorption maximum band around 515–528 nm. It is therefore a useful reagent for evaluation of antioxidant activity of compounds. In the DPPH test, the antioxidants reduce the DPPH radical to a yellow-colored compound, diphenyl picryl hydrazine, and the extent of the reaction depends on the hydrogen-donating ability of the antioxidants. The methanol extract of both *Philenoptera violacea* and *Xanthocercis zambesiaca* demonstrated a concentration-dependent scavenging activity by quenching DPPH radicals (Conforti et al. 2008). The hydrogen-donating activity, measured using DPPH test, showed that the concentration of *Xanthocercis zambesiaca* needed for 50% scavenging (SC50) was found to be 2.5 mg/ml, for *Philenoptera violacea* was >2.5 mg/ml (Shirwaikar et al. 2006).

19.4.2 Anticarcinogenesis

Polyphenols particularly are among the diverse phytochemicals that have the potential in the inhibition of carcinogenesis (Liu 2004). Phenolic acids usually significantly minimize the formation of the specific cancer-promoting nitrosamines from the dietary nitrites and nitrates. Glucosinolates from various vegetable sources such as broccoli, cabbage, cauliflower, and brussels sprouts exert a substantial protective support against the colon cancer. Regular consumption of brussels sprouts by human subjects (up to 300 g day^{-1}) miraculously causes a very fast (say within a span of 3 weeks) and appreciable enhancement in the glutathione S-transferase, and a

subsequent noticeable reduction in the urinary concentration of a specific purine metabolite serves as a marker of DNA degradation in cancer. Isothiocyanates and the indole-3-carbinols do interfere categorically in the metabolism of carcinogens, thus causing inhibition of procarcinogen activation and thereby inducing the "phase II" enzymes, namely, NAD(P)H quinone reductase or glutathione S-transferase, that specifically detoxify the selected electrophilic metabolites which are capable of changing the structure of nucleic acids. Sulforaphane (rich in broccoli) has been proven to be an extremely potent phase II enzyme inducer. It predominantly causes specific cell-cycle arrest and also the apoptosis of the neoplasm (cancer) cells. Sulforaphane categorically produces d-D-gluconolactone which has been established to be a significant inhibitor of breast cancer. Indole-3-carbinol (most vital and important indole present in broccoli) specifically inhibits the human papillomavirus (HPV) that may cause uterine cancer. It blocks the estrogen receptors specifically present in the breast cancer cells as well as downregulates CDK6 and upregulates p21 and p27 in prostate cancer cells. It affords G1 cell-cycle arrest and apoptosis of breast and prostate cancer cells significantly and enhances the p 53 expression in cells treated with benzopyrene. It also depresses Akt, NF-kappaB, MAPK, and Bel-2 signaling pathways to a reasonably good extent. Phytosterols block the development of tumors (neoplasms) in colon, breast, and prostate glands. Although the precise and exact mechanisms whereby the said blockade actually takes place are not yet well understood, yet they seem to change drastically the ensuing cell-membrane transfer in the phenomenon of neoplasm growth and thereby reduce the inflammation significantly. Cancer is one of the most prominent diseases in humans. Plants still remain a prime source of drugs for the treatment of cancer and can provide leads for the development of novel anticancer agents (Williams et al. 2004). The pace of research in the continuing discovery of new anticancer agents from natural product sources has been staggering lately (Rahman and Choudhary 2001). Recently, intensive research has been focused on developing tumor therapies from saponins. *Xanthocercis zambesiaca* extract had saponins and glycosides. Saponins exhibit potent anticancer activity in several human cancer cells through apoptosis-inducing pathways (Kinghorn et al. 2003), and glycosides are compounds that strongly influence the anticancer activity of the plant extract (Kaskiw et al. 2009). *Xanthocercis zambesiaca* have been proven to have isoflavones (Yan et al. 2009), and this compound regulates estrogen levels. It already has been proven that estrogen reduces risks of ovarian and endometrial cancer (Liao et al. 2009).

19.4.3 Antimicrobial Activity

Phytoconstituents employed by plants to protect them against pathogenic insects, bacteria, fungi, or protozoa have found applications in human medicine. Some phytochemicals such as phenolic acids act essentially by helping in the reduction of particular adherence of organisms to the cells lining the bladder and the teeth, which ultimately lowers the incidence of urinary tract infections and the usual dental caries.

Plants can also exert either bacteriostatic or bactericidal activity of microbes (Calderon-Montano et al. 2011). The volatile gas phase of combinations of *cinnamon* oil and clove oil showed good potential to inhibit growth of spoilage fungi, yeast, and bacteria normally found on intermediate moisture foods when combined with a modified atmosphere comprising a high concentration of CO_2 (40%) and low concentration of O_2 (<0.05%). *A. flavus*, which is known to produce toxins, was found to be the most resistant microorganism. It is worthy of note that antimicrobial activity results of the same plant part tested most of the time varied from researcher to researcher. This is possible because concentration of plant constituents of the same plant organ can vary from one geographical location to another depending on the age of the plant, differences in topographical factors, the nutrient concentrations of the soil, extraction method as well as method used for antimicrobial study. It is therefore important that scientific protocols be clearly identified and adequately followed and reported (Monte et al. 2014).

19.5 Conclusions and Future Prospects

Researches on specific phytochemicals in foods and their effects on disease risks are limited, but there's enough evidence from the association between foods rich in phytochemicals and disease risks which strongly suggests that consuming foods rich in these compounds may help to prevent diseases. However, it isn't known whether the health benefits are the result of individual phytochemicals, the interaction of various phytochemicals, the fiber content of plant foods, or the interaction of phytochemicals and the vitamins and minerals found in the same foods. The consumption of fruits, vegetables, and whole grains, as well as dietary patterns such as the Mediterranean diet that emphasize these foods, have been associated with a reduced risk of several types of cancer including breast, lung, and colon. Increase of three servings per day of whole grains is associated with a lower risk (17%) of colorectal cancer. Studies also have looked at the intake of specific phytochemicals and found a link to a reduced cancer risks. One study found that only specific flavonoid subgroups were associated with a decreased risk of breast cancer. These have found the reduced risk of cancer wasn't as strong for individual phytochemicals as for the foods rich in phytochemicals. The consumption of cruciferous vegetables such as broccoli, cabbage, and cauliflower has been associated with a decreased risk of prostate, lung, breast, and colon cancers. Isothiocyanate phytochemicals found in cruciferous vegetables, especially sulforaphane in broccoli, which has been studied extensively, are believed to offer some degree of prevention. It is quite clear from the above discussion that phytochemicals play a very important role in fighting many diseases like diabetes, cardiovascular diseases, nervous system disorder, cancer, and other diseases. Nowadays, the successful treatment for cancer has become a challenge to the whole world. Almost all the countries are doing several investigations on the prevention and treatment of cancer, so that the lives of billions of people can be saved.

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Chapter 20
Anticancer Plants and Their Conservation Strategies: An Update

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20.1 Introduction

Cancer is an abnormal growth of cells which divide irrepressibly by deviating the usual rules of the cell division. Normal cells obey the signals which direct them to undergo division and differentiation into new cells or decease. Cancerous cells advance to become independent from these signals and result to grow and proliferate relentlessly. Cancer disease involves several tempo-spatial deviations in the physiology of cells leading to precancerous (premalignant) or cancerous (malignant) state. The major cause for the disease condition and death in cancer patients is due to the invasion of tumor cell into the surrounding tissues and distant organs (Seyfried et al. 2014). According to Hanahan and Weinberg (2000), cancer cell genotypes are caused by the altered cellular physiology which as a group directs the tumor malignancy. These physiological variations include (i) self-sufficiency in signals produced for cell growth, (ii) insensitivity to signal molecules that inhibit cell growth, (iii) evasion of apoptosis (programmed cell death) mechanism, (iv) ability to multiply rapidly, (v) a continuous process of angiogenesis, and (vi) spread to other tissues (metastasis). Attainment of the above physiological variations or unusual competences reached at some point of the tumor progression signifies the success of violating tumor defensive mechanisms of a cell.

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The most widely used treatment methods for cancer are surgery, chemotherapy, and radiotherapy or a combination of treatments, such as surgery and chemotherapy or surgery and radiotherapy. The conventional treatment for cancer is surgery, which is effective in the treatment of localized primary tumor and related lymphatic system. Surgery is performed for different purposes such as diagnosis, staging, primary treatment, debulking, palliation, etc. The side effects of surgery include pain (often temporary), fatigue, the risk of infection at the surgical site, scaring, and numbness. Radiation therapy (radiotherapy) is another form of cancer treatment, which uses the high-energy rays (ionizing radiation) to kill or damage cancer cells. The objective of radiation therapy is to kill as many cancer cells as possible without destroying the healthy tissue. It is of two types: (a) external radiation therapy is given through a machine which delivers the high-energy rays at the specific area of the body to be treated and (b) internal radiation therapy involves the placement of a radiation source inside the body. Radioactive seeds, capsules, or ribbons are positioned in or near cancer cells to deliver a dose of radiation directly to the tumor site. Internal radiation can also be given in a liquid form, which is swallowed by the mouth or injected through a vein, while chemotherapy involves the use of drugs to destroy the cancer cells. Chemotherapy is a systematic (whole body) treatment. It can destroy cancer cells almost anywhere in your body. However, some healthy and normal cells may also be damaged by this treatment (Sbeity and Younes 2015). Surgery, when used as a sole treatment, cures more patients compared to other cancer therapies, because surgery operates through zero-order kinetics, in which 100% of cancer cells are killed, whereas radiotherapy and chemotherapy are able to destroy a portion of tumor cells by each therapy (Urruticoechea et al. 2010). The recent cancer treatments include the hormonal therapy involving certain hormones made in the body and the targeted therapy to destroy cancer cells using the body's immune system. The possible side effects of the above therapies are risks of infection, anemia (low red blood cells), bruising and bleeding, hair loss, tiredness (fatigue), sour mouth and ulcers, feeling sick (nausea) or being sick (vomiting), loss of appetite, taste changes, constipation, diarrhea, skin color changes, and hormonal changes (Aslam et al. 2014).

To avoid the side effects of various cancer therapies, there is a growing interest among oncologists to make use of naturally derived anticancer compounds from plants. Drugs derived from plants have offered exceptional input to the contemporary therapeutics; for instance, the *serpentine* compound extracted from the roots of *Rauwolfia serpentina*, an Indian medicinal herb, is used for treating hypertension and to lower the blood pressure. Likewise, other pharmacologically active phytocompounds include reserpine, rescinnamine, vinblastine and vincristine, etoposide, guggulsterone, teniposide, plaunotol, nabilone, artemisinin, ginkgolides, and many more. In fact, some of the major and currently used anticancer compounds such as paclitaxel (Taxol), topotecan, vinblastine, vincristine, podophyllotoxin, and camptothecin are primarily derived from medicinal plants (Pandey et al. 2011). The major advantages of using plant-derived anticancer drugs are that they are relatively nontoxic and available in an ingestive form (Amin et al. 2009). Further, recent studies have witnessed the possible application of many such medicinal plants containing bioactive compounds such as alkaloids, flavonoids, saponins, etc. as anticancer

agents. As a result, isolation and identification of such bioactive compounds from medicinal plants are gaining a momentum in recent times, and the list of plants having such compounds is steadily increasing.

In India, a very rich biodiversity can be seen with more than 130,000 scientifically documented species of plants and animals. Biological diversity is defined as "the variability among living organisms from all sources, including, 'inter alia', terrestrial, marine, and other aquatic ecosystems, and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems" (UNEP 1992). In terms of plant diversity, India occupies the 10th rank in the world and 4th rank in Asia. India is represented by about 11% of the plant diversity around the world with more than 45,500 floral species (Khandekar and Srivastava 2014). Major causes for the loss of biodiversity in India include habitat destruction, overexploitation, pollution, and species introduction. Other factors involved are fires, which adversely affect regeneration in some cases, and such natural calamities such as droughts, diseases, cyclones, and floods. Unrestrained utilization of natural resources, unplanned expansion of high input farming, transforming rich biodiversity locations for human shelter and to develop industries, and the destruction of coastal areas are the major threats to biodiversity (Kulkarni 2012).

The conservation of biodiversity is essential as it provides material goods (e.g., food, medicines, timber, and fiber), helps in controlling flood, regulates the climate and recycling of nutrients, and provides other benefits such as recreation. In agriculture, biological diversity plays a key role on pollination, control of pests, and storage and sequestration of carbons. Overall, biodiversity has a positive influence on the human health both physically and mentally. Biodiversity in nature also assures long-term benefits by resisting the natural disturbances and ecological changes. It has been reported that the economic value of returns from natural biota may be about 10–100 times the cost required for its maintenance (Rands et al. 2010). Some of the strategies which help in the prevention of biodiversity loss include habitat restoration and rejoining fragmented habitats; controlling evasive species; reducing climate change and global warming; controlling ozone layer depletion and thinning; sustainable fishing, hunting, grazing, and use of forest products; bio-conservation and use of protected areas; global adoption of biotechnology in agriculture; and legislation on releasing of toxic pollutants in air and water bodies, bush burning, and wildfires (Aguoru et al. 2015). Considering the importance of medicinal plants with immense therapeutical significance, their habitat loss, or near-to-extinction status, the present chapter highlights various methods of plant conservation including micropropagation and cryopreservation of the major anticancer plants.

20.2 About Cancer and It's Types

Cancer commences inside a cell, which is the basic building block of all living organisms. In normal course, older cells die off and are replaced by newer cells through a regulated cell division process. However, in cancer patients, this

organized procedure of forming new cells breaks down, and cells continue to divide uncontrollably even when new cells are not required and develop to form a tumor, an extra mass of cells. Further, the changes occurred in tumor cells over a period of time trigger them to invade nearby normal tissues and obstruct their function (NIH 2003).There are more than 150 types of cancers which are mainly categorized based on the tissues or organs that are affected (Michael 2003). Some of them include carcinomas, sarcomas, leukemia, etc. Carcinomas are solid tumors affecting the lung, breast, prostate, skin, stomach, and colon. Sarcomas are the cancers characterized by rare and deadly solid tumors that are formed in the bone and the soft tissues surrounding the organs. Leukemia is a group of different cancers of the blood cells and the bone marrow. It is characterized by an unusual formation of white blood cells (leukocytes). The malignancies of lymph nodes are known as lymphomas. They are of two types, Hodgkin's and non-Hodgkin's. A rare kind of tumor formed by malignant plasma cells producing immunoglobulins is called as myeloma.

20.2.1 Signs of Cancer

Natural therapies can be used to treat cancer and work better if detected at its primary stages (Michael 2003).Though it is difficult to identify cancer, the following signs could be useful: (a) a swelling or stiffening in the testicles or breasts needs additional examinations; (b) the appearance of warts or a change in warts could be an indication of skin cancer or squamous carcinoma; (c) melanoma can be indicated by a chronic skin sores that do not heal; (d) gastrointestinal cancer is indicated by changes in bowel or bladder habits including constipation, abdominal pain, chronic diarrhea, and rectal and urinary bleeding; (e) a chronic symptom of cough and coughing up blood is an indication of bronchial tree damages; (f) a persistent indigestion and difficulty to swallow are commonly associated with esophagus, colorectal, or stomach cancer; (g) sudden loss of body weight as cancer cells use more energy source; (h) uterine, endometrial, or cervical cancers are indicated by unusual bleeding or vaginal discharge; and (i) a chronic weakness is an indicator of rapidly progressing cancers.

20.2.2 Causes of Cancer

Substances that are known to cause cancer are known as carcinogens. Some of the factors that increase cancer risk include smoking, alcohol consumption, exposure to UV and ionizing radiation, exposure to chemicals during job and some viruses and bacteria, pesticides, etc. Cigarette smoking, chewing of tobacco, and contact to

environmental tobacco smoke escalate the risk of cancer. The risk of mouth, esophagus, and voice box cancers is found in increased numbers among smokers who also consume above two alcoholic drinks per day (Saha et al. 2007). Melanoma and other associated skin cancers are caused due to premature aging of the skin cells and DNA damages because of the exposure to ultraviolet (UV) radiations from the sun or tanning beds (Narayanan et al. 2010; D'Orazio et al. 2013). Ionizing radiations, the invisible high-frequency radiations, can damage nucleic acids or genes of the body cells. Everyone is exposed to ionizing radiations coming from cosmic rays. However, only about 1% cancer risk is associated with ionizing radiation (Narayanan et al. 2010).

Microorganisms (viruses and bacteria) causing infections in humans also contribute to several types of cancer developments. Human papillomavirus (HPV), a sexually transmitted virus, is linked to cancers of the cervix, vagina, vulva, penis, and anus (Braaten and Laufer 2008). Liver cancer is caused by hepatitis B (HBV) and hepatitis C (HCV) viral infections (Bartosch 2010; Schinzari et al. 2015). Some types of lymphomas are linked to the infections caused by Epstein-Barr virus (EBV) in majority of the adults (Gandhi 2006; Grywalska and Rolinski 2015). *Helicobacter pylori*, a widespread bacterium, is the major causative agent of the peptic ulcers and inflammation of the stomach (chronic gastritis). This bacterium can add to the progress of gastric cancer, also known as stomach cancer (NIH 2003; Wroblewski et al. 2010; Correa and Piazuelo 2011).

Pesticides and herbicides, used in the agriculture for controlling pests and weeds, are known to cause cancer. The farmers, agricultural workers, and pesticide manufacturers who are exposed to DDT, amitrole, ethylene oxide, chlorophenoxy herbicides, dimethylhydrazine, hexachlorobenzene, lindane, mirex, lead acetate, nitrofen, etc. will have a high risk of cancer (Bassil et al. 2007). The pesticides leach into water and accumulate in fish, cattle, and poultry and enter into the food chain, and this bioaccumulation affects the fatty tissues in the brain, sexual organs, and breasts. Applications of toxic heavy metals in industries find their way into the human body. The metallic compounds of lead, mercury, arsenic, copper, aluminum, nickel, cadmium, etc. have a tendency to build up in fat cells affecting the brain and central nervous system (Beyersmann and Hartwig 2008). Exposure to these heavy metals contributes in the development of peripheral neuropathy, anemia, kidney damage, skin diseases, and several types of tumors including liver, lung, skin, and kidney neoplasms (Rousselot et al. 1999; Yedjou and Tchounwou 2007; Tchounwou et al. 2012). A number of solvents such as paint thinners, grease removers, carbon tetrachloride, benzene, chloroform, tetrachloroethylene, and dichloromethane are scientifically proven to cause cancer in animal models (Clapp et al. 2008). Exposure to various fibers (asbestos and ceramic fiber), fine particles, and dusts (silica dust and wood dust) increases the risk of cancers. Exposure to chemicals such as dioxins, polycyclic aromatic hydrocarbons (PAHs), metallic compounds, diesel exhaust particles, toxins from fungi (aflatoxins), vinyl chloride, benzidine increases the risk of cancers (NIH 2003; Ledda et al. 2017).

20.3 Medicinal Plants as Anticancer Agents: Current Scenario

Plant-based natural products are extensively used throughout the world as herbal medicines for treating various human ailments (Swamy and Sinniah 2015, 2016). Plant-based natural compounds include therapeutic compounds, coloring agents, essential oils, and cosmetics (Swamy and Sinniah 2016; Mohanty et al. 2017). The plants which impart medicinal values are generally called as medicinal plants and considered as rich resources of ingredients for both drug discovery and manufacturing drugs (Arumugam et al. 2016; Swamy and Sinniah 2015; Swamy et al. 2017). Plants such as ginger, green tea, walnuts, and many more are considered as an important source of nutrition and thus recommended for their therapeutic values. Most of the pharmaceutical drugs such as antibiotics, blood thinners, laxatives, and antimalarial and antidiabetic medications are made from the ingredients of plants. Moreover, plants such as *Vinca minor*, *V. major*, *Catharanthus roseus*, *Digitalis purpurea*, *Taxus baccata*, *Papaver somniferum*, *Nothapodytes nimmoniana*, and *Ophiorrhiza mungos* are used for isolating some of the anticancer compounds including vincristine, Taxol, morphine, and camptothecin (Rasool Hassan 2012; Prakash et al. 2013, 2016; Kaushik et al. 2015). More than 100 phytocompounds including deserpidine, reserpine, rescinnamine, vincristine, and vinblastine were introduced in the US drug market during the period between 1950 and 1970. Later, several novel drug molecules were introduced into the global market between 1971 and 1990. Some of the important drugs included nabilone, E-guggulsterone, teniposide, etoposide, plaunotol, artemisinin, lectinan, and ginkgolides. Approximately 2% of the new drugs, such as toptecan, paciltaxel, irinotecan, gomishin, etc., were introduced from 1991 to 1995. The plant *C. roseus* consists of the compounds vinblastine and vincristine. Vinblastine is effective against Hodgkin's disease, choriocarcinoma, leukemia in children, non-Hodgkin's lymphoma, and cancer of the testis and neck, while vincristine is advocated for the treatment of acute lymphocytic leukemia, lymphosarcoma, and cervical, lung, and breast cancers. Podophyllotoxin, another anticancer agent isolated from *Podophyllum emodi*, is presently used to treat lymphomas and lung and testicular cancers. Drugs derived from *N. nimmoniana* (*Mappia foetida*), a tree indigenous to India, are widely used against cervical cancer (Pandey et al. 2011). Likewise, various bioactive compounds such as alkaloids, flavonoids, saponins, etc. isolated from other medicinal plants have proven to be effective against different cancer types.

Medicinal plants include about 8000 species and account to nearly 50% of all the higher flowering plants in India. In other words, among 400 different plant families of the flowering plants, a minimum of 315 are found in India (Khan 2014). At present, medicinal plants found in the Himalayan region contribute majorly for the herbal industries both in India and other countries. At higher altitudes, plants are subjected to numerous testing circumstances comprising of physiological drought, higher doses of mutagenic UV radiation, strong winds, and desiccation. Plants living in such stressful environments produce a spectrum of secondary metabolites

through physiological adaptations and altered biochemical profiles in their tissues (Umadevi et al. 2013). Herbs have been widely used in several ancient folk practices of medicine like Ayurveda, homeopathy, and Unani practices in India, Chinese traditional system of medicine, and other folk medicines practiced in other countries. Herbal drugs are the preparations made from plants or their ingredients for preventing and treating diseases or to support health. The herbal drug is the chief constituent of all the traditional medicines and is usually regarded as safe. However, allopathic medicines possess higher toxicity and side effects, and hence the use of herbal drugs has increased rapidly in recent times and increased the number of herbal drug manufacturers. Few drug molecules have been abandoned because of higher toxicity effect, while some other drugs are chemically modified or sometimes administered in combination with additional herb-based drugs to overcome side effects. Some advantages of herb-based drugs include the enhanced tolerance, low cost, and a high potency and efficiency with negligible or no side effects. Some of the disadvantages of herbal drugs include the inability to cure sickness rapidly, risks with self-dosing, and complexity in standardizations (Prakash et al. 2013; Pathak and Das 2013).

Currently, medicinal plants play vital role in both developed and developing countries of the world. The people in many Asian countries generate income for their living by selling the materials collected from the wild or through cultivation (Swamy et al. 2015a; Swamy and Sinniah 2016). Collection of naturally occurring medicinal plant resources has been practiced in many countries with the objective of using them in the folk medicines or to process them in to pharmaceutical products. This activity provides revenue to the native individuals and delivers rare raw materials at a lesser price, compared to the raw material procured through cultivation (Upadhyay et al. 2011). However, it leads to habitat loss and disturbs the biodiversity of the region. In a report by the National Medicinal Plants Board (NMPB), through the Foundation for Revitalisation of Local Health Traditions (FRLHT), Bengaluru, India, it has been stated that about 3.19 lakh metric tons (MTs) of medicinal herbs have been traded for the year 2005–2006. Among the major 960 medicinal plants traded, 178 species have a yearly consumption of over 100 metric tons (MTs) (Ganesan et al. 2016). As per the report of the World Health Organization (WHO), the demand for herbs is rising steadily (15–25%) per annum, and the current global market for plant-based products is around US$62 billion. By the year 2050, this demand is believed to grow further up to US$5 trillion (Swamy and Sinniah 2016; Mohanty et al. 2017). There is a high demand for medicinal and aromatic herbs' raw materials from nutraceutical, pharmaceutical, flavor, and aroma industries to manufacture different herbal products (Swamy and Sinniah 2016).

The growing demand for medicinal plants has resulted in the uncontrolled harvesting that leads to the biodiversity loss and many important species extinction. Further, the reasons for loss of biological resources and reduced biodiversity include an inadequate cultivation of herbs in fields, lack of scientific information on cultivation, inappropriate management of harvesting or overharvesting, etc. (Swamy et al. 2015b; Swamy and Sinniah 2016). The Food and Agriculture Organization of the United Nations has reported that flora endures permanent losses and results in the

ecological imbalances. About 4000–10,000 medicinal plant species are in the verge of disappearing or endangered during the last few years (Tasheva and Kosturkova 2012; IUCN 2017). In this regard, various initiatives have been developed and directed by many international organizations to conserve rare and endangered medicinal plant species using both *in situ* and *ex situ* conservation approaches. In 2012, as a global initiative, the Global Strategy for Plant Conservation (GSPC 2010) was engaged by over 180 nations with an aim of plant conservation and for improving plant diversity. Likewise, the United Nations Development Program (UNDP 2010a), the United Nations member states, and other international bodies have initiated the Millennium Development Goals (MDGs; UNDP 2010b) which have been established to conserve plant species (Reed et al. 2011). The other option is to cultivate the medicinal plants at a larger scale on the wasteland, degraded land, and problematic land without affecting the area sown for food grain production, because food security is also important. To achieve this objective, several bottlenecks of cultivation such as nutrient management, water management, and weed management need to be addressed (Upadhyay et al. 2011; Swamy et al. 2015b; Mohanty et al. 2017). Medicinal plant cultivation has been achieved successfully in many commercial crops (Swamy and Sinniah 2016). For instance, Biswas (2010) reported that cultivating two medicinal plants *Aloe vera* and *Moringa oleifera* obtained a profit of INR 7.19 lakhs/ha/year, while *Aloe vera* cultivation resulted in a profit of INR 2.72 lakhs/ha/year (Biswas 2010). Interestingly, the cultivation of medicinal plants has certainly improved the socioeconomic status of the farmers (Swamy and Sinniah 2016). Even though plant conservation strategies are in process and succeeding through using *in situ* safeguarding and *ex situ* conservation in seed banks and botanical gardens, other methods of conservations are much appreciated for many endangered plant species (Reed et al. 2011). These complementing conservation strategies including *in situ* and *ex situ* conservation, seed banks, micropropagation, and cryopreservation methods are required to preserve the biological diversity of numerous endangered and threatened medicinal herbs. All these methods are discussed below giving more importance to the conservation of endangered anticancer plant species.

20.4 Methods of Conservation of Anticancer Plants

The rich biological diversity gives food, medicines, and other useful items like timber, rubber etc., and livelihood to people. In recent years, it is declining due to destruction of habitat, overexploitation, collection for research, introduction of exotic species, control of pests and predators, pollution, and deforestation. Other factors include the distribution range, degree of specialization, reproductive rate, outbreaks of diseases, loss of gene flow, and substitutions. The increasing demand for anticancer plant parts such as roots, bark, and stem and the whole plants in the case of herbs has led to the depletion of genetic resources. It is estimated that more than 90% of raw materials for pharmaceutical companies is drawn from the wild, as less than 20 plant species are under commercial cultivation (Siwach et al. 2013). In

many cases, the cultivation/plantation of medicinal herbs is limited due to the low seed viability, variations in season, weather, climate, diseases and pests, constrains in marketing, lack of awareness in cultivation practices, etc. As a result, the anticancer plant resources are getting depleted, and those who are harvesting/collecting the plants are not taking care to replenish the resources leaving a huge gap between production and supply of anticancer plants. To bridge the gap between the supplies of natural compounds, there is a need to conserve the anticancer plants. Alternatively, the nondestructive production of anticancer compounds will play an important role in the conservation of anticancer plants. There are different approaches for conservation of anticancer plants such as *in situ* and *ex situ* conservation and biotechnological approaches.

20.4.1 In Situ *Conservation*

It is the method of conservation of plants in their natural habitats or in the area where it is grown naturally. Biosphere reserves, national parks, sacred sites, and sacred grooves, etc. will fall under this category (Akshay et al. 2014).

20.4.1.1 Biosphere Reserves

Biosphere reserves comprise marine, coastal, and terrestrial ecosystems. Each of these reserves chiefly promotes to resolve the biodiversity conservation with more accountability and foster the ecological balance. Biosphere reserve is not foreseen to interchange the existing conservation descriptions, but rationally to improve the societal and environment association by the sustainable uses (Reed and Egunyu 2013; Coetzer et al. 2014). The biosphere reserves are well admired by the developing nations with the objectives focused on both conservation and sustainable development, which encourage socioeconomic developments in these protected lands (Coetzer et al. 2014). There are 18 biosphere reserves in India in different states, out of which 9 biosphere reserves, viz., Gulf of Mannar (Tamil Nadu), Nilgiri (Kerala, Tamil Nadu, and Karnataka), Sundarbans (West Bengal), Nanda Devi (Uttarakhand), Pachmarhi (Madhya Pradesh), Similipal (Orissa), Nokrek (Meghalaya), Achanakmar-Amarkantak (Chhattisgarh and Madhya Pradesh), and Great Nicobar, are included in the World Network of Biosphere Reserves of UNESCO (MOEF 2015).

20.4.1.2 National Parks

An area within a nature reserve is notified as a national park by the state government with an aim of protecting and propagating or to improve wildlife within or its surroundings. These are the most extensive type of safe zones globally which prohibits the direct usage of any natural resources in the national parks for commercial

purposes (Muhumuza and Balkwill 2013). National parks are very crucial to conserve the ecological, floral, faunal, or zoological associations. It is an area in which activities like hunting, poaching, and grazing on cultivation are prohibited, and it is strictly earmarked for improving the wildlife and biodiversity (Muhumuza and Balkwill 2013). They are well demarcated and emphasized to preserve the flora and fauna of that area. As of June 2017, there were 103 national parks covering an area of 40,500 km^2 (15,600 sq.m) and comprising 1.23% of India's total surface area (ENVIS 2017).

20.4.1.3 Sacred Groves

Sacred groves are small or large patches of vegetation protected on the basis of cultural and traditional practices on the religious background. Hunting and logging are usually strictly prohibited within these patches. In India there are about 13,720 sacred groves present in different states (Akshay et al. 2014). Sacred groves acted as a repository for Ayurvedic medicines and also replenishable resources like fruits and honey. The groves are often associated with ponds and streams and meet the water requirements of local communities. In modern times, sacred groves have become biodiversity hotspots, as various species seek refuge in the areas due to progressive habitat destruction and hunting. Sacred groves are classified into (a) local village sacred groves, managed by the entire village; (b) regional sacred groves, managed by temple trust; (c) pan-Indian sacred groves, which are large and managed by temple trust; (d) and sacred groves as the abode of ancestral spirits, which are both a burial ground and location of deity and ancestor worship. Many species that have become extinct in other parts of the country are well preserved in these groves. These areas also provide natural habitat, water, and nesting sites for many species of wildlife and birds. They also act as a gene bank of various plants and animals, thus ensuring that these species do not become extinct (Amirthalingam 2016).

20.4.2 Ex Situ *Conservation*

It is the conservation of critically endangered medicinal herbs away from their natural habitats. In *ex situ* conservation, biotechnological approaches are much appreciated as they have several advantages such as (1) better-quality plants can be produced in large scale which are free from pests and diseases through micropropagation; (2) secondary metabolites can be produced through callus cultures, cell suspensions, and hairy roots by using limited plant materials; and (3) cryopreservation helps in the long-term conservation of endangered plant species and also potential cell lines identified for production of anticancer compounds/secondary metabolites.

20.4.2.1 Gene Banks

Gene banks are biorepositories used to preserve genetic materials. The different types of gene banks include seed banks, tissue banks, cryobanks, and field gene banks. Seed banks are the best and compact techniques to conserve orthodox seeds. In medium-term storage facilities, seeds are kept in containers or packets and stored between 0 and 5 °C temperature with a relative humidity of 15–20%. For long-term storing, most of the materials are kept at very low temperatures between −20 and −180 °C. Based on the initial seed quality and plant species, most seed samples can be kept viable until 20–30 years in medium-term storage facilities, while seeds can be stored viable for up to 100 years in long-term storage facilities (Kasagana and Karumuri 2011). In tissue banks, buds, protocorms, and meristematic cells are preserved through particular light and temperature arrangements in a nutrient medium. This technique is used to preserve seedless plants and plants that reproduce sexually. While in the cryobank technique, a seed or embryo is preserved at very low temperatures. It is usually preserved in liquid nitrogen at −196 °C. This is helpful for the conservation of endangered species/species facing extinction. For example, sensitive seeds, pollen, vegetative tissues, and selected orthodox seeds of plant species were conserved in the national cryobank at the NBPGR (National Bureau of Plant Genetic Resources), India. About 30,000–40,000 samples of varied germplasms can be stored in six large capacity cryotanks at the national cryobank at NBPGR. At present, nearly 6000 accessions are cryo-conserved in the form of seeds, shoot apices, embryonic axes, embryos, and pollens (Kasagana and Karumuri 2011). On the other hand, field gene bank is used to conserve genes in an artificially created ecosystem. It needs more land, adequate soil, weather, etc. Germplasms of important commercial and medicinal crops are conserved through this method. For instance, the field gene bank of Tropical Botanic Garden and Research Institute (TBGRI) has covered 30,000 accessions of 250 medicinal and aromatic plant species which include 100 endemic, rare, and endangered medicinal and aromatic plants of the tropical regions of India.

20.4.2.2 Micropropagation (In Vitro Propagation)

Micropropagation is the technique of production of plants in sterile conditions under controlled environment of light and temperature. Micropropagation can be initiated from any part of the plant (seeds, leaves, nodes, tubers, etc.). In this method, the plants are produced by the formation of multiple shoots through shoot tips or axillary buds by using different plant growth regulators (Sudipta et al. 2011; Swamy et al. 2014). The other method of micropropagation is the somatic embryogenesis and organogenesis in which the plants are produced through callus. Initially callus is produced from leaf, seed, shoot, and root explants. When the shoots are produced directly from the explants, it is called direct organogenesis, and in the indirect way, first the callus is produced from explants and later shoots are formed from the callus. The elongated shoots are excised and are kept for rooting and the plantlets are

produced. In somatic embryogenesis, the bipolar structures called somatic embryos are formed. These somatic embryos germinate to form plantlets avoiding the rooting stage. When the somatic embryos are formed on the explants, it is called direct somatic embryogenesis; when the somatic embryos are formed through the callus, it is called indirect somatic embryogenesis. These somatic embryos are kept generally on basal medium for germination and the plantlets are produced.

The plants produced through micropropagation are free from nutritional disorders, pests, diseases, etc. By using shoot apex/shoot tip/nodal culture, true-to-type plants can be produced, while meristem culture produces virus-free plants (Kaushik et al. 2015; Prakash et al. 2016; Safarpour et al. 2017). From a small plant tissue, over one million plants can be produced in 1 year (Rai 2010). In vitro techniques have been increasingly applied to mass propagate and conserve germplasms as it has superiority over conventional method of propagation and offer some distinct advantage over alternative strategies (Swamy et al. 2009, 2010; Noor et al. 2017; Bustam et al. 2017). Some of the advantages are as follows: (1) propagation can be achieved at any time independent of flowering period of species (this assumes that seed material is not required); (2) virus elimination can be achieved through meristem cultures; (3) elite genotypes can be maintained through clonal material production; (4) rapid multiplication is possible; (5) difficult-to-germinate or immature seeds or embryos may be facilitated for breeding programs; and (6) distribution across the border may be safer and easier, in terms of germplasm health status using in vitro cultures. The plants derived from callus cultures display vast genetic disparity that could be exploited in vegetatively propagated plant species for developing superior clones/varieties (Kumaraswamy and Anuradha 2010; Sudipta et al. 2011; Sharma and Thokchom 2014).

The development of micropropagation protocols for various anticancer plants using various plant parts such as nodal explants, leaves, shoots, petioles, embryos, cotyledons, epicotyls, tubers, shoot tips, mature embryos, and rhizome buds is listed in Table 20.1. Rajora et al. (2013) reported the multiple shoot production from the nodal explants of *C. roseus* using 6.0 mg/l benzylaminopurine (BAP) and 6 mg/l kinetin (KIN) supplemented to Murashige and Skoog (1962) (MS) media. In BAP-supplemented media, bud stimulation was noted within 4 days, whereas in KIN-containing media, bud breaking took about 12 days. In vitro rooting was better obtained in MS media containing 10 mg/lindole-3-butyric acid (IBA). Rooted plants were successfully acclimatized at room temperature. The established plants were observed to flower and set seeds in the greenhouse after 3 months. In an experiment conducted by Pandey et al. (2014) using the axillary nodes, BAP at 1.0 mg/l gave the highest number of shoots (2.4 ± 0.3), shoot length (5.2 ± 0.4 cm), and nodes/shoot (6.1 ± 0.1), and kinetin at 0.75 mg/l gave the highest number of leaves/shoot (14.2 ± 1.7) in *C. roseus*. Guo et al. (2012) reported the highest rooting percentage of in vitro produced shoots from seeds of *Podophyllum hexandrum* on WPM (woody plant medium) fortified with 1.5 mg/indole-3-acetic acid (IAA) and 0.5 mg/l naphthaleneacetic acid (NAA). The rooting occurred after 20 days, and maximum percentage (60.1%) of the shoots rooted after 30 days with a mean root length of 0.5–1 cm. After 45 days, about six to eight roots with an average length of 2.5 cm

Table 20.1 In vitro propagation methods of some important anticancer plants

Name of plant	Plant parts used	Type of culture obtained	References
Taxus baccata	Nodal segments	Plantlets	Abbasin et al. (2010)
Salvia officinalis	Leaves and leaves from in vitro raised young plants	Callus and somatic embryos	Ioja-Boldura et al. (2010)
Campotheca accuminata	Auxiliary buds	Plantlets	Kang et al. (2011)
Solanum nigrum	Nodal explants with axillary buds	Plantlets	Padmapriya et al. (2011)
Glycyrrhiza glabra	Shoot tips	Plantlets	Sawaengsak et al. (2011)
Hypericum perforatum	Hypocotyl	Plantlets	Banerjee et al. (2012)
S. nigrum	Shoot tips, nodal explants	Plantlets	Kavitha et al. (2012)
Withania somnifera	Nodal and leaf explants	Plantlets and callus	Rao et al. (2012)
Nothapodytes foetida	Mature embryos	Plantlets	Tejavathi et al. (2012)
Tinospora cordifolia	Leaf, young nodal explants	Callus and plantlets	Bhalerao et al. (2013)
S. nigrum	Shoot segments	Plantlets	Rathore and Gupta (2013)
Scutellaria barbata and *S. racemosa*	Shoot tip, nodal explants	Plantlets	Brearley et al. (2014)
Colchicum hierosolymitanum	Seeds	Callus and regenerated somatic embryos	Daradkeh et al. (2012a, b)
Gloriosa superba	Sprouted tuber node	Microtubers and plantlets	Selvarasu and Kandhasamy (2012)
Aloe vera	Shoots with and without sheath	Plantlets	Abdi et al. (2013)
T. baccata	Leaf, stem, shoot tip	Callus, plantlets	Baharak et al. (2014)
Aegle marmelos	Nodal explants	Plantlets	Bindhu (2013)
Curcuma longa	Rhizome buds	Plantlets	Ghosh et al. (2013)
N. foetida	Leaf and stem segments	Plantlets	Khadke and Kuvalekar (2013)
Allium sativum	Branches with nodal explants	Plantlets and callus	Mehta et al. (2013)
A. marmelos	Nodal segments with axillary buds	Plantlets	Puhan and Rath (2013)
Catharanthus roseus	Nodal segments	Plantlets	Rajora et al. (2013)
Azadirachta indica	Node, internode, shoot tip explants	Callus, plantlets	Shivanna et al. (2013)
A. indica	Nodal segments	Plantlets	Gehlot et al. (2014)

(continued)

Table 20.1 (continued)

Name of plant	Plant parts used	Type of culture obtained	References
Annona muricata	Shoots, petioles	Callus, shoots	Inwanna et al. (2014)
Salvia santolinifolia	Young shoots	Plantlets	Jan and Khatoon (2014)
C. roseus	Axillary nodal explants	Plantlets	Pandey et al. (2014)
W. somnifera	Shoot apex	Plantlets	Rani et al. (2014)
T. cordifolia	Nodes, internodes, and shoot tips	Plantlets	Sivakumar et al. (2014)
Andrographis paniculata	Leaves	Plantlets	Al-Mamun et al. (2015)
Podophyllum hexandrum	In vitro raised multiple shoots	Plantlets	Guo et al. (2015)
A. indica	Embryo with cotyledons	Callus, plantlets	Houllou et al. (2015)
N. foetida	Mature embryos	Plantlets	Kaveri and Rao (2015)
T. baccata	Leaf	Callus	Mahdinejad et al. (2015)
Cannabis sativa	Cotyledon and epicotyl explants	Plantlets	Movahedi et al. (2015)
W. somnifera	Nodal segments	Plantlets	Autade et al. (2016)
Zingiber officinale	Rhizome buds	Plantlets	David et al. (2016)
C. sativa	Nodal segments	Plantlets	Lata et al. (2016)
Indigofera tinctoria	Nodal explants	Plantlets	Nair et al. (2016)
Plumbago zeylanica	Nodal explants	Plantlets	Vijay et al. (2016)
Z. officinale	Young buds	Plantlets	Zuraida et al. (2016)

were evidenced. In *T. baccata*, the use of 2 mg/l BAP induced better shoot multiplication rate and morphogenesis of shoot tips. Combination of BAP (2 mg/l) and NAA (0.3 mg/l) improved the callus formation and increased multiple shoots to eight per cultured *T. baccata* embryo. Shoots elongated on ½ strength MS medium supplemented with 8 mg/l IBA (Abbasin et al. 2010).

The high frequency bud break was obtained in *Aegle marmelos* (L.) when the nodal explants were cultured on MS medium with 0.5 mg/l BAP. The highest number of shoots (3.6 ± 0.33) and shoot length (2.8 ± 0.20 cm) was recorded on the MS medium containing 0.5 mg/l BAP, 0.1 mg/l KIN, and 0.5 mg/l gibberellic acid (GA_3). Better rooting of the elongated shoots was obtained in ½ MS medium supplemented with 2.5 mg/l IBA and 0.5% activated charcoal (Puhan and Rath 2012). Similarly, a maximum number of shoots (5.36 ± 1.47) were obtained from the nodal explants of *A. marmelos* when cultured on MS medium fortified with 1.0 mg/l BAP and 0.1 mg/l IAA. The excised shoots rooted well in the ½ strength MS medium contained with 10 mg/l IBA and 0.1 mg/l IAA. The in vitro raised plantlets were acclimatized with a success rate of 60% (Bindhu 2013). Likewise, the shoot tips of

Annona muricata in the½ strength MS medium consisting of 0.5 mg/l BAP and 0.05 mg/l NAA recorded an average of 1.6 shoots within 1 month. The rooting was also well established on the same media, and the in vitro raised plantlets showed 60% survival rate under the greenhouse condition (Inwanna et al. 2016).

In *Azadirachta indica*, 100% shoot proliferation with an average of 3.95 shoots having a length of 3.60 cm was obtained from the nodal explants on MS medium supplemented with 8.88 μM BAP. The in vitro raised shoots, when cultured on MS medium with 4.44 μM BAP, resulted in 4.95 shoots with an average length of 3.95 cm. The excised shoots were rooted in the medium having tryptophan (146.89 μM) within 6–7 days. The plantlets were hardened in the greenhouse and showed 95% survival in field conditions (Gehlot et al. 2014). Similarly, the nodal explants of *A. indica* showed shoot regeneration in MS medium supplemented with BAP (1.0 mg/l), GA_3(0.1 mg/l), and IAA (0.05 mg/l) at 150 mM saline concentration producing 3.0 shoots/explant with about 80% regeneration frequency. Increasing the saline concentration to 250 mM reduced the shoot regeneration frequency to 25.33% with only one shoot/explant (Shivanna et al. 2013). For the first time, Lata et al. (2016) used meta-topolin (mT) {6-(3-hydroxybenzylamino)purine} for both shoot multiplication and rooting in *Cannabis sativa.* The highest number of shoots (13.44 ± 1.38) was obtained with the use of 2 μM mT concentration with a maximum shoot length of 11.44 ± 0.8 cm. In mT, the average number of shoots and length of shoots was found to be higher compared to the thidiazuron (TDZ) treatments. The vigorously growing shoots in MS medium with 1–4 μM mT were transferred to fresh medium, resulting in root initiation within 3–4 weeks, and the fully developed healthy roots were formed in 6 weeks. Among different concentrations of mT tested for rooting, MS medium with 2 μM mT gave the best rooting response.

The effect of different media (MS, Gamborg (B5) and WPM) on the shoot multiplication and rooting was studied in *G. glabra* shoot tip culture by Sawaengsak et al. (2011). MS medium with 0.5 mg/lBAP supported the shoot multiplication with the highest number of shoots (4.75) per explant. The ½ strength B5 medium supplemented with 5 mg/l of IAA or IBA gave the highest frequency of root formation, whereas the roots were more elongated in the above medium supplemented with IBA. In *Plumbago zeylanica*, the highest number of shoots (19.4 ± 0.96) from nodal explants with a mean length of 6 ± 0.94 cm was obtained on the MS medium supplemented with 4.44 μM BAP. The maximum mean number of roots (12 ± 1.56) with an average length of 8.92 ± 0.75 cm was observed in the ½ MS medium with 125 mg/l activated charcoal. The in vitro raised plantlets were hardened in 1:1:1 ratio of sand/soil/vermicompost and transplanted successfully in soil (Vijay et al. 2016).

Jan and Khatoon (2014) reported the shoot multiplication from nodal explants in *Salvia santolinifolia* and observed that the shoot multiplication was slow in primary explants and increased during subcultures. The maximum number of shoots (1.8 ± 0.7) was produced in the medium with BAP 3.0 mg/l, while the highest rooting was observed in MS medium with 2.5 mg/l IBA, and the longest roots were obtained in MS medium with 3.5 mg/l IBA. Brearley et al. (2014) studied the influence of six cytokinins, such as BAP, KIN, TDZ, mT, 2iP (2-isopentenyladenine),

and zeatin [6-(4-hydroxy-3-methylbut-2-enylamino)purine]; eight different carbon sources, 0.1 M each of sucrose, D-maltose, fructose, sorbitol, D-glucose, D-mannose, myoinositol, and D-mannitol; and two incubation periods (14 and 21 days) on the in vitro regeneration of *Scutellaria barbata* and *Scutellaria racemosa*. The nodal explants of *S. barbata* D. Don. produced four shoots when inoculated in MS medium supplemented with 5 μM meta-topolin and 0.1 μM NAA (shoot induction medium) after 21 days of incubation. Similarly, nodal explants of *S. racemosa* produced four and five shoots after 14 and 21 days of incubation in the above medium. However, nine and ten shoots were recorded in the medium supplemented with fructose and glucose, respectively, after 14 days, whereas *S. barbata* recorded 19 shoots after 21 days in glucose-supplemented medium. However, *S. racemosa* after 14 days of incubation produced only five shoots in maltose and four shoots in medium with sorbitol, whereas the medium with sucrose maltose produced five shoots after 21 days of incubation; fructose, glucose, and sorbitol produced four shoots (Brearley et al. 2014).

In a study, multiple shoots were formed in *Solanum nigrum* by using different concentrations of BAP (5–25 μM) or kinetin (5–25 μM) in MS media. The nodal explants with axillary bud produced maximum number of shoots (49 ± 1.32) with a height of 9.46 cm in 15 μM KIN supplemented MS media. The maximum number of roots (47 roots/shoot) with a root length of 5.5 cm was formed in 10 μM IBA in the MS medium (Padmapriya et al. 2011), while Rathore and Gupta (2013) reported the formation of the maximum number of multiple shoots (8.0 ± 0.57) and higher shoot length (5.57 ± 0.81 cm) in MS medium containing 2 μM BAP. The elongated shoots were kept for rooting in the ½ MS with different concentration of IBA. The best IBA concentration for rooting was found to be 10 μM IBA which induced 4.5 ± 0.5 roots with a mean length of 3.35 ± 0.57 cm. In another study, Kavitha et al. (2012) reported the use of 1.0 mg/l BAP as the best plant growth regulator concentration for inducing multiple shoots (8.4 ± 0.22) in *S. nigrum.* They used MS media with NAA (1.0 mg/l) to induce better rooting. In vitro rooted plantlets were hardened and transferred to soil successfully.

In *Tinospora cordifolia,* induction of shoots from nodal explants was achieved with BAP (8 μM) and KIN and BAP (12 μM and 8 μM) supplemented MS media. The regenerated shoots were rooted in medium with 8 μM NAA (Bhalerao et al. 2013). In contrast, Sivakumar et al. (2014) used kinetin at 4.36 μM to induce higher shoot regeneration frequency of *T. cordifolia* nodal explants. To avoid the phenolic problem, silver nitrate (20%) was used with kinetin (4.36 μM), which gave 100% response with an average 2.01 ± 0.1 shoots. Multiple shoots were produced from the in vitro raised nodal explants in medium containing BAP alone or BAP with NAA or IAA. It was observed that a better growth response was observed in MS media containing BAP (8.82 μM) alone and produced about 4.81 ± 0.2 shoots/explant having an average length of 3.1 ± 0.1 cm after 20 days. The elongated shoots when transferred to ½ strength MS medium fortified with 3% sucrose and 6.43 μM of IBA produced 5.2 ± 0.2 roots per shoot with an average root length of 3.2 ± 0.1 cm. Rooted plantlets were transplanted ex vitro and approximately 80% of the plantlets survived.

Rani et al. (2014) recorded the formation of the highest number of shoots (7.56 ± 0.59) from the shoot apex explants of *W. somnifera* in MS medium fortified with 4 mg/l BAP and 1.0 mg/l NAA. However, the average shoot length (4.22 ± 0.41 cm) was higher in the medium consisting of 0.5 mg/l BAP and 3.0 mg/l NAA. MS medium supplemented with 0.5 mg/l IBA induced maximum number of roots (10.06 ± 0.53) with an average root length of 5.07 ± 0.47 cm. The rooted plantlets were acclimatized in plastic pots containing sand: FYM (1:1) with 68% survival. Likewise, Autade et al. (2016) obtained the maximum multiple shoots (5.3 ± 0.41) with mean shoot length of 6.5 ± 0.12 cm in MS medium having BAP (0.5 mg/l) and NAA (1.5 mg/l) using the nodal explants of field grown *W. somnifera*. Rooting was well established in MS media augmented with IBA (2.0 mg/l). About 12 ± 0.20 roots with a mean length of 9.8 ± 0.26 cm were obtained. In our unpublished data, a standardized in vitro multiplication protocol has been established for *W.somnifera* by using shoot tips of in vitro raised seedlings as the explants. The seeds showed better germination on MS basal medium. Multiple shoots were obtained in MS medium augmented with 0.5 mg/l BAP, and the excised shoots readily rooted in 0.5 mg/l IBA-supplemented MS media.

Aloe vera in vitro plantlets were produced using two types of explants (with and without sheath, types A and B, respectively) in different media (MS, B5, and SH) added with different hormones such as NAA, BAP, and Kin either alone or in combination. The highest frequency of shoot induction was recorded in MS media containing 0.2 mg/l NAA and 4 mg/l BAP in type A explants. On the other hand, 11.2 shoots were induced in MS medium having 4 mg/l BAP. The highest number of roots (8.1) with an average root length of 15.7 cm was obtained in B5 medium contained with 2 mg/l NAA. The rooted plantlets in vitro were transferred to plastic bags with 1:1 ratio of soil and sand or 1:1:1 ratio of soil, sand, and perlite for acclimatization. Ninety-five percent of the plantlets survived in the greenhouse. More importantly, all in vitro regenerated plants behaved normally without any observed phenotypic variations (Abdi et al. 2013). From the nodal segments of *Allium sativum,* Mehta et al. (2013) induced 3.43 ± 0.39 shoots having 7.84 ± 0.31 cm height in MS medium fortified with 1 mg/l kinetin. In vitro rooting was obtained in MS medium added with 2 mg/l IBA.

In *Curcuma longa,* multiple shoots were formed from the rhizome buds in MS medium fortified with 2.5 mg/l BAP and 1.5 mg/l NAA which yielded 9.00 ± 0.57 shoots with a mean shoot length of 7.20 ± 1.01 cm. The 1/2 strength MS medium with 2.0 mg/l IBA produced higher in vitro rooting percentage and roots (9.66 ± 1.20). The hardened plantlets showed about 86% survival rate (Ghosh et al. 2013). Likewise, David et al. (2016) studied the effect of BAP and NAA concentrations ranging between 1 and 3 mg/l on the shoot multiplication and rooting in *Zingiber officinale* Rosc. "Tambunan" using rhizome buds. Their study revealed that BAP (3.0 mg/l) and NAA (1.0 mg/l) supplemented MS media recorded shoot induction within 7 days. Further, the same medium promoted the shoot proliferation rate and yielded 6.14 ± 0.91 shoots with mean length of 1.69 ± 0.17 cm after 10 weeks. The in vitro rooting was better achieved in the medium augmented with 2 mg/l NAA. About 34.40 ± 1.81 roots per shoot were formed with an average root length

of 4.52 ± 0.20 cm after 10 weeks. The rooted plantlets were acclimatized in sand: clay (1:4) with 64% of survivability observed after 3 weeks (David et al. 2016). Later, Zuraida et al. (2016) studied the production of in vitro shoots from the young buds of *Z. officinale* var. *Rubrum* in MS medium fortified with BAP and NAA. They reported that MS medium consisting of 3 mg/l BAP and 0.5 mg/l NAA gave the maximum number of shoots (19.5 ± 2.3). Interestingly, micro-shoots maintained on MS medium supplemented with 4.5% sucrose and 3 mg/l BAP and 0.5 mg/l NAA resulted in the formation of 23 ± 2.5 plantlets and roots / explant (15.4 ± 2.4) , but reduced the length of lateral roots (2.6 ± 0.2 cm).

20.4.2.3 Secondary Metabolite Production Using Plant Tissue Culture

An alternative approach to propagation/cultivation and extraction of important medically valued compounds is the plant tissue culture. In vitro methods are successfully engaged for the production of many secondary metabolites from plants. This approach is very useful for those plants which are scarcely available, difficult to cultivate, and facing the danger of extinction or those with no agronomical information is available. The accumulation of secondary metabolites in vitro gives an opportunity for production of plant-derived anticancer compounds in large scale using bioreactors, in shorter period without depleting the plants from their natural habitats. Through genetic engineering technique, secondary metabolite production can be enhanced in the engineered hairy roots. The following section highlights the production of anticancer compounds using callus, cell, and root cultures. The secondary metabolites such as vinblastine and vincristine, podophyllotoxin, camptothecin, Taxol, cannabinoids, alliin, colchicine, β-sitosterol, thymol, and stigmasterol were produced in vitro using various explants through callus culture, cell suspension culture, and hairy root culture and also in plantlets (Table 20.2).

Cannabinoid production was observed in *Cannabis sativa* in the hairy roots initiated from callus culture in solid B5 medium augmented with 4 mg/l NAA in darkness at 25 °C. Liquid cultures of roots were achieved by incubating root tips in B5 liquid medium supplemented with 4 mg/l NAA. The roots were kept on rotary shaker at 110 rpm and incubated in dark at 25 °C. The roots were transferred to fresh medium every 30 days and maintained in liquid medium for 3 years. The quantity of cannabinoids in hairy roots was analyzed using high-performance liquid chromatography (HPLC), and the observed cannabinoid production was below 2 μg/g dry weight of roots (Farag and Kayser 2015). Nasim et al. (2010) reported the increased alliin production in various explants (clove, leaf, and root) of *Allium sativum* by supplementing the MS medium with 4, 8, 16, 32 g/l gypsum ($CaSO_4$) as sulfur source. All the tissues and explants showed increase in the alliin content compared to the control, the highest being observed in leaves (3.74 ± 0.41 μg/g dry weight) compared to the control (2.22 ± 0.41 μg/g dry weight) in the medium with 16 mg/l of sulfur. The impact of different concentarations of polyethylene glycol (PEG 4000) 0%, 3%, 6% and 12% on the production of vinblastine and vincristine in 13 week old callus cultures of *C.roseus* by Iskander and Iriawati (2016). They observed

Table 20.2 In vitro secondary metabolites production from anticancer plants

Plant name	Explants used	Type of in vitro culture	Secondary metabolite produced	References
C. roseus	Young leaf	Embryogenic and non-embryogenic calli	Vinblastine	Aslam et al. (2010)
A. sativum	Leaf, root	Non-embryogenic and embryogenic callus, proliferated, matured and germinated embryos	Alliin	Nasim et al. (2010)
G. superba	Leaf, stem, and cormlets	Callus culture	Colchicine	Pandurangan and Philomina (2010)
T. baccata	Stem sections	Cell culture	Taxol	Rezaei et al. (2011)
C. hierosolymitanum	Seeds	Cell suspension	Colchicine	Daradkeh et al. (2012a)
T. baccata	Leaf	Cell cultures	Taxol	Kajani et al. (2012)
Nigella sativa	Leaf, epicotyls, hypocotyls, root	Cell suspension culture	Thymol	Chaudhry et al. (2014)
Ophiorrhiza mungos	Fruit	Shoots from callus	Camptothecin	Namdeo et al. (2012)
P. hexandrum	Zygotic embryos	Somatic embryos	Podophyllotoxin	Rajesh et al. (2014)
C. sativa	Callus	Hairy roots	Cannabinoids	Farag and Kayser (2015)
N. foetida	3-week-old germinated seedlings	Callus culture	Camptothecin	Fulzele et al. (2015)
Salvia santolinifolia	Nodes	Callus culture	β-Sitosterol, stigmasterol	Jan et al. (2015)
C. accuminata	Hypocotyl	Hairy roots	Camptothecin	Ni et al. (2015)
C. roseus	Young leaf	Callus culture	Vincristine and vinblastine	Iskandar and Iriawati (2016)

that no significant reduction in the production of vinblastine and vincristine and also no change in protein profile in the presence of PEG.

Pandurangan and Philomina (2010) reported the influence of nutritional factors and light intensity on the production of colchicine content in *Colchicum hierosolymitanum* and *Gloriosa superba* tissue cultures. They observed a higher content of colchicine (0.095 mg/g dry weight) accumulation in *C. hierosolymitanum* callus grown under dark conditions compared to the callus grown in light conditions (0.070 mg g^{-1} DW). The highest colchicine content was recorded in medium MS medium supplemented with sulfate ions (40 mM), and the precursor (40 μM)

tyrosine also stimulated the formation of colchicine. Likewise, different ratios of NH^{+4} and NO^{-3} were used to study their effect on colchicine content in *C. hierosolymitanum* by Daradkeh et al. (2012a, b). About 0.070 mg/g dry weight of colchicine was observed with the use of 40 mM NH^{4+} (ammonium nitrate as nitrogen source), and 0.090 mg/g dry weight was recorded when 3% sucrose was used in the media after 4 weeks of incubation. Supplementing the B5 medium with salicylic acid (50 mg/l) has improved the Taxol production in suspension cultures established from the callus induced from longitudinally sectioned stem segments of *T. baccata.* The increase in the production of Taxol was fourfold as compared to control treatments. The combination of ultra-sonication and salicylic acid (50 mg /l) further enhanced the total Taxol production. About 4, 1.2, and 8 times higher accumulation of Taxol was observed with the ultra-sonication, salicylic acid, and control treatments, respectively (Rezaei et al. 2011). Later, Kajani et al. (2012) studied the effect of five basal media compositions such as Gamborg, MS, WPM, Schenk and Hildebrandt (SH), and Driver and Kuniyuki (DK) on the various taxane compounds' production and secretion in *T. baccata*. They observed maximum yields of baccatin III (10.03 mg/l) and 10-deacetyl baccatin III (4.2 mg/l) in DK basal media, but the yield of Taxol was maximum (16.58 mg/l) in the WPM basal media. The extracellular yield of Taxol (7.81 mg/l), baccatin III (5.0 mg/l), and 10-deacetyl baccatin III (1.45 mg/l) was also obtained by using DK basal medium compared to any other media tested. Rajesh et al. (2014) observed the highest accumulation of podophyllotoxin (2.8 mg/l) in the germinated somatic embryos of *P. hexandrum* on 3/4 strength MS medium supplemented with 8% sucrose.

The impact of gamma irradiation in low doses (5–30 Gy) on camptothecin production in *N. foetida* was studied by Fulzele et al. (2015), and it has been found that the camptothecin and 9-methoxy-camptothecin yields significantly increased by 20-fold at 20 Gy and only ~2-fold and ~9-fold at 10 and 15 Gy compared to the non-irradiated callus cultures. Namdeo et al. (2012) reported that the maximum camptothecin content was recorded in in vitro plantlets (0.0768% w/w) and the minimum camptothecin was observed in the adventitious buds (0.0026% w/w) compared to naturally grown *O. mungos* (0.0030% w/w) plants. Kaushik et al. (2015) used the axillary and terminal buds to study the direct organogenesis in *O. mungos* Linn. They observed that the maximum number of 63.1 ± 1.35 shoots/explant with a shoot length of 2.8 ± 1.15 cm was produced in MS medium supplemented with 0.25 mg/l BA and 0.25 mg/l kinetin in 4 weeks. Shoots were elongated in the medium supplemented with 1mg/l GA_3 by 2.33-fold in 91% of shoot clusters within 3 weeks. The rooting of the elongated shoots occurred in ½ MS medium with 100 mg/l activated charcoal. Acclimatization was achieved successfully in 95% of the rooted plantlets. The camptothecin from mother plants and the in vitro raised plants were studied using HPLC, and the comparable chemical profiles were observed. Prakash et al. (2016) studied the plant regeneration in *N. nimmoniana* from various explants from the in vitro regenerated plants from embryos in various media (MS, B5, and WPM) and compared the camptothecin produced in in vitro raised plants with that of mother plant. Radical elongation was recorded in 92.8%

of embryos on ½ strength B5 medium, and among them, 98.21% showed complete plantlet formation as compared to control in which only 11.66% of radical elongation was observed and complete plantlets were not formed. Among the various explants (root, hypocotyls, cotyledonary node, axillary buds, and terminal bud) used for shoot bud induction from in vitro raised embryo explants, 97% of shoot bud regeneration frequency was observed in axillary buds at 0.2 mg/l BAP. The maximum multiple shoot induction was observed from axillary bud cultures on MS medium fortified with 0.2 mg/l BAP and 0.1 mg/l IBA with a mean of 10.24 shoots per explant in 8 weeks of culturing. The excised shoots were kept on different concentrations of IBA and NAA. The best rooting medium was ½ strength MS medium with 0.1 mg/l NAA and 0.1 mg/l IBA which resulted an average of 6.23 roots with mean root length of 5.62 cm after 3 weeks. The camptothecin production was estimated in different parts of mother plants (leaves, stem, and roots) of field-grown *N. nimmoniana* through HPLC, in which the maximum camptothecin content (0.12% w/w) was recorded in roots. The concentration of CPT in leaves and stem was 0.0013% w/w and 0.026% w/w, respectively. HPLC studies showed that the randomly selected regenerants showed consistency in retaining the chemical potency in CPT production compared to the CPT from the roots of elite mother stock. Ni et al. (2011) observed that 1.5-fold increase in camptothecin production was achieved in the hairy roots of *C. accuminata* containing overexpression of ORCA3 gene.

20.4.2.4 Cryopreservation

Cryopreservation is the method of the storage of living materials at very low cryogenic temperature (−196 °C in liquid nitrogen). It is an alternative method to in vitro germplasm and conventional field collection which facilitate the storage of plant genetic resources safely and cost effectively for decades and need minimum spaces and routine maintenance (Engelmann 2004, 2011; Cruz-Cruz et al. 2013). In this process the metabolic rates and the biochemical activities are considerably reduced. During cryopreservation, the ice crystal formation in cells might injure the cell structure integrity and physically damage the cells. Cryogenic strategies include freeze dehydration, osmotic dehydration, use of cryoprotective (penetrating and non-penetrating) substances, and hardening metabolism or combinations of these processes (Cruz-Cruz et al. 2013). Cryopreservation contributes in storing various genetic materials including seeds, meristematic apices, pollens, and buds for long-term and allows their easy access across the nations (Dixit et al. 2005; Cruz-Cruz et al. 2013). Thus, cryopreservation assists in the modern plant breeding programs. Moreover, it supports the consistently growing area of phytochemical production through biotechnological approaches by cryopreserving embryogenic and non-embryogenic cell lines and roots to ensure their biochemical and genetic stability (Sen-Rong and Ming-Hua 2012). This will certainly avoid the possible somaclonal variations due to long-term culture maintenance in the lab. Storage of seeds through cryopreservation is the most preferred method for conserving many species (Kaviani 2011; Sen-Rong and Ming-Hua 2012).

Petijová et al. (2012) studied the cryopreservation of shoot tips of *Hypericum perforatum* L., grown in BAP, by using two pre-culture agents 0.3 M sucrose and 0.076 μM abscisic acid. They observed that exposure of plants for long-term to BAP delayed the protective role of abscisic acid during pre-culture, which led to reduce the ability to withstand cryopreservation as a consequence of the uncovered meristematic zone. On the other hand, short-term influence of BAP induced anatomical and morphological alterations causing higher levels of meristem compactness and protection by leaf primordia, which resulted in increased tolerance of low temperatures. The cryopreservation for *A. asphodeloides* Bunge. germplasms was achieved by using vitrification of embryogenic calli (Sen-Rong and Ming-Hua 2012). They found that more than 60% of the vitrified embryogenic calli exhibit the regrowth. Perez et al. (2015) studied various cryopreservation techniques (vitrification of cell suspensions, encapsulation-dehydration of apices, vitrification, and seed desiccation) for ex situ conservation of *Uncaria tomentosa*. They observed that 89.5% of the seeds were germinated after exposure to cryopreservation using desiccation and 67.6–78.1% using vitrification technique. Good cell multiplication and callus formation were observed in 61.1% of the cultures following vitrification. Survival of shoot apices ranged between 31.8% and 52.9% after cooling in liquid nitrogen. Embryos of *N. nimmoniana* were dehydrated for 2 h in laminar air flow chamber and preserved in liquid nitrogen at −196 °C for week. These embryos after 1 week were rewarmed in hot water bath at 40 °C for 1–2 min. The rewarmed embryos were kept MS medium with 12 h photoperiod at 25 ± 2 °C for 8 weeks for recovery. Embryos dehydrated for 2 h showed 60% of the germination. The prolonged desiccation time (2.5–3 h) showed reduction in the survival of embryos from 19.6 to 15.4% (Radha et al. 2010). Embryogenic cell suspension culture of *C. roseus* pretreated with glycerol (5% or 10%) and DMSO (5% or 10%) either alone or in combination was stored in liquid nitrogen for 1 h. On re-culture, the cells pretreated with 5% DMSO have showed the highest number of cell colonies (10.06 ± 0.55). The regrowth of calli was observed in an optimized medium, supplemented with 6.62 μM 6-benzyladenine (BA) and 5.37 μM NAA, and somatic embryos were produced similar to those from nonfrozen embryogenic cultures. These somatic embryos were regenerated into plantlets, and all the plantlets exhibited normal morphology (Fatima et al. 2009).

20.5 Conclusion and Future Prospects

The discovery of anticancer compounds from plants had created a tremendous impact in cancer research, and the list of anticancer plants and the natural sources of anticancer compounds is increasing day by day. The herb based companies and locals are concentrating on the collection of the anticancer plants from their natural habitats, but are not paying attention in replacing/replanting the plants in their natural habitats. This is leading to the depletion of anticancer plant resources. Biotechnological approaches can help in the conservation of anticancer plants, as these approaches utilize small portions of plants as explants and large number of

plants can be produced continuously irrespective of the season in the smaller space and time avoiding the destructive collection of anticancer plants. The cost of micropropagated planting material is the bottleneck to cultivate anticancer plants on a commercial scale. In this regard, the development of low-cost technologies in tissue culture will help in conserving the anticancer plants. Governments should make replanting mandatory for the companies/individuals who are collecting the anticancer plants from natural habitats, which will help in conserving the anticancer plants and passing on these resources to the future generations. Protocols are developed for cryopreservation of many medicinal plants for long-term storage of various plant parts such as seeds, in vitro raised shoot tips, somatic embryos, cell suspension cultures, etc. will help in the long-term conservation (preservation) of anticancer plant germplasm. Government initiatives in establishing the biosphere reserves, gene banks, and national parks and creating awareness among individuals on the conservation of medicinal plants and using them as home remedies by growing them in home gardens will help in the conservation of medicinal plants and passing on the medicinal plant resources to the future generations.

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Chapter 21
Anticancer Plants: Chemistry, Pharmacology, and Potential Applications

V. D. Ravichandra, C. Ramesh, Mallappa Kumara Swamy, B. Purushotham, and Gudepalya Renukaiah Rudramurthy

21.1 Introduction

Cancer is an abnormal group of cells which undergoes uncontrolled division and capable of invading other tissues through circulatory (lymphatic and/or blood) systems (Oleg and Denis 2015). Normal diploid human cells multiply for a finite number of generations, but cancer cells can proliferate indefinitely. Nearly about 12.7 global cancer cases and 7.6 million deaths were observed in 2008. While, nearly 56% the global cancer cases and 64% of cancer deaths were recorded to occur in the economically developing world (Abidemi et al. 2015). It is estimated that nearly 1.1 million new cancer cases are observed only in Indian population which would push India as the single largest contributor (7.8%) of the global cancer burden. The mortality figures were 6,82,830 contributing to 8.33% of the global cancer deaths; and the 5-year prevalence was 1.8 million individuals with cancer corresponding to 5.52% of global prevalence. The cancer cases are increasing constantly in recent times around the globe which is mainly attributed for the modern lifestyle activities (Dhananjaya and Aparna 2014; American Cancer Society 2016). Globally, the mortality rate due to cancer is considered as the second highest only after cardiovascular diseases (Siegel et al. 2016; Singh et al. 2016). Moreover, the availability of specific and safe anticancer drugs is a major challenge for clinical practitioners, even though there is a remarkable achievements/progress in the medical science field (Nitesh et al. 2014). The enormous research on cancers has produced several new and

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significant solutions. Unfortunately, and so far, the drugs used in the cancer treatment possess several limitations. Hence, it is projected that in the future days, cancer could become the primary cause of death (Sankappa et al. 2015). Among various forms of human cancer, the breast, lung, and colorectal cancers are very frequent types. Men are largely affected by lung cancer, while in women, breast cancer is very common (Ferlay et al. 2010; Horn et al. 2012).

Cancer is mainly caused by two factors:

(a) Risk factors which include genetic, environmental, cultural, and geographical factors and lifestyle modifications
(b) Acquired causes like chemical carcinogens (alkylating agents, cyclophosphamide; metals, arsenic, cadmium, nickel, etc.), physical agents (trauma, injury, heat, mechanical injury to DNA, radiations), and biological carcinogens (bacterial, viral, and fungal infections) (Singh et al. 2016)

Chemical carcinogens damage the DNA, produce mutation, and convert the normal cell into initiated cell which later gets converted into a cancerous cell. Physical agents cause mechanical injury to the DNA and produce mutation, finally resulting in cancer. Certain bacterial infection on chronic persistence can also result in cancer. For instance, *Helicobacter pylori* infection causes cancer of the stomach (Kavishankar et al. 2011). The normal cell's growth and differentiation are coordinated through several numbers of genes. Any alterations of these genes in normal cells prompt an aberrant expression leading to carcinogenesis. Most of the anticancer therapeutics are developed by targeting these mutated genes (Biswas SK 2015; Biswas et al. 2015). Likewise, anticancer drugs are developed by controlling the epigenetic processes of a cell that involve the growth and differentiation of cells. Interestingly, most of the plant-based antitumor drug molecules such as polyphenols, alkaloids, and terpenoids function by modifying the epigenetic processes. Some examples of such drugs include azacitidine, romidepsin, and vorinostat (Schneider-Stock et al. 2012; Singh et al. 2016).

The treatment of cancer includes surgical removal, radiation therapy, and chemotherapy. The chemotherapy results in a cure and/or prolonged remission. Cancer therapy, which involves the application of chemical drugs (drug therapy), suppresses the disease, and subsequently relapse and/or future complications may lead to death (Rang et al. 2003). Chemotherapy causes the complications like bone marrow depression, lymphocytopenia, hemorrhages, alopecia, teratogenesis, hyperuricemia, etc. (Tripathi 2008). Surgery is useful in removing the visible tumors but may leave smaller nests of cancer cells in the patient which continue to proliferate. However, radiation therapy is relatively imprecise as it can kill both cancer cells and normal cells and thus has toxic side effects which may themselves be lethal to the patients (Abidemi et al. 2015). Therefore, an alternative adjuvant therapy has arisen in recent times (Mishra et al. 2013). In this regard, natural products in particular plant based constitute an important and significant source of anticancer molecules. Moreover, natural products are widely explored for their wide-ranging biological properties (Swamy and Sinniah 2016; Arumugam et al. 2016; Swamy et al. 2016; Mohanty et al. 2017).

In human cells, certain endogenous factors lead to the formation of reactive oxygen species (ROS) such as hydroxyl, superoxide, and peroxyl radicals. The ROS induces extensive oxidative damage leading to several age-related and degenerative conditions such as cancer, atherosclerosis, cardiovascular disease, ischemia/reperfusion, hypertension, diabetes mellitus, hyperoxaluria, and neurodegenerative disease such as Parkinson's disease, Alzheimer's, and aging (Shyamala et al. 2014; Swamy et al. 2017). Antioxidants protect the body from oxidative stress/damage induced by free radicals, and these compounds found in food act as health-protecting factors. Food antioxidants obtained from plants including vitamin E, vitamin C, carotenes, phytic acid, and polyphenols and phytoestrogens have been recognized as the potential risk reducers of diseases including cancer. Antioxidants prevent the onset of cancer in the early stage of carcinogenesis and are generally, very beneficial to cells (Valko et al. 2007; Aboul-Enein et al. 2012; Raghavendra et al. 2013). Likewise, many bioactive compounds derived from plant leaves, roots, bark, flowers, and stems, such as alkaloids, phenolics, flavonoids, tannins, terpenoids, glycosides, essential oils, etc., effectively perform numerous biological functions (Singh et al. 2013; Mohanty et al. 2014; Swamy and Sinniah 2015; Swamy et al. 2017). Some of the phytocompounds and their chemical derivatives that have significant anticancer potential include vincristine, vinblastine, Taxol, etoposide, elliptinium, colchicinamide, camptothecin, 10-hydroxycamptothecin, gossypol, curcumol, ipomeanol, tetrandrine, lycobetaine, monocrotaline, homoharringtonine, and curdione (Singh et al. 2016).

The present chapter provides a detailed summary of various factors that cause cancers, plant-based anticancer compounds and their pharmacological activities. This information will certainly allow further exploration of the useful medicinal plants and their compounds with more effective therapeutic anticancer potential.

21.2 Causes and Management of Cancer

Cancer is a mass of tissue produced due to abnormal, uncontrolled, and autonomous proliferation of cells with the capability of invading and/or spreading to other tissues and/or body parts known as metastasis. Cancer is a complex disease with several possible causative factors. Most cancers are related to hereditary, lifestyle, environmental, or behavioral exposures (Blackadar 2016). Exact causes of cancer are not certainly known, but cancer mainly developed due to the mutation in the DNA caused by various physical, chemical, and biologically harmful agents (Blackadar 2016).

21.2.1 Physical Carcinogens

Physical carcinogens are substances or agents that directly damage cellular DNA and produce mutations resulting in the development of malignancy. Physical carcinogens are categorized as radiation and non-radiation agents.

21.2.1.1 Non-radiation Causes

Substances, which cause cancer by physical nature through direct damage or trauma, rather than by chemical effects, are known as non-radiation agents. For instance, asbestos inhalation and natural mineral fiber dust responsible for causing cancer of the serous membrane surrounding the lungs (mesothelioma), wollastonite, glass wool, attapulgite, and rock wool. Nonfibrous particulate materials, such as crystalline silica (cristobalite, quartz, and tridymite) and finely powdered metallic cobalt and nickel, are known to induce cancer (Curtis 1991; Harsh 2006; Blackadar 2016).

21.2.1.2 Radiation Causes

Ionizing radiations include cosmic rays, gamma rays, UV rays, X-ray, etc. Ionizing radiations ionize molecules of cells by transferring sufficient energy, and this can lead to chemical changes, including DNA damage and mutation in cells. The various sources of radiations to which human are exposed normally are sunlight, radiological diagnostic techniques, radioisotope therapy, electronic devices, and many more (Curtis 1991; Harsh 2006).

21.2.2 Biological Carcinogens

The biological carcinogen is an agent which causes mutation by damaging DNA through their physiological mechanisms such as enzymatic hydrolysis of cellular components, release of inflammatory mediators, and many more. They include microorganisms such as bacteria, fungi, virus, and many more (Rajeev et al. 2012; Chen et al. 2014). For instance, *Helicobacter pylori* is a Gram-negative bacterium that specifically infects the duodenum and develops duodenal cancer (Rajeev et al. 2012). Mycotoxins produced by certain fungi such as *Aspergillus flavus* (aflatoxins) are known to induce liver cancer (Bouvard et al. 2009; Wild and Gong 2009). Hepatitis B and C viruses can damage liver cells and develop hepatoma or hepatocellular carcinoma, while human papillomaviruses are responsible for 1.9 million cancer cases globally, which mainly encompass liver, gastric, and cervical cancer (Oh and Weiderpass 2014). Several different viruses known as oncoviruses are known to induce different types of cancer, for example, Epstein-Barr virus, nasopharyngeal carcinoma; human papillomavirus, cervical carcinoma and Kaposi's sarcoma; hepatitis B and hepatitis C viruses, hepatocellular carcinoma; herpes virus, Kaposi's sarcoma/primary effusion lymphomas; human T-cell leukemia virus-1, T-cell leukemia; and many more (Chen et al. 2014; Morales-Sánchez and Fuentes-Pananá 2014; Oh and Weiderpass 2014).

21.2.3 Chemical Carcinogens

Chemical carcinogens are substances that are free radical in nature or converted to free radicals after the metabolic activation in the body and thereby induce mutation in cells (Loeb and Harris 2008). Carcinogenesis induced by the chemicals happens in two stages, i.e., initiation and promotion. Initiation is the process of converting normal cell into mutated (initiated) cell, and the promotion is conversion of initiated cell into cancer cell. The direct initiators, without metabolic activation in the body, damage the DNA leading to mutation (examples are alkylating and acylating agents), and indirect initiators (paracetamol) damage the DNA after metabolic activation in the body and produce mutation (Stojan et al. 2004; Harsh 2006; Loeb and Harris 2008).

21.2.4 Hormonal Agents

Several studies reported that exposure to endogenous estrogen and progesterone (by ovaries) influences high risk of breast cancer; increased exposure may also lead to late menopause and problems related to pregnancy (Folkerd and Dowsett M (2010). As per 'National Cancer Institute', oral contraceptives in the form of "combined oral contraceptive" (containing both estrogen and progesterone) and "minipill" (contains only progestin) promote the development and growth of ovarian cancer, endometrial cancer, liver cancer, and cervical cancer as well as the risks associated with cancer (Papac 1998; Harsh 2006).

21.2.5 Approaches in Cancer Treatment

The main approaches for the treatment of cancer include surgical removal, chemotherapy, irradiation, and targeted cancer therapy (Baudino 2015). The role and significance of these approaches depend on different factors such as type of tumor, stage of its development, site of the tumor, and many more.

21.2.5.1 Surgical Excision

Surgery is the primary approach for the treatment of solid tumors (isolated), which plays an effective role in palliation and prolongs life expectancy. Since biopsies are essential to detect the stage of the tumor and also in the definitive diagnosis, surgical excision becomes very significant. The localized surgery is aimed at removing the complete tumor mass alongside the surrounding lymph nodes (Tripathi 2008).

21.2.5.2 Irradiation

Radiation therapy involves the use of ionizing radiations to cure or improve cancer symptoms. Irradiation acts by causing damages to DNAs of tumorous cells and leads to their killing. To protect the skin and organs (normal tissues) from irradiation, shaped beams of radiation passed through different angles so that their exposure will intersect at the cancer mass. The effect of radiation therapy depends on the type and stage of tumors. Radiation therapy is the most widely used therapy radiation sources include brachytherapy (internal source) or external radiation sources. The low-energy sources such as X-rays are widely employed in the treatment of skin cancers, while high-energy X-ray beams are employed for treating cancers inside the body. Moreover, radiations are particularly used in addition to chemotherapy and/or surgery except for early neck and head cancer (Tripathi 2008; Rajamanickam et al. 2012).

21.2.5.3 Chemotherapy

Chemotherapy is the method of using cytotoxic/chemotherapeutic drugs to treat cancers. Chemotherapy with antiproliferative agents, including alkylating agents, antimetabolites, antibiotics, and hormones, apart from being complementary to surgical intervention and radiotherapy, is essential in cases of metastasis (Tripathi 2008).

21.3 Cancer Therapy: Current Scenario

Cancer has become a significant healthcare problem globally, with an estimated incidence of ten million new cases per year worldwide, among which 46% are observed in the developed countries. There is an increase in mortality rate, with more than seven million cases of deaths per year. The National Cancer Registry Programme has estimated that 700,000–900,000 new cancer patients are diagnosed in India every year. As per the estimation of the World Health Organization (WHO), approximately 15 million new cases of cancer may be diagnosed per year globally by 2020. Also, overall mortality from cancer has increased by 104%, which is five times higher in developing countries compared to developed countries. The success rates of cancer therapy still remain unsatisfactory in spite of great advancements such as irradiation and chemotherapy, which may be due to their associated toxicities. Currently, one of the major approaches to treat cancer is chemotherapy which is accompanied by toxic effects, drug resistance, and recurrence of cancer. Consequently, there is a dire need for the development of novel healing approaches against cancers utilizing natural sources (Colledge et al. 2010; Longo et al. 2012).

Recently, targeted cancer therapies are practiced to overcome the conventional chemotherapy-associated health problems. In specific, targeted cancer therapy is

targeted to suppress the growth of only tumorous cells at the targeted sites of the body using specific and effective anticancer agents (Baudino 2015; Allahyari et al. 2017). The target includes precise protein molecules that are known with carcinogenesis activities. Major targeted cancer therapies that are having some hope in the future include small-molecule inhibitors, monoclonal antibodies, and immunotoxins (Allahyari et al. 2017). Also, research developments have allowed us to examine and modify treatment methods against various tumor types (Baudino 2015).

21.4 Cancer in Traditional Medicines

The cancer was described with different terminologies, and treatment guidelines were given in several systems of traditional medicine such as Ayurveda, Chinese traditional medicine, traditional Korean medicine, Kampo, Unani, and many others (Yuan et al. 2016).

21.4.1 Ayurveda

Ayurveda, a traditional system of Indian medicine, uses natural drugs successfully since ancient times to prevent or to suppress various cancers using various lines of treatment. *Charaka Samhita* and *Sushruta Samhita*, the two well-known Ayurvedic scripts, have described cancer as an inflammatory/noninflammatory lesion and described them as either minor neoplasm (Granthi) or major neoplasm (Arbuda) (Premalatha and Rajgopal 2005; Longo et al. 2012). In benign tumor, there will be a failure of one or more than one body system (Pittaja, Vataja, or Kaphaja), and thus the body still will be fighting to control the coordination of the systems, and thus is not too harmful, where as malignant tumor is very harmful and all the body systems (Tridosaja) may affected (Premalatha and Rajgopal 2005; Pal 2014; Yuan et al. 2016).

21.4.2 Traditional Chinese Medicine (TCM)

TCM is a complex system of medicine. So it is very difficult for allopathic physicians to test its effectiveness in treating or preventing cancer including testing TCM using randomized clinical trials and comparing the results to conventional medicine. As TCM is tailored to the patient, it can't be tested by giving a group of patients the same medicine for a certain duration (Kapoor 1990; Balachandran and Govindarajan 2005; Yuan et al. 2016). Instead of looking at the medical effects, a TCM practitioner measures how well a treatment works by looking at how the patient feels and the balance of the patient's yin and yang. The TCM techniques are

most commonly based on acupuncture and Chinese herbal remedies. Some studies reveal that acupuncture is useful for a number of different cancer conditions. There are few scientific evidences showing that some Chinese herbal medicine can control cancer symptoms (Macek 1984; Ernst and Cassileth 1998; Premalatha and Rajgopal 2005; Mary and Susan 2012; Prakash et al. 2013; Yuan et al. 2016).

21.4.3 Unani

Sartan is an Arabic word which means *crab* (cancer), and according to Unani (Greco-Arab) medicine, *sartan* is essentially a disease condition associated with excessive production and collection of *sauda* (black bile). Sartan mostly develops in soft tissues (Az'ae Ratab) like the intestine, breast, oral cavity, uterus, stomach, pancreas, prostate, and lungs. According to Unani philosophy, cancer is the end stage of the degeneration of metabolic efficiency of the body, the extinguishing of the innate heat due to incorrect diet, and other imbalances in various aspects of the patient's lifestyle usually occurring over a period of time. The principle of management of sartan explained in Unani medicine is mainly stressed upon to prevent the collection of sauda with the help of venesection or phlebotomy (fas'd), use of melanogogue drugs like *Cuscuta reflexa (*giant dodder), *Citrullus colocynthis (*bitter apple) along with some dietary remedy like kashkusshaeer (easily digestible food), nabeez (arisht), and maul jubn (cow's churned milk) (Shan et al. 2012; Qamar et al. 2015).

21.5 Role of Plants in Cancer Treatment

It is well known, since over centuries, the use of plant materials having suitable history of use in folklore for treating various human ailments including malignancy (Swamy et al. 2016, 2017; Arumugam et al. 2016; Yuan et al. 2016). Recent studies often resulted in obtaining few active molecules, is a prominent attempt to know about various phytoconstituents with antitumor activity (Mohanty et al. 2014, 2017). Later in the 1950s, the National Cancer Institute (NCI) of the United States funded a major screening program, thus resulting in an intensive survey of plants, microorganism, and marine animals for antitumor activity. Moreover, the knowledge on the presence of novel compounds either from plant or animal kingdom helped in the adoption of "random selection screening program" (Saad et al. 2008). The list of various plants and their taxonomy which are used in the traditional and other system of medicines is mentioned in Table 21.1 (Rajandeep et al. 2011; Negi et al. 2011; Akbar et al. 2011; Harpreet et al. 2011; Umadevi et al. 2013; Ngeh and Rob 2013; Savithramma et al. 2014; Sharma and Majee 2015; Sejal 2016; Rastogi et al. 2016; Shaikh et al. 2016; Alic et al. 2016).

Table 21.1 The list of traditional plants that possess anticancer properties

Common name	Sources	Family	Plant part	Nativity
Wormwood	*Artemisia monosperma*	Asteraceae	Whole plant	Asia
Meadow saffron	*Colchicum autumnale*	Colchicaceae	Corms	Great Britain, Ireland
Celery	*Apium graveolens*	Umbelliferae	Aerial part	North America
Bilwa	*Aegle marmelos*	Rutaceae	Fruits	India
Garlic	*Allium sativum*	Liliaceae	Bulb	India
Hairy agrimony	*Agrimonia pilosa*	Rosaceae	Herb	China, India
Aloe	*Aloe vera*	Liliaceae	Offset	Asia
Kalmegh	*Andrographis paniculata*	Acanthaceae	Aerial parts	India
Apamarg	*Achyranthes aspera*	Amaranthaceae	Herb	India
Neem	*Azadirachta indica*	Meliaceae	Whole plant	India
Rasna	*Alpinia galanga*	Zingiberaceae	Rhizome	Europe
Soursop	*Annona muricata*		Aerial parts	India
Cow's paw	*Bauhinia variegata*	Fabaceae	Aerial parts	China
Downy birch	*Betula alba*	Betulaceae	Herb	Canada, North America
Barberry	*Berberis vulgaris*	Berberidaceae	Berries	Europe
–	*Brucea antidysenterica*	Simaroubaceae	Aerial parts	Africa
Flame of the forest	*Butea monosperma*	Fabaceae	Leaves, bark	India
Crown flower	*Calotropis gigantea*	Asclepiadaceae	Whole plant	India, Cambodia
Green tea	*Camellia sinensis*	Theaceae	Leaves	China
Cilantro	*Coriandrum sativum*	Umbelliferae	Leaves, fruits	Asia
Happy tree	*Camptotheca acuminata*	Cornaceae	Root, leaf, stem	China, Tibet
Cannabis	*Cannabis sativa*	Cannabaceae	Herb	Central Asia
Karra	*Cleistanthus collinus*	Euphorbiaceae	Aerial part	India
Vinca, periwinkle	*Catharanthus roseus*	Apocynaceae	Leaves, flowers	Mexico, India
Cinnamon	*Cinnamomum cassia*	Lauraceae	Bark	China
Turmeric	*Curcuma longa*	Zingiberaceae	Rhizome	India, China
Spurge olive	*Daphne mezereum*	Thymelaeaceae	Aerial part	Europe
–	*Dysoxylum binectariferum*	Meliaceae	Bark, wood	India
Amla	*Emblica officinalis*	Euphorbiaceae	Fruits	India
Fritillaria	*Fritillaria thunbergii*	Liliaceae	Bulb, aerial parts	China, Japan
Liquorice	*Glycyrrhiza glabra*	Fabaceae	Stolon	Asia
Mexican cotton	*Gossypium hirsutum*	Malvaceae	Aerial parts	America, Mexico
Ginkgo	*Ginkgo biloba*	Gingkgoaceae	Seeds, leaves	China
Soya bean	*Glycine max*	Fabaceae	Beans	North America

(continued)

Table 21.1 (continued)

Common name	Sources	Family	Plant part	Nativity
Pink lapacho	*Handroanthus impetiginosus*	Bignoniaceae	Bark	Argentina, Mexico
Saint John's wort	*Hypericum perforatum*	Hypericaceae	Herb	Asia, Europe
Centella, Brahmi	*Centella asiatica*	Apiaceae	Aerial parts	Asia
Indian mulberry, noni	*Morinda citrifolia*	Rubiaceae	Root, leaves, green fruits	Asia, Australia
–	*Oldenlandia diffusa*	Rubiaceae	Herb	China
–	*Origanum dayi*	Lamiaceae	Whole plant	Europe
Tulsi	*Ocimum sanctum*	Lamiaceae	Aerial part	India
Kutki	*Picrorhiza kurroa*	Plantaginaceae	Rhizome	Nepal
Babchi	*Psoralea corylifolia*	Fabaceae	Whole plant	India, China
Ceylon leadwort	*Plumbago zeylanica*	Plumbaginaceae	Aerial parts	North America, Sri Lanka
Sinopodophyllum	*Podophyllum hexandrum*	Berberidaceae	Resin from roots	USA, Southern Canada
American mandrake	*P. peltatum*	Berberidaceae	Resin from roots	Eastern USA, Canada, India
Ginseng	*Panax ginseng*	Araliaceae	Whole plant	China, Korea
Heal-all plant	*Prunella vulgaris*	Lamiaceae	Herb	Asia, Europe
English yew	*Taxus baccata*	Taxaceae	Bark	Europe, Africa
Tylophora	*Tylophora indica*	Asclepiadaceae	Root, leaf	India
Indian madder	*Rubia cordifolia*	Rubiaceae	Roots	India
Night shade	*Solanum nigrum*	Solanaceae	Stem, leaf, fruit	South Asia, Europe
Costus	*Saussurea lappa*	Asteraceae	Roots	South Asia
Milk thistle	*Silybum marianum*	Asteraceae	Seeds	Asia, Europe
Nux vomica	*Strychnos nux-vomica*	Loganiaceae	Seeds	Asia
Arjuna	*Terminalia arjuna*	Combertacae	Bark	India
Bitter leaf	*Vernonia amygdalina*	Asteraceae	Shrub	Africa
Simpleleaf chastetree	*Vitex trifolia*	Verbenaceae	Leaf	East Africa
European mistletoe	*Viscum album*	Santalaceae	Leaves, young twigs	Europe, Asia
Ashwagandha	*Withania somnifera*	Solanaceae	Root, leaves, berries	India
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome	Asia

21.6 Occurrence and Chemistry of Plant-Derived Anticancer Agents

Nature has gifted a rich source of compounds which have remarkable therapeutic values. The marked structural diversity of these natural compounds and their potentials with respect to their bioactivity lead to the isolation of active principles from plants, animals, marine flora and fauna, and microorganisms. These chief compounds can be further improved for their therapeutic potential by molecular modifications. In the field of cancer, since 1940 onwards, out of around 155 small molecules, 73% are natural, with 47% actually being derived naturally or their derivatives.

Efforts of drug innovations have produced notable anticancer drug molecules from plants (vinblastine, vincristine, etoposide, camptothecin, paclitaxel, irinotecan, and topotecan), microorganisms (dactinomycin, bleomycin, and doxorubicin), and marine creatures (aplidine, cytarabine, and dolastatin 10). Apart from these, few compounds occurring in vegetables and fruits also possess anticancer activities. These agents include curcumin (from turmeric), genistein (from soya bean), resveratrol (from peanuts, red grapes, and berries), cysteine and diallyl sulfide (from allium), beta carotene (from carrots), lycopene (from tomato), allicin (from garlic), capsaicin (from red chilli), 6-gingerol (from ginger), diosgenin (from methi), ellagic acid (from pomegranate), silymarin (from milk thistle), ursolic acid (from apple, prunes, and pears), catechins (from green tea), indole-3-carbinol (from cruciferous vegetables), eugenol (from cloves), and limonene (from citrus fruits). Among various herbal drugs for cancer, few important classes of phytoconstituents have been discussed below. The source and chemistry of phytochemicals with anticancer potentials are given in the following Table 21.2.

21.6.1 Catharanthus *Alkaloids*

Vinca alkaloids are naturally occurring or semisynthetic nitrogenous compounds made up of carbon, hydrogen, nitrogen, and oxygen. Vinca alkaloids are having a common chemical structure with an asymmetric dimer consisting of a vindoline ring linked to a catarantine ring through carbon-carbon bonds. Vinblastine and vincristine are powerful anticancer drugs obtained from *Catharanthus roseus*, commonly known as periwinkle (Moudi et al. 2013). Ever since from the last five decades, they are used widely for treating cancerous patients which is due to their exceptional mode of action and efficiency. The *Catharanthus* alkaloids constitute more than 130 indole alkaloids. As a major lead, vinblastine paved a way for other drug developments and discovery. Later due to increased demand of natural anticancer drugs, related alkaloid vincristine and its pharmaceutical importance are studied; thus *C. roseus* became one of the widely explored anticancer plants. As a result, it is now used as a model for studying biosynthetic pathways involved in the plant secondary

Table 21.2 Botanical source and phytoconstituents reported for anticancer properties from plants

Class	Source	Parts used	Phytoconstituents	Basic nucleus present	References
Catharanthus alkaloids	*Catharanthus roseus*	Leaves and flowers	Vincristine, vinblastine	Indole	Tafur et al. (1975) and Robert van et al. (2004)
Taxols	*Taxus brevifolia*, *T. baccata*, *Taxomyces andreanae*, *Pestalotiopsis microspora*, *Phyllosticta citricarpa*	Bark	Paclitaxel, docetaxel	Benzylamine with phenylpropanolamine	Wani et al. (1971) and Bissery et al. (1991)
Epipodophyllotoxins	*Podophyllum hexandrum*	Resins	Etoposide, teniposide, tafluposide, Etopophos	Tetrahydrofurfuryl	Nerendra (2011), Liu and Hou (1997) and Gordaliza et al. (2004)
Camptothecins	*Camptotheca acuminata*	Bark	Topotecan, irinotecan	Pyran, indole, and quinoline	Muqeet and Qudsia (2014) and Antony et al. (2004)
Steroids	*Azadirachta indica*, *Brassica napus*, *Glycine max*	Aerial parts, seeds	Stigmasterol	Steroid	Huma et al. (2015)
Stilbenes	*Polygonum cuspidatum*		E-Stilbene, resveratrol	Phenol	Crozier et al. (2006)
					Seigler (1998)
Chalcones	*Ficus microcarpa*	Aerial roots	Phloretin, arbutin	Phenol	Seigler (1998) and Nowakowska (2007)
	Helianthus annuus	Aerial parts			
	Artocarpus spp.	Aerial parts			
	Bidens spp.	Aerial parts			
	Coreopsis spp.	Aerial parts			

Coumarins	*Dipteryx odorata*, *Anthoxanthum odoratum*, *Galium odoratum*, *Hierochloe odorata*, *Cinnamomum zeylanicum*, *Dichanthelium clandestinum*, *Verbascum* spp., *Prunus* spp.	Leaves, flowers, and seeds	Hydroxycoumarin, dicoumarin, warfarin, esculetin, psoralidin	Phenol	Kalili and Villiers (2011)
Hydroxycinnamic acids	*Coffea arabica*, *Eucalyptus globulus*, *Salvinia molesta*, *Phellinus linteus*		Caffeic acid, cinnamic acid	Phenol	Dewick (2002) and Spencer (2012)
Hydroxybenzoic acids	*Daucus carota*, *Vitis vinifera*, *Vitex negundo*, *Elaeis guineensis*, *Pterocarpus santalinus*, *Areca catechu*, *Olea europaea*, *Citrus paradise*	Leaves, bark, wood, and seeds	Gallic acid, vanillic acid, syringic acid, protocatechuic acid	Phenol	Lüttge (2011) and Dey and Harborne (1997)
Combretastatins	*Combretum caffrum*		Combretastatin A1, combretastatin A4, combretastatin B-1, combretastatin B-4, combretastatin D-1, combretastatin D-4	Phenol	Chung et al. (1998), El-Zayat et al. (1993) and George et al. (1988)
Curcuminoids	*Curcuma longa*	Rhizomes	Curcumin, demethoxycurcumin, bisdemethoxycurcumin	Diphenyl	Akram et al. (2010), Simay et al. (2008) and Chainani-Wu (2003)
Cyanogenic alkaloids	*Prunus amygdalus*	Seeds	Amygdalin	Glucopyranosyl with phenol and CN bridge	Zuoqing and Xiaohong (2014)

metabolism. Vinca alkaloids have been generally given with other drugs as combination chemotherapy regimens for cancer treatment. Earlier reports established that these drugs exhibit different mechanisms of action by inhibiting spindle formation during the cell division, and they do not exhibit cross-resistance with drugs that alkylate DNA (Tafur et al. 1975; Robert van et al. 2004; Moudi et al. 2013).

21.6.2 Taxanes

Taxanes, widely used chemotherapeutic agents, belong to diterpene class originally identified from yew plants of the genus *Taxus* and recently found in the shells and leaves of *Corylus avellana* (the common hazel plant). Paclitaxel and docetaxel, diterpene alkaloids (Taxol) isolated from *Taxus* plant, have been very successful anticancer molecules. Taxol was also later identified from the fungal species (Wani et al. 1971). *Pestalotiopsis microspora*, *Taxomyces andreanae*, and *Phyllosticta citricarpa* are major source of Taxol from fungi category (Wani et al. 1971; Bissery et al. 1991; Gary et al. 1996).

21.6.3 Podophyllotoxins

Podophyllotoxin is a naturally occurring aryltetralin lignin and was first obtained from *Podophyllum peltatum*, a North American plant commonly known as American mandrake. Later podophyllotoxin was isolated from several other *Podophyllum* spp. like *Podophyllum hexandrum* and *Podophyllum pleianthum*. Podophyllotoxin and its chemical derivatives (teniposide and etoposide) belong to lignan family of natural products and are widely used in a variety of cancer chemotherapy protocols. So far, it is observed that the antitumor properties of etoposide and teniposide are due to their interaction with DNA and inhibition of DNA topoisomerase II (Liu and Hou 1997; Gordaliza et al. 2004; Nerendra 2011), and they are not the inhibitors of microtubule assembly.

21.6.4 Camptothecins (CPT)

CPT, a cytotoxic quinoline alkaloid, has a pentacyclic ring. It was first isolated by Wall and Wani in the year 1966 from the tree bark of *Camptotheca acuminata*, which is commonly used in the preparations of TCM (traditional Chinese medicine). The compound is also isolated from the plants such as *Nothapodytes foetida* and *Ophiorrhiza* species and some of the endophytic fungi belonging to *Botryosphaeria*, *Fusarium*, and *Trichoderma* genera (Pu et al. 2013; Ding et al. 2013; Kaushik et al. 2015; Prakash et al. 2016). Later, due to major disadvantage of

natural camptothecin regarding its low solubility, synthetic camptothecins have been developed. The two CPT analogues which are used in the chemotherapy of cancer are the topotecan and irinotecan. Camptothecin and its derivatives have become a promising group of chemotherapeutic agents for cancer. The FDA has approved its derivatives topotecan and irinotecan for the second-line treatment of ovarian carcinoma and metastatic colorectal carcinomas, respectively (Gordaliza et al. 2004; Antony et al. 2004; Muqeet and Qudsia 2014).

21.6.5 Plant Phenols

Phenolic compounds are widely distributed throughout the kingdom of plants representing about 8000 different phenolic structures. As other secondary metabolites, they also exhibit growth and development as well as a defensive role in plants (Carocho and Ferreira 2013). Phenolic compounds are known to possess anticancer potentials due to their antioxidant properties. Several anticancer molecules consisting of phenol ring have been reported (Kang et al. 2004; Boudet 2007; Carocho and Ferreira 2013).

21.6.5.1 Stilbenes

The members of the stilbene groups showed a C6-C2-C6 structure and derived from the same biosynthetic pathway as flavonoids. Although small, these compounds are widely distributed among plant kingdom. About 200 stilbenes and stilbene glycones are reported in higher plants, among which the important one is resveratrol (chemically 3,4′,5-trihydroxystilbene) that was originally identified as a phytoalexin. This natural stilbene has been found in about 185 plant species and is present in foods and beverages derived from them such as peanuts, mulberries, and grapes and has been extensively studied in recent years due to its anticancer potential (Seigler 1998; Crozier et al. 2006). Pterostilbene (3,5-dimethoxy-4′-hydroxystilbene) is a natural analogue of resveratrol with higher bioavailability. However, pterostilbene exhibits a higher antioxidant activity compared to resveratrol (Sirerol et al. 2016).

21.6.5.2 Chalcones

Chalcones are intermediates in the formation of all flavonoids and exhibit non-heterocyclic C-ring, C6-C3-C6 structure. They possess an open-chain conformation with two aromatic rings linked by a three-carbon unsaturated carbonyl system. These compounds are mostly yellow colored, commonly occurring in flowers, and also found in other plant organs. Although most of chalcones are found as free aglycones, some occur as glycosides. Chalcones possess antiproliferative and antioxidant properties (Seigler 1998; Nowakowska 2007). The most abundant chalcones

like flavokawain, isoliquiritigenin, and xanthohumol are commonly observed in the plants belonging to the families of Fabaceae, Cannabaceae, Piperaceae, and Moraceae. These chalcones have been proved as promising lead antitumor agents mainly by three different activities: cytotoxic, antioxidant, and apoptosis induction. Recent studies on SAR contributed regarding the improvement of anticancer properties of chalcones by aryl ring substitution and introducing heterocyclic moieties.

21.6.5.3 Xanthones

Xanthones are mainly found in higher plant families like Leguminosae, Moraceae, Loganiaceae, Polygalaceae, Gentianaceae, Lythraceae, Rhamnaceae, and Guttiferae. Structurally they are heterocyclic compounds with C6-C1-C6 skeleton. Xanthones are classified in five major categories, namely, xanthone glycosides, xanthonolignoids, simple oxygenated xanthones, prenylated and related xanthones, and miscellaneous xanthones. Among them, xanthones are mostly identified from plants. Recently, few tumor-suppressive xanthone derivatives are also identified on the marine fungi. Chemotherapeutic drugs interfere with the enzymes associated with the metabolic activation and elimination processes (detoxification) of xenobiotics (Demirkiran 2007).

21.6.5.4 Coumarins

Coumarins are derived from phenolic acids and exhibit a benzene ring fused with an oxygen heterocycle. Among the 1000 naturally occurring coumarins in higher plants, the very common coumarins are coumarin, scopoletin, umbelliferone, and esculetin. Free coumarins are found in plants; most of them are simple and glycosylated. The higher quantities of coumarins are present in roots and seeds but have also been described in all the different parts of plants (Seigler 1998; Kalili and Villiers 2011).

21.6.5.5 Hydroxycinnamic Acids

The hydroxycinnamic acids are compounds that are derived from cinnamic acid through the phenylpropanoid biosynthetic pathway. They exhibit C6-C3 skeleton of trans-phenyl-3- propenoic acid with one or more hydroxyl groups attached to the phenyl ring, some of which may undergo methylation. The precursors for hydroxycinnamic acids are the amino acids L-phenylalanine and L-tyrosine. Hydroxycinnamic acids show a defensive and UV-protective effect in plant models. The most common compounds within this group are p-coumaric, caffeic, and ferulic acids (Dewick 2002; Crozier et al. 2006; Spencer 2012).

21.6.5.6 Hydroxybenzoic Acids

The hydroxybenzoic acid group is composed of seven carbon molecules with a C6-C1 skeleton and is derived from the shikimic acid pathway. These molecules are distributed in the plant kingdom as gallic, syringic, vanillic, and protocatechuic acids, the four most abundant compounds. Among this group of compounds, there is a subgroup known as tannins, which vary greatly from small to large molecules. There are two classes of tannins, the hydrolysable and the non-hydrolysable or condensed tannins (phlobaphenes). Hydrolysable tannins have a central core of polyhydric alcohol such as glucose and hydroxyl groups which can be esterified by gallic acid, while structural complexity is more in condensed tannins and are the products of polymerization of some flavonoids (Dey and Harborne 1997; Chung et al. 1998; Dewick 2002; Crozier et al. 2006).

21.6.5.7 Combrestatins

Combretastatins are a class of phytoconstituents belonging to natural phenols. Several types of natural combrestatin molecules are isolated from *Combretum caffrum* (bark), commonly known as South African bush willow (George et al. 1998). Despite having a similar name, combrestatins are not related to statins, a family of cholesterol-reducing agents. Few important combrestatins isolated from the plant and reported for anticancer activity are combretastatin A-1, combretastatin A-4, and combretastatin B-1. Molecules that come under combretastatin family generally possess three common structural features: a trimethoxy "A"-ring, a "B"-ring consisting substituents often at C3′ and C4′, and an ethene bridge (often) between the two rings which gives necessary structural rigidity. Molecules with such an ethene bridge are also stilbenoids; molecules with a non-ethene bridge are dihydrostilbenoids. The compounds belonging to the class of combrestatin have varying abilities, causing vascular disruption in cancers (inhibition of tumor angiogenesis). Combretastatin A-1, combretastatin A-4, and combretastatin B-1 are most potent naturally occurring and are currently being investigated in a number of clinical trials for their anticancer potentials (George et al. 1987, 1988; El-Zayat et al. 1993).

21.6.6 Phytosterols

Phytosterols are specific phytochemicals structurally similar to cholesterol but are exclusively found in plants. Though structurally similar to cholesterol, they differ in the arrangement of the side-chain structures. Commonly found phytosterols include β-sitosterol, campesterol, and stigmasterol. As a food additive, phytosterols reduce the absorption of cholesterol in the intestine and could function as tumor-preventive agents. Chemically, sterols are characterized by the hydroxyl group in position C-3 of the steroid skeleton, and unsaturated bonds in positions 5–6 of the B-ring, and

positions 22–23 in the alkyl substituent commonly present in edible oil-containing plants and also present in various vegetables, legumes, nuts, seeds, and unpasteurized milk. Research studies have indicated that stigmasterol isolated from neem, rapeseed, and soya bean is found useful in the prevention and treatment of certain cancers, including breast, ovarian, prostate, and colon cancers (Bruce and Grattan 2013; Huma et al. 2015).

21.6.7 Curcuminoids

Curcuminoids belong to terpenoid class comprised of curcumin, demethoxycurcumin, bisdemethoxycurcumin, and dihydrocurcumin. The rhizomes of turmeric are the major source of curcuminoids. They are the natural phenols accountable for turmeric's pronounced yellow color. Curcumin is an odorless powder having a melting point of 184–186 °C and shows poor solubility in water, benzene, and petroleum ether, however, readily soluble in glacial acetic acid, ethyl alcohol, and propylene glycol. They are highly soluble in ethyl ether and acetone. Their chemical structure has two aromatic ring systems containing o-methoxy phenolic groups, connected by a seven-carbon linker consisting of α,β-unsaturated β-diketone moiety. They exist in multiple tautomeric states in which the diketone is stable in the enol state while being easily deprotonated to the keto state (Tønnesen et al. 1982).

21.6.8 Cyanogenic Glycosides

Amygdalin also known as vitamin B17 belongs to a group of cyanogenic glycosides derived from phenylalanine, an aromatic amino acid. Amygdalin is very common among Rosaceae, Poeaceae, and Polygonaceae families and in the genera *Prunus* and *Fagopyrum.* Although amygdalin has been scientifically proved for certain anticancer mechanisms, furthermore clinical studies are essential to support the data of claiming its beneficial effects in cancer patients (Chen et al. 2013; Zuoqing and Xiaohong 2014).

21.7 Pharmacology of Plant-Derived Anticancer Agents

Medicinal plants and their active constituents are being increasingly identified as beneficial complementary therapy for cancer. Several clinical researches have revealed the useful effects of phytoconstituents on the survival, immune modulation, and improvement of health condition of cancer patients, when the herbal medicines are used in combination with conventional therapeutics. Several therapeutic drugs have been discovered through screening of products originated from plant,

animals, microbes, and marine organisms. Vinblastine, vincristine, topotecan, irinotecan, paclitaxel, etoposide, stigmasterol, and phenolic compounds are examples of plant-derived agents that are being employed in cancer chemotherapy. The mechanism of action, therapeutic uses, and adverse effects of phytochemicals as anticancer agents are mentioned in Table 21.3.

21.7.1 Catharanthus *Alkaloids*

The mechanism of action of *Catharanthus* alkaloids for their cytotoxic property is due to their interactions with polymerization of tubulin protein and disruption of mitotic spindle and directly arrests cell division at metaphase arrest. However, *Catharanthus* alkaloids do many other biochemical mechanisms that may or may not be associated to their effects on microtubules. Many of the effects that do not include interruption of microtubule happen only after treating cells with vinca alkaloids in clinically irrelevant doses. Nevertheless, the *Catharanthus* alkaloids and other anti-microtubule drugs also have an effect on both malignant and nonmalignant cancer cells in the non-mitotic cell cycle, because microtubules are involved in several non-mitotic functions. The *Catharanthus* alkaloids bind to target sites on tubulin protein that differ from those of the taxanes, colchicine, podophyllotoxin, and guanosine-5′-triphosphate. Existing evidence suggests the existence of two vinca alkaloid binding sites per mole of tubulin dimer. It is observed that in each microtubule, nearly about 16–17 high-affinity binding sites are located at the ends of per microtubule and binding of vinca alkaloids to these sites destroys microtubule formation, but one of the most important effects of low drug concentrations can be reducing the rates of both growth and shortening at the microtubule assembly which can cause “kinetic cap” and suppresses function. Earlier reports established that these drugs do not exhibit cross-resistance with drugs that causes alkylation of DNA and exhibited different mechanism of action by inhibiting spindle formation during cell division (Tafur et al. 1975; Robert van et al. 2004).

21.7.2 Taxanes

Both paclitaxel and docetaxel (taxanes) act by suppressing and inhibiting the growth, differentiation, and proliferation of malignant cells and become the most desired antitumor medicines by a chemotherapist. Taxanes inhibit the division of cell, the separation of chromatids, and the growth of cells, eventually causing cell death. They prevent depolymerization of tubulin protein and cause stabilization of mitotic spindle during cell division and are commonly known as inhibitors of mitosis or microtubule. Hence they are also sometimes called as mitotic poisons. According to research studies, they are equipotent antineoplastics, and their activity lies in the disruption of microtubules, which are the crucial components of mitotic spindle

Table 21.3 Mechanism of action, indications, and adverse effects of various classes of phytoconstituents from plants

Phytoconstituent	Mechanism of action	Indications	Adverse effects	References
Vincristine, vinblastine	Inhibits polymerization of tubulin, prevents spindle formation during mitosis, and thus arrests cell division	Hodgkin's lymphoma, nephroblastoma, leukemia	Neuropathy, hypertension, GIT upset, leukopenia, alopecia, paralysis	Robert van et al. (2004) and Tafur et al. (1975)
Paclitaxel, docetaxel	*Taxanes* are known to suppress and inhibit cell growth; differentiation and proliferation in indefinitely known cancer cell lines are the most preferred anticancer drugs by physicians	Melanoma; cancer of the breast, prostate, bladder, and ovaries; cancer of the intestine, stomach, liver, and lungs; head and neck cancer	Local redness, bruising, hand-foot syndrome, sore throat, dizziness, cough, dyspnea, female infertility	Wani et al. (1971) and Bissery et al. (1991)
Etoposide, teniposide, tafluposide, Etopophos	Inhibits depolymerization of tubulin, stabilizes formed mitotic spindle during mitosis, and thus arrests cell division	Kaposi sarcoma, lung cancer, lymphoma, leukemia glioblastoma,	Bone marrow depression, alopecia, GIT toxicity, hypersensitivity	Nerendra (2011), Liu and Hou (1997) and Gordaliza et al. (2004)
	Inhibition of the formation of the mitotic spindle, resulting in an arrest of the cell division process in metaphase and a clumping of the chromosomes (c-mitosis). Later it was shown that, at the subcellular level, this is due to binding of podophyllotoxin to tubulin, preventing these macromolecules to form a microtubules, which constitute the fibers of the mitotic spindle			
	Block the catalytic activity of DNA topoisomerase II by stabilizing a cleavage enzyme–DNA complex in which the DNA is cleaved and covalently linked to it			
Topotecan, irinotecan	Camptothecins binding to the topoisomerase I-DNA complex, leading to an accumulation of DNA strand breaks upon replication, ultimately causing cell death during the S-phase of the cell cycle	Ovarian cancer, lung cancer, cervical cancer, colon cancer, rectal cancer	Bone marrow depression, immune suppression, diarrhea, asthenia	Muqeet and Qudsia (2014) and Antony et al. (2004)

Stigmasterol	Stigmasterol is known to act through multiple *mechanisms of action to treat cancer which include* inhibition of carcinogen production, *cancer* cell proliferation, angiogenesis, invasion, and metastasis and through the promotion of process of apoptosis of cancerous cells	Ovarian cancer, bladder cancer, cervical cancer, prostate cancer, colon cancer		Huma et al. (2015)
Stilbenes, chalcones, xanthones, coumarins, hydroxycinnamic acids, hydroxybenzoic acids	Natural phenols possess in vivo potentials which are required to protect cell against free radical-mediated DNA damage and mutation Natural phenols also show ability to destroy DNA in vitro which shows their cytotoxic property against cancer cells Induction of apoptosis in vivo and in vitro cells	Leukemia, liver cancer, ovarian and breast cancer, testicular cancer, pancreatic cancer, lung cancer	Adverse effects are still under observation	Boudet (2007), Crozier et al. (2006), Seigler (1998), Nowakowska (2007), Demirkiran (2007), Kalili and Villiers (2011), Dewick (2002), Spencer (2012), Dey and Harborne (1997), Chung et al. (1998), George et al. (1987), El-Zayat et al. (1993) and George et al. (1988)
Curcuminoids	Curcuminoids possess antioxidant potentials and have the ability to neutralize free radicals. And thereby curcuminoids protect cells against oxidative damage caused by various chemicals and free radicals	Breast cancer, colon cancer		Akram et al. (2010) and Simay et al. (2008), Elattar TM and Virji AS (2000)
Cyanogenic alkaloids	Amygdalin belongs to cyanogenic alkaloids, induces apoptosis in cancer cells, and shows cytotoxic property	Colon and prostate cancer	Not reported	Zuoqing and Xiaohong (2014)

fibers and responsible for transcription, transportation, posttranslational modifications, and other cellular mechanisms (Nogales 2000; Zhou and Giannakakou 2005).

Previously, it was believed that both taxanes act by either excessive polymerization or depolymerization of microtubules; however, after a series of experimental trials, it was put forward that they reversibly and tightly bind to β-tubulin (Rao et al. 1999; Snyder et al. 2001) and stabilize microtubules by enhancing the rate of nucleation, growth, and elongation stages of polymerization (Derry et al. 1995; Yvon et al. 1999; Jordan and Wilson 1998). Among the most promising anticancer agents, taxanes are important phytoconstituents from natural source in Asia since cancer has become a major health complication in theses counties. Irrespective of its demerits such as side effects and poor solubility, the results of several ongoing experimental and clinical research studies have shown that taxanes are still the first-line drugs for treating lung, ovary, breast, and other metastatic carcinomas. Taxanes are helpful in prolonging the life and improving the lifestyle of cancer patients.

21.7.3 Podophyllotoxins

The mechanism of cytotoxic property of podophyllotoxins at the cellular level is the inhibition of the spindle formation during mitosis leading to cell division arrest and proliferation at the metaphase and forms clumping of the chromosomal proteins. Later, it is proved that the mode of action of podophyllotoxins is due to their capability to bind to tubulin, preventing these macromolecules from forming microtubules (mitotic spindles). Podophyllotoxin functions by arresting the cell division at metaphase through preventing microtubule assembly. These podophyllotoxins stabilize a cleavage of enzyme DNA complex where DNA cleavage takes place and blocks DNA topoisomerase II activity by covalently linking to them. It binds at the colchicine site of the tubulin to inhibit DNA topoisomerase. With some chemical modifications, etoposide/teniposide was derived from podophyllotoxin, and they exhibit their cytotoxicity by inhibiting DNA topoisomerase II enzyme activity. Podophyllotoxin is an inhibitor of microtubule during mitosis, while etoposide and teniposide are DNA topoisomerase II inhibitor agents (Berger et al. 1996; Desbene and Giorgi-Renault 2001).

21.7.4 Camptothecins (CPT)

Camptothecin acts as an anticancer agent by inhibiting topoisomerase I enzyme that relieves topological strain in DNA due to introduction of single-strand breaks. A nick in the single-stranded DNA by topoisomerase I allows DNA to freely rotate during replication. Camptothecins possess active lactone ring which intercalates between DNA bases in the topoisomerase I cleavage complex. This binding of

camptothecins and its derivatives in the cleavage complex prevents topoisomerase I enzyme from re-ligating the nicked DNA strand after relieving the topological strain. This intercalation of camptothecins traps the topoisomerase I enzyme in the cleavage complex bound to the DNA. As the progressing DNA replication fork strikes with the confined topoisomerase I enzyme, DNA breaks and replication process is hindered. If continuous DNA strands are broken during the replication process, the repair mechanisms found in mammalian cells cannot repair them and lead to cell death. This amassed and trapping of DNA-topoisomerase I enzyme complexes is the major reason for the stimulation of cell apoptosis. Overall, this intercalation disrupts the progress of DNA replication process and eventually leads to cause cell mortality. This complex formation mechanism affects the breaking of DNA strands and encourages apoptotic pathways of cell inhibition (Hsiang et al. 1985).

21.7.5 Plant Phenols

Plant phenolic compounds have the ability to reduce the risk of carcinogenesis as they interfere with the cellular mechanisms at all stages (initiation, promotion, and progression). With similarities and particularities, the number of targets and mechanisms where they are involved paved the way to their protective or therapeutic effects against cancer (Kang et al. 2004; Boudet 2007). The chalcones have been proved as promising lead anticancer-chemopreventive agents mainly by three different activities: cytotoxic, antioxidant, and apoptosis induction. Recent studies conducted on structure-activity relationship (SAR) contributed toward the improvement of anticancer potentials of chalcones by substituting aryl rings and introducing heterocyclic moieties (Seigler 1998; Nowakowska 2007).

Xanthones are known to act on the enzymes associated with the metabolic activation and elimination process (detoxification) of xenobiotics. Enzymes specifically involved in phase I biotransformation reactions of xenobiotics which enter the body also increase the risk of production of carcinogens, which can interfere with DNA structure, leading to development of cancer. In vitro and in vivo anticancer potentials of xanthones have been the object of a considerable number of research studies in recent days (Demirkiran 2007).

Coumarin, a derivative of plant origin, can possess both cytostatic and cytotoxic properties. Studies have shown that coumarin and 7-hydroxycoumarin can inhibit the growth of various human cancer cell lines such as A549 (lung cancer cells), ACHN (renal cancer cells), H727 (lung cancer cells), MCF7 (breast cancer cells), and HL-60 (leukemia cells). Clinical findings have proved their antiproliferative activity in prostate carcinoma, malignant melanoma, and renal cell carcinoma (Emami and Dadashpour 2015). Coumarin has also shown dose-dependent cytotoxic effect against Hep2 cells (human epithelial type 2 cancer cells) and showed some typical features of apoptosis with loss of microvilli of membrane, cytoplasmic hyper-vacuolization, and karyorrhexis. Coumarins are found to be effective for treating radiotherapy-related toxicities, which demonstrated a combinational ther-

apy of coumarin/troxerutin in protection of mucous and salivary glands in patients undergoing radiation therapy (Tamer et al. 2014; Emami and Dadashpour 2015).

21.7.6 Phytosterols

Phytosterols such as stigmasterol, campesterol, and β-sitosterol are abundantly found in the diet. They play a significant role in controlling the levels of blood cholesterol and possess the ability to mitigate cancer risks at initial stages (Bruce and Grattan 2013). According to epidemiological studies, phytosterol content of the food is associated with the reduced risk of common cancers including cancers of the prostate, colon, and breast (Bradford and Awad 2007). Phytosterols affect host cellular systems potentially enabling more robust anticancer molecular responses. The mechanisms include the improvement of immune system to recognize cancer cells, affecting the hormonal-dependent development of endocrine tumors and sterol biosynthesis (Bruce and Grattan 2013). In addition, phytosterols directly inhibit tumor growth by slowing of cell division, cell cycle arrest, enhancing oxidative stress, inducing of apoptosis, and inhibiting metastasis of tumor (Bradford and Awad 2007; Bruce and Grattan 2013; Huma et al. 2015).

21.7.7 Curcuminoids

Curcumin is a potential agent in cancer therapy and is gaining wide acceptance as a preventive measure due to its safety (Lin et al. 2007). It affects several steps in the development of cancer process, which is important in preventing chemoresistance. Clinical trials have indicated its effectiveness for therapy of cancer as a single agent or in combination. In vivo animal researches involving mice and rats and in vitro assays employing cancer cells have revealed the potency of curcuminoids to suppress tumorigenesis process at three phases: tumor promotion, angiogenesis, and tumor cell growth. Curcuminoids inhibit the proliferation of colon cancer cells (Akram et al. 2010). Curcuminoids are also capable of suppressing the ability of several common mutagenic and carcinogenic agents in a variety of cell types in both in vitro and in vivo models. The anticarcinogenic properties of curcuminoids are due to direct antioxidant and free-radical scavenging potentials, as well as their potency to indirectly enhance glutathione (endogenous antioxidant) levels, thereby serves in detoxification of mutagenic and carcinogenic agents in the liver (Chainani-Wu 2003; Simay et al. 2008; Akram et al. 2010).

Curcumin inhibits the STAT3 and NF-κB signaling pathways, which play important roles in the development of cancer and progression. It also inhibits Sp-1, and its housekeeping gene expressions may serve as an important hypothesis to prevent malignant formation, migration, and invasion. Recent studies have proved that curcumin acts by inhibiting the Sp-1 activation, and it downregulates genes, including

ADEM10, calmodulin, EPHB2, HDAC4, and SEPP1 in a dose-dependent manner in cancer cell lines of colorectal carcinoma. These results are consistent with other research studies, which have suggested that curcumin could suppress the activity of Sp-1 in urinary bladder cancer and could decrease DNA binding activity of Sp-1 in non-small cell lung cancer cells. Recent data show that the curcumin analogue B19 plays an important role in apoptotic process by inducing ER stress and autophagy in an epithelial ovarian tumor cell lines (Mehta et al. 1997; Hanif et al. 1997; Mukhopadhyay et al. 2001; Siwak et al. 2005; Lin et al. 2007).

21.7.8 Cyanogenic Glycosides

Several hypotheses are derived to explain the mechanism of amygdalin for its anticancer properties. Hypothesis (1) proposed that cancerous cells contained plenty of beta-glucosidases, which release HCN from laetrile through hydrolysis. Normal cells will not be affected as they contained low amount of beta-glucosidases and high concentrations of rhodanese, which converts HCN to thiocyanate which is less toxic. It is proved that both cancerous and normal cells contain only trace concentration of beta-glucosidases and similar amounts of rhodanese. Hypothesis (2) suggests that, after ingestion, amygdalin was hydrolyzed to mandelonitrile, transported intact to the hepatic system, and converted to a beta-glucuronide complex, which was then carried to the cancer cells and hydrolyzed by beta-glucuronidases to release mandelonitrile and then HCN. Hypothesis (3) suggests that laetrile is the discovered vitamin B17 and further asserted that cancer is a result of "B17 deficiency." It postulated that regular dietary administration of laetrile prevents all incidence of cancer. The term "vitamin B17" is not approved by the Committee on Nomenclature of the American Institute of Nutrition Vitamins. There is no credible evidence to support this conjecture (Zuoqing and Xiaohong 2014; Chen et al. 2013; Sakarkar and Deshmukh 2011).

21.8 Conclusions and Future Prospects

Due to increased incidences of cancer cases worldwide, there is an accelerated scope in the field of drug development to mitigate cancer. Synthetic drugs that are presently available to treat cancers cause high toxicity to the normal cells leading to the development of secondary cancers. In this regard, antitumor agents derived from natural resources play a major role, and current research is focused on exploring various medicinal plants to develop novel anticancer lead molecules. Among various phytoconstituents, *Catharanthus* alkaloids, taxanes, podophyllotoxins, and camptothecins act by inhibiting the cancer cell proliferation by disruption of DNA or spindle formation during mitosis. Hence, the cytotoxic potential of these agents may increase the risk of adverse effects, while plant phenols, curcuminoids,

stigmasterol, and amygdalin show anticancer activity by protecting the cells against free radical-mediated destruction or by inducing apoptosis in cancer cells, and therefore these drugs have less risk of adverse effects. Hence, the research on the development of phytochemicals from plant and other sources which are more effective and less toxic needs more attention.

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Chapter 22
Botany, Chemistry, and Pharmaceutical Significance of *Sida cordifolia*: A Traditional Medicinal Plant

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22.1 Introduction

The use of plants for the treatment of various human ailments is not new and known for the past several centuries. Herbal formulations were used by early human days to the modern era for the remedy against many forms of diseases. Plants have been recognized as a curative agent since from many ancient civilizations such as Indians, Chinese, Egyptians, Romans, and Greeks (Gaidahani et al. 2009; Kumara et al. 2012; Mohanty et al. 2017). Plant products in the form of extracts or dry powder are chiefly employed in Ayurveda, Chinese medicine, homeopathy, naturopathy, Native American, Siddha, Tibetan, and Unani medicines (Erok 2013; Mohanty et al. 2015; Atanasov et al. 2015; Swamy et al. 2015; Arumugam et al. 2016). According to the assessment report of the World Health Organization (WHO), around 80% of the global population mainly rely upon herb-based medicines to meet their basic health-care needs

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(Kumara Swamy et al. 2011, 2015; Kumara et al. 2012; Swamy and Sinniah 2015; Arumugam et al. 2016; Mohanty et al. 2017). Moreover, currently available modern therapeutic drugs are discovered primarily from the knowledge on herbs from the traditional practices of treatments. Today's drug discovery is largely focused on utilizing natural resources and their compounds to combat the danger of persistently growing human illnesses (Mohanty et al. 2014; Arumugam et al. 2016; Swamy et al. 2016, 2017). Many pharmaceutical industries often rely on plants as a source of essential ingredient and raw material (Swamy and Sinniah 2016; Arumugam et al. 2016; Tariq et al. 2017). Hence, to meet the growing demand for herbal raw materials, several medicinal and aromatic plants have been cultivated commercially for obtaining bioactive metabolites (Sudipta et al. 2011; Swamy and Sinniah 2016; Mohanty et al. 2017).

The genus *Sida* belonging to the family Malvaceae includes over 200 herbaceous species with ethnomedicinal significance (Sivarajan and Pradeep 1996; Dinda et al. 2015). These plants are distributed pantropically throughout the world. *Sida cordifolia,* also well known as "Malva Branca" is utilized in Brazilian folk medicines for treating inflammatory diseases, blennorrhea, asthma, nasal congestion, and stomatitis (Franzotti et al. 2000; Franco et al. 2005; Dinda et al. 2015). In many parts of the African countries, it is used against several health problems, especially for treating respiratory diseases (Galal et al. 2015; Dinda et al. 2015). The plant possesses anti-inflammatory (Kanth and Diwan 1999), hypoglycemic, analgesic (Dinda et al. 2015; Siddiqui et al. 2016), anti-protozoan (Khare 2004), antiulcer (Philip et al. 2008; Akilandeswari et al. 2013), antimicrobial (Masih et al. 2014; Halilu et al. 2016), antihelminthic (Pawa et al. 2011; Nathaniel et al. 2014), and anticancer (Dassonneville et al. 2000; Mallikarjuna et al. 2013; Srinithya et al. 2016) properties. The presence of the constituent, ephedrine, imparts psychostimulative properties to the plant and affects the heart and central nervous system. Also, ephedrine acts as a bronchodilator and decongestant (Santos et al. 2005; Galal et al. 2015; Adam and Steven 2006). In India, the root of *S. cordifolia* is popularly recognized by the name *Bala* and used widely as an ingredient for preparing various formulations of Ayurvedic medicines. The remedial properties of *S. cordifolia* are because of the presence of various bioactive phytoconstituents such as 2-carboxylated trytamines, β-phenylamines, quinoline, quinazoline, indole, ephedrine, vasicinone, 5-hydroxy-3-isoprenyl flavone, 5, 7-dihydroxy-3-isoprenyl flavone, 6-(Isoprenyl)-3-methoxy-8-C-*β*-D-glucosyl-kaempferol 3-O-*β*-D-glucosyl [1–4]-α-D-glucoside, and many others (Ghosal et al. 1975; Prakash et al. 1981; Sutradhar et al. 2007b, 2008; Chaves et al. 2013).

In recent years, cancer disease has been widely spread across the world, and its incidences are increasing constantly. In 2016, American Cancer Society estimated about 1,685,210 number of new cancer cases worldwide. In Asian countries (constitutes 60% total global population) one-third of deaths were related to cancer, and the death rate was projected to geometrically increase to 16 million in 2025 (Shin et al. 2012; Ferlay et al. 2010). According to World Cancer Research Foundation, Denmark has the highest cancer rate among the nations, while Nigeria has the lowest rate in 2012 (Ferlay et al. 2010). Cancer cells develop rapidly and progressively

invade normal body tissues in a stepwise manner, hence making its early detection and prevention difficult. The cause for cancer includes chemicals, radiations, ingestion of carcinogens, immune failure, and pathogenic and genetic factors (Chacko et al. 2015).

For decades, traditional herbal medicine has played a vital role in the development of anticancer drugs with minimum side effects worldwide (Nath et al. 2013). Exploration of botanical natural products for cancer prevention has resulted in the development of more than 300 anticancer drugs (Abdel-Kadir et al. 2007). Though herbal medicine is natural and safe, it can be detrimental or toxic to humans when taken in specific conditions such as pregnancy, lactation, ulcer, and hepatic problems or at high doses. For instance, root bark extract of *Annona senegalensis* exhibited 50% cellular toxicity against HeLa, PANC-1, and 293-T cells at 211.35, 166.07, and 125.89 μg/ml (Okoye et al. 2011). In contrast, some active compounds including piperine from *Piper nigrum*, *Cicer arietinum*, and *Plantago major* at 5000 mg/kg, 600 μg/ml, and 2000 mg/kg were reported to be nontoxic on mammary tumorigenesis and peripheral blood mononuclear cells and nonmortal (Garcia et al. 2003; Damanhouri and Ahmad 2014; Kumar et al. 2014). Interestingly, pharmacological study ascertained negligible or low-toxicity effect of *S. cordifolia* extract when administered orally on mice and exhibited good anti-inflammatory and analgesic activity (Franzotti et al. 2000; Konaté et al. 2012; Quedraogo et al. 2012). The weak toxicity level of *S. cordifolia* to biological system gives an interesting feature and suggests its potential safety for therapeutic applications. Thus considering the above facts, the present chapter aims to provide detailed information about the ethnobotanical, phytochemical, and pharmacological aspects of *S. cordifolia*. The information will be useful for the development of novel drugs against various deadly diseases including cancer.

22.2 Botanical Description of *Sida cordifolia*

22.2.1 Morphological Features

S. cordifolia is an erect perennial herb that grows up to 1.5 m height. It can tolerate drought as well as high rainfall conditions and strive in a wide variety of soil types. Leaves are simple heart truncate and serrate shaped with 2.5–7 cm long and 2.5–5 cm broad, ovate with entire leaf blade, and dense stellate hairs (Jain et al. 2011). Stems are ascending, stout and strong, and tall with densely stellate pubescent. A well-developed tap root system is up to 60 cm. Peduncle bears yellow flowers produced in clusters jointly above the panicle, flowers bisexual are paired or solitary, small axillaries or subterminal with cup-shaped calyx and yellow corolla, mericarps with awns (Dinda et al. 2015). Fruits are slender mericarp, 5-carpeled, 6–8 mm diameter across and sub-discoid with each carpel having two long linear and setaceous awns; seeds are flattened, grayish black, smooth, and produced in capsules divided into ten segments (Khurana et al. 2016).

22.2.2 Taxonomy

Sida cordifolia is a flowering plant belongs to the Malvaceae family. The order Malvales taxa consisting of four families (Bombacaceae, Malvaceae, Sterculiaceae, and Tiliaceae) comprises about 250 genera and 4230 species which are widely spread across the world (Tate et al. 2005). Chromosomal evolution of the family has been dynamic with haploid numbers ranging from $n = 5$ to 36; generic alliance of *Sida* contains 11 genera (*Akrosida*, *Allosidastrum*, *Dendrosida*, *Krapovickasia*, *Malvella*, *Maximalva*, *Rhynchsida*, *Sida*, *Sidasodes*, *Sidastrum*, and *Tetrasidas*) which are characterized by morphology and basic chromosome numbers of pollen and fruits with a varying haploid chromosome numbers from $n = 6$ to 28 (Tate et al. 2005; Carolina et al. 2010). Common names includes country mallow, flannel weed, heart leaf shape *Sida*, Brazil "Malva Branca," Malaysia "Pokok kelulut puteh," Chinese "Ke dong," Nigeria (Hausa) "Garmani," Hindi "Kungyi," Sanskrit "Bala," Tamil "Mayir-manikham," Bengali "Brela," and Punjab "Simak."

22.2.3 Geographical Distribution

Sida cordifolia, a native to the northeast part of Brazil, is popularly identified as "Malva Branca" and broadly distributed in more than 82 countries worldwide, mainly throughout the tropical and subtropical regions including Australia; North and South America; Central America and Caribbean; Europe; West, East, and Central Africa; and Asia (Bonjardim et al. 2011; Dinda et al. 2015).

22.3 Ethnomedicinal Uses

An ethnobotanical investigation has shown that many species of the genus *Sida* are traditionally used to treat various kinds of diseases. Among such plants, *S. cordifolia* L. and *S. acuta* Burn f. contribute to the modern system of herbal medicine and drug development (Jain et al. 2011). In India, *S. cordifolia* has been used widely since historic periods as one of the important raw material for preparing various Ayurvedic medicines (Galal et al. 2015). Some of the ethnobotanical importance of different parts of *S. cordifolia* and their preparations are detailed in Table 22.1. Its medicinal significance includes the treatment of illnesses such as bronchial asthma, nasal congestion, headache, aching joints and bones, edema, cough, gastrointestinal and urinary infections, debility, skin ailments, weight loss, asthma, rheumatism, stomatitis, stomach upset, diarrhea, dysentery, premature ejaculation, miscarriage, malaria, fever, bronchitis, variola, and sciatica; its use in childbirth; and anti-gonorrhea, antituberculosis, anti-obesity, anticancer, antibacterial, anti-ulcer, antipyretic, diuretic, aphrodisiac, and hepatoprotective properties (Sutradhar et al. 2007a;

Table 22.1 Ethnobotanical importance *S. cordifolia* uses and its mode of preparation against difference ailments

Plant part used	Ethnomedicinal use	Country	Mode of preparation	References
Root	Rheumatism and sciatica	India	Decoction of fresh root bark	Vasudevan Nair (2004)
	Hemiplegia, Parkinson's disease, weight loss, weakness, mental exhaustion	India	–	Divakar et al. (2013)
	Facial paralysis	India	–	Khatoon et al. (2005) and Nagashayana et al. (2000)
	Sunstroke	India	Paste of root with sugar orally	Kapoor and Lakhera (2013)
	Leucorrhoea	India	Powdered root bark mixed with milk and sugar orally	Kapoor and Lakhera (2013)
	Jaundice	India	Mixture of half cup root juice and half tablespoon sugar candy is given once daily till cured	Sarkar and Das (2010)
	Eye inflammation	Burkina Faso and Tanzania	Maceration	Brink and Achigan-Dako (2012)
	Abortion	Kenya and Central African Republic	Extract orally	Brink and Achigan-Dako (2012)
	Urinary tract problem and fever, dysentery	Bangladesh and Benin	–	Brink and Achigan-Dako (2012) and Rahmatullah et al (2010)
	Menstruation	Kenya and Central African Republic	Chewing	Brink and Achigan-Dako (2012)
Leaf and twig	Hair loss, constipation, and fever	Colombia	Decoction	Ballesteros et al. (2013)
Leaf	Cuts	India	Pounded leaves	Kapoor and Lakhera (2013)
	Syphilis and pneumonia	Rwanda	Leaves	Boily and Puyvelde (1986)
	Ophthalmic diseases	India	Paste of leaves externally	Ajithabai et al. (2012)
	Fever; prevent miscarriage	Burkina Faso	Decoction	Nacoulma (1996)
	Dysentery, sprains, swellings, and intestinal worms	Burkina Faso, Kenya, Philippines, Burundi, Senegal	Poultice to sprains and swelling, decoction orally for control of intestinal worms	Brink and Achigan-Dako (2012)

(continued)

Table 22.1 (continued)

Plant part used	Ethnomedicinal use	Country	Mode of preparation	References
	Fever, rheumatism and lung disorder	Republic of Congo	Infusion, aqueous extract of whole plant	Yusuf and Kabir (1999); Brink and Achigan-Dako (2012)
	Cystitis, diuretic, and astringent	Mauritius	Decoction	Brink and Achigan-Dako (2012)
Flowers and fruits	Urinary tract problem	India	Paste of flowers and unripe fruits with water orally	Kapoor and Lakhera (2013)
Seeds	Bowel complaints and gonorrhea	India	–	Kapoor and Lakhera (2013)
Root and seed	Anti-paralytic and aphrodisiac	India	–	Dinda et al. (2015)
Whole plant	Toothache and diarrhea	India	–	Rahmatullah et al. (2010)
	Spermatorrhea, urogenital disorder, pile, asthma, gonorrhea, rheumatism, hay fever	Nigeria	–	Olowokedejo et al. (2008)
	Asthma and nasal congestion	Brazil	–	Balbach (1989)
	Throat inflammation	Brazil	Infusion	Breitbach et al. (2013)
	Stomach upset and bacterial infections	Nigeria	Aqueous leave extract	Halilu et al. (2016)
	Cough, rheumatic, and abdominal pain	Burkina Faso	–	Nacoulma (1996)
	Skin cancer	Kenya	Leaves powder applied	Tariq et al. (2017)
	Cancer and leukemia	Benin	–	Brink and Achigan-Dako (2012)
	Week sperm count	Bangladesh	Leaf and bark of root	Nawaz et al. (2009)
	Pregnancy and labor pain	Cameroon and Ghana	Maceration orally for 6 months	Lutterodt (1988) and Yemele et al. (2015)
	Vaginitis and cancer	Cameroon	–	Pieme et al. (2014)

Nagashayana et al. 2000; Jain et al. 2011; Srivastava et al. 2013; Dinda et al. 2015; Halilu et al. 2016). Seeds are commonly considered to be aphrodisiac, while the juice of the whole plant is useful in preventing premature ejaculation (Konaté et al. 2012), controlling neurodegenerative (Alzheimer's, Parkinson's) diseases, loss of memory, degeneration of nerves, and other neurological disorders (Auddy et al. 2003). Traditionally its root extract is used to promote wound healing, crumpled leaves serve as an astringent dressing of wounds and treatment of skin injuries, stomach problems, nervous system, muscular pain and as a cardiac tonic (Pawar et al. 2016), analgesic, depressive effect on central nervous system, cancer treatment and anti-inflammatory and regeneration of liver cells (Franco et al. 2005; Jenny et al. 2005; Nunes et al. 2006). In Brazil, *S. cordifolia* is used in their folk medicines for treating inflammatory diseases, blennorrhea, asthma, nasal congestion, and stomatitis (Franzotti et al. 2000; Franco et al. 2005; Dinda et al. 2015). In many parts of the African countries, it is used against several health problems, especially for treating respiratory diseases (Galal et al. 2015; Dinda et al. 2015). Methanol extract of *S. cordifolia* at 6–8 μg/ml was observed to have cytotoxic effect on HeLa cell line and was also effective against phytopathogens such as *Bacillus subtilis*, *Escherichia coli*, *Enterobacter aerogenes*, *Mycobacterium* spp., *Pseudomonas aeruginosa*, and *Micrococcus* spp. (Joseph et al. 2011). Seeds are useful for vowel complaints; roots possess tonic, astringent, and diuretic properties, hemiplegia, urinary disorder, and facial paralysis (Kanth and Diwan 1999)**.** In China, it is used as an equivalent to ephedra, an herbal preparation from the plant *Ephedra sinica*, while it is employed against dental problems in Kenya (Galal et al. 2015).

22.4 Phytochemistry

The survey of literature showed the presence of several pharmacologically important chemical compounds in *S. cordifolia* plant parts. Current research is focused at the isolation of specific pure phytocompounds of *S. cordifolia* and to appreciate their biological significance. For many decades extracts from different parts of *S. cordifolia* have been reported to be used for a variety of therapeutic purposes. Generally the genus *Sida* is well known to possess high amount of alkaloids, flavonoids, and steroids (Table 22.2). Some of the identified and isolated compounds of *S. cordifolia* are discussed below.

22.4.1 Alkaloids

The aerial parts of *S. cordifolia* are reported to contain ephedrine and Ψ-ephedrine and appreciable quantities of water soluble alkaloids (Dutta and Crane 1963). The total alkaloid content is reported to be 0.085%. Reverse-phase high-performance

Table 22.2 The known volatile constituents of *S. cordifolia*

Compounds	Plant part	Method used	References
5-hydroxy-3-isoprenyl flavones	Aerial parts	TLC/UV/IR/NMR	Sutradhar et al. (2006), (2008)
3′-(3″,7″-dimethyl-2″,6″-octadiene)-8-C-β-D-glucosyl-kaempferol-3-O-β-D-glucoside	Aerial parts	TLC/UV/IR/NMR	Sutradhar et al. (2006, 2007b)
6-(isoprenyl)-3-methoxy- 8-C-*β*-D-glucosyl-kaempferol 3-O- *β*- D-glucosyl [1→4]- α- D-glucoside	Aerial parts	TLC/UV/IR/NMR	Sutradhar et al. (2007b)
Ephedrine	Aerial parts/roots/ whole plant	HPLC-PDA/GC-MS	Ghosal et al. (1975), Khatoon et al. (2005), and Joseph et al. (2011)
Ψ-ephedrine	Aerial parts/roots	HPLC-PDA	Khatoon et al. (2005)
(*S*)-(+)-N-methyl-tryptophan methyl ester,	Roots	–	Ghosal et al. (1975), Prakash et al. (1981), and Khatoon et al. (2005)
β-phenethylamine			
Hypaphorine	Roots	GC-MS	Ghosal et al. (1975), Prakash et al. (1981), and Khatoon et al. (2005)
Vasicine	Roots	RP-HPLC/HPTLC	Ghosal et al. (1975), Prakash et al. (1981), Khatoon et al. (2005), and Dhalwal et al. (2010)
Vasicinone			
Vasicinol			
Arachidic acid	Seeds	GLC/UV/IR/NMR	Rao and Lakshminarayana (1984)
Linoleic acid			
Malvalic acid			
Myristic acid			
Oleic acid			
Sterculic acid			
Dihydrosterculic acid			
Palmitic acid	Seeds/leaves	GLC/UV/IR/NMR/ LC-MS	Rao and Lakshminarayana (1984) and Ahmed et al. (2017)
Stearic acid			
β-sitosterol	Whole plant	UV/NMR	Sutradhar et al. (2008)
Stigmasterol			

TLC thin-layer Chromatography, *UV* ultraviolet, *IR* infrared, *NMR* nuclear magnetic resonance, *GC-MS* gas chromatography-mass spectroscopy, *LC-MS* liquid chromatography-mass spectroscopy, *HPLC* high-pressure liquid chromatography, *HPTLC* high-performance thin-layer chromatography, *GLC* gas liquid chromatography

liquid chromatography (RP-HPLC) and HPTLC methods were adopted to simultaneously quantify vasicine and vasicinone occurring in *Sida* species (Dhalwal et al. 2010). The results revealed the presence of vasicine (0.010 ± 0.031%) and vasicinone (0.0061 ± 0.022%). Likewise, a simple high-performance thin-layer chromatography (HPTLC) method was developed to quantify ephedrine content in the *Sida* species (Khatoon et al. 2005). The maximum ephedrine content (0.112%) was found in *S. cordifolia* whole plant, while the roots of *S. cordata* recorded with the least amount (0.005%). Joseph et al. (2011) identified ephedrine and vasicinol, vasicinone, and hypaphorine from the whole plant of *S. cordifolia* using gas chromatography–mass spectrometry (GC-MS) analysis. In addition, aerial parts of *S. cordifolia* also contain 5′-hydroxymethyl-1′-(1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazoline-1-yl)-hepta-1-one(a quinazoline alkaloid), and this compound is reported to possess anti-inflammatory and analgesic activities (Sutradhar et al. 2007b). In addition, cryptolepine (indoloquinoline alkaloid) was also reported from this plant (Matsui et al. 2007; Galal et al. 2015).

22.4.2 Flavonoids

The aerial parts of *S. cordifolia* were detected with a C-flavonol glycoside, namely, 3′-(3″,7″-dimethyl-2″,6″-octadiene)-8-C-β-D-glucosyl-kaempferol 3-O-β-D-glucoside, and two flavones such as 5-hydroxy-3-isoprenyl flavones and 5,7-dihydroxy-3-isoprenyl flavones (Sutradhar et al. 2007b, 2008; Chaves et al. 2013). Similarly, three more flavonol C-glycosides, namely, 6-(isoprenyl)-3-methoxy-8-C-*β*-D-glucosyl-kaempferol 3-O-*β*-D-glucosyl [1→4]-α-D-glucoside, 3′-(3″. 7″-dimethyl-2″0.6″-octadiene)-8-C-β-D-glucosyl-kaempferol-3-O-β-D- glucoside, and 3′-(3″. 7″-dimethyl- 2″0.6″-octadiene)-8-C-β-D-glucosyl-kaempferol-3-O-β-D-glucosyl[1→4]-α-D-glucoside, were isolated from the same plant source (Sutradhar et al. 2007b, 2008; Chaves et al. 2013).

22.4.3 Fatty Acids

Rao and Lakshminarayana (1984) and Ahmed et al. (2017) identified the following fatty acids such as arachidic acid, linoleic acid, malvalic acid, myristic acid, oleic acid, palmitic acid, sterculic acid, stearic acid, dihydrosterculic acid, and sterculic acid from the seed oils of *S. cordifolia.* The occurrence of a hydroxyl unsaturated fatty acid, (10E, 12Z)-9-hydroxyoctadeca-10,12-dienoic acid, was obtained from the bioassay-guided fractions of methanolic leaf extracts of *S. cordifolia* (Nune et al. 2006).

22.4.4 Steroids

To date, no extensive study on the steroids in *S. cordifolia* has been recorded; however, Sutradhar et al. (2008) reported that the dried powder of the aerial parts of *S. cordifolia* contain two steroid compounds, namely, β-sitosterol and stigmasterol.

22.5 Pharmacological Significances

In this section, the significant outcomes from the previous research studies on the pharmacological activities of different isolated compounds and plant extracts of *S. cordifolia* are reviewed (Tables 22.3 and 22.4).

22.5.1 Anticancer Activity

Joseph et al. (2011) evaluated the cytotoxic activity of the ethanolic extract of *S. cordifolia* whole plant and found the dose-dependent cell toxicity in HeLa cell lines. Also, they observed that cells treated with *S. cordifolia* extract have arrested the cells by apoptosis. Likewise, the ethanolic extract (70%) of *S. cordifolia* at 250 and 500 mg/kg bw dose had significantly restored necrotic cells, by exhibiting antioxidant and anticancer activities (Mallikarjuna et al. 2013). In another study, it has been reported that cryptolepine, an alkaloid compound of *S. cordifolia*, hinders the growth of mutated osteosarcoma (MG63) cells at ≥0.5 μM within 72 h of incubation. Further, it promotes anticancer activity of a potent cyclin-dependent kinases

Table 22.3 Phytochemical compounds of *S. cordifolia* with pharmacological activates

Compounds	Activity	References
5′-Hydroxymethyl-1′- (1,2,3,9-tetrahydro -pyrrolo [2,1-b] quinazolin-1-yl)-heptan-1-one)	Analgesic, anti-inflammatory	Sutradhar et al. (2006)
1,2,3,9-tetrahydro-pyrrolo [2,1-b] quinazolin-3-ylamine	Inhibit writhing	Sutradhar et al. (2007a)
5, 7-dihydroxy-3-isoprenyl flavone	Analgesic, anti-inflammatory	Sutradhar et al. (2008)
3′-(3″. 7″-dimethyl-2″6″-octadiene)-8- C-*β*-D-glucosyl-kaempferol-3-O-*β*-D- glucoside	Analgesic, anti-inflammatory	Sutradhar et al. (2006, 2007b)
Di-(2-ethylhexyl) phthalate	Anti-inflammatory	Preethidan et al. (2013)
Rasin, mucin, sympathomimetic amines, saponins		Franzotti et al. (2000), Franco et al. (2005), and Kubavat and Asdaq (2009)
Cryptolepine	Anticancer	Matsui et al. (2007)

Table 22.4 Biological activities of *S. cordifolia*

Activity	Microorganisms	Plant part	References
Antibacterial	*Staphylococcus aureus*, *Basillussubtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*	Ethanol leave extract	Halilu et al. (2016)
	Klebsiella pneumonia, *Pseudomonas aeruginosa*	Ethanol leaf extract	Kalaiarasan and John (2010)
	Bacillus subtilis, *Escherichia coli*, *Pseudomonas fluorescens*,	Methanol leaf and root extracts	Mahesh and Satish (2008) and Masih et al. (2014)
	Staphylococcus aureus, Xanthomonas axonopodis pv. Malvacearum, and *Enterococcus faecalis*		
Antifungal	*Candida albicans*, *C. parapsilosis*, *C. krusei*, and *C. tropicalis*	Acetone aerial extract	Quedraogo et al. (2012)
Antifungal	*C. guilliermondii*, *C. albicans*, *C. tropicalis*, *C. krusei*, and *Trichosporon inkin*	Leaf powder	Nune et al. (2006)
Hypoglycemic and analgesic	Mice	Root and aerial methanol extract	Kanth and Diwan (1999), Dinda et al. (2015), and Siddiqui et al. (2016)
Anthelmintic	*Pheretima posthuma* (earthworm)	Aqueous and methanol extract	Pawa et al. (2011) and Nathaniel et al. (2014)
Anti-protozoan	*Ascaridia galli* and *Hymenolepis nana*	Root chemical isolate (vasicinone)	Khare (2004)
Antiulcer	Albino rat	Aerial methanol extract, ethanol leave extract	Philip et al. (2008) and Akilandeswari et al. (2010)
Anti-inflammatory	Male Wistar rats	Ethyl acetate and methanol extract	Kanth and Diwan (1999)
Anticancer	Swiss mice (HT-24 cell lines)	Aqueous leaf extract	Srinithya et al. (2016)
	Female Wistar rat	Ethanol leaf extract	Mallikarjuna et al. (2013)
	Human colon cancer cells (HCT-116), human leukemia cells (LH-60)	Cryptolepine (whole plant extract isolate)	Dassonneville et al. (2000)
	Human LH-60 cells leukemia	Cryptolepine	Matsui et al. (2007)

inhibitor (p21 WAF1/CIP1) expression in human colon cancer HCT116 cells hence could be suitable for chemotherapy for osteosarcoma (Matsui et al. 2007). Likewise, cryptolepine was previously reported to inhibit cell cycle and induce apoptosis in human LH-60 leukemia (Dassonneville et al. 2000). The p21 WAF1/CIP1 suppressed G1 and G2 M-phase cell cycle, differentiation and growth of cells in vivo and in vitro (Gartel et al. 1996; Matsumoto et al. 1998), and because it rarely mutates in human cancer cells, it thus serves as an attractive molecular target to suppress cell growth in cancer cells. Recently, aqueous leaf extract of *S. cordifolia* was capped with silver nitrate solution to form nanoparticles (AgNPs) which showed anticancer activity against EAC and HT-29 cell lines with IC_{50} value of 204.7 and 129.3 μg/ml, respectively (Srinithya et al. 2016).

22.5.2 Anti-inflammatory Activity

As a physiological response to tissue injury produced by mechanical stress, microbial or chemical stimuli such as cyclooxygenase and lipoxygenase cause significant increase in inflammation (Swathy et al. 2010). Kanth and Diwan (1999) reported that *S. cordifolia* has mild to moderate anti-inflammatory activity at a dose of 0.6 g/kg and without ulcerogenicity effect equivalent to indomethacin, and such desirable properties require gastrointestinal safety. It was suggested that the extract of *S. cordifolia* may be acting through increasing the release of insulin by stimulating the β-cells of the pancreas.

22.5.3 Neurodegenerative Activity

Many progressive loss and degenerative functions of neuron tissues cause adverse effect on health and well-being of millions of people globally leading to diseases such as Parkinson's, Alzheimer's, schizophrenia, amyotrophic lateral sclerosis, Huntington's, and many more. *S. cordifolia* had been previously reported to be administered for neuron disorders such as facial paralysis, Parkinson's disease, and hemiplegia by the ancient Ayurveda (Nagashayana et al. 2000). Recently, emphases on exploration of herbal drugs for their antioxidative and neuroprotection potentials are on the increase. Aqueous extract and different fractions (hexane, chloroform, and aqueous) of *S. cordifolia* were evaluated against rotenone-induced histopathological, neurochemical, biochemical, and behavioral alterations in a rat model of Parkinson's disease (PD).The results indicated the therapeutic potential of these polar fractions in curing PD through antioxidative activities. A dosage of 100 mg/kg of its aqueous fractions was observed to improve biochemical, histopathological, and neurochemical performance similar to L-deprenyl drug (monoamine oxidase-B inhibitor) and protected against oxidative stress-induced dopaminergic

neurodegeneration (Khurana and Asmita, 2013). Importantly, *S. cordifolia*, containing a number of phytochemicals compounds with great pharmacological significance such as ephedrine, vasicinol, vasicine, vasicinone, asparagine, mucin, hypaphorine, phytosterol, gelatin, and ferulic acid (Pattar and Jayarai 2011; Khurana and Asmita 2013; Joseph et al. 2011), were reported to have positive activity against oxidative damage and neurodegeneration (Zhang et al. 2010).

22.5.4 Cardioprotective Activity

One of the major reasons for the regeneration of neurons is the inability of a biological system to ready detoxify imbalance production of reactive oxygen species. Swathy et al. (2010) reported that ethanol aerial and root extract of *S. cordifolia* possess effective free radical scavenging activities. *S. cordifolia* root is the major ingredient used in the treatment of neurological disorder in Ayurveda medicine, and Sutradhar et al. (2007a) validated *S. cordifolia* ethanol extract for its neurological, antioxidant, and anti-inflammatory activities and found comparable results to that of a commercial standard drug, deprenyl (Swathy et al. 2010).

22.5.5 Myocardial Infarction

Coronary diseases may lead to irreversible necrosis of heart muscles and adversely heart attack. Hence the use of alternative and complementary medicine in treatment of ailments such as myocardial infarction (MI) and other cardiac-related morbidities are gaining attention worldwide; however, many efforts were relented to provide scientific evidence on ethnopharmacology efficacy of different herbs. Histopathological observations and biochemical findings revealed therapeutic potential of hydroalcoholic leaf extract of *S. cordifolia* doses at 100 and 500 mg/kg significantly increased endogenous antioxidants superoxide dismutase (SOD) and catalase in heart tissue homogenate (HTH); this ascertains its potency and protective activity in the treatment of MI in traditional medicine (Kubavat and Asdaq 2009). Simultaneous increase in SOD and catalase are the indicators of cardioprotection (Yim et al. 1990).

22.5.6 Antibacterial Activity

The ethanolic leaf extract of *S. cordifolia* showed a higher antibacterial activity in the Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) compared to Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) with an inhibition range of 10–16 mm at 5 and 20 mg/ml concentration (Halilu et al. 2016). The

methanol leaf and root extracts of *S. cordifolia* exhibited a significant activity against *B. subtilis*, *E. coli*, *P. fluorescene, S. aureus*, and *Xanthomonas axonopodis* pv. malvacearum with an inhibition zone range of 12–18 mm at 100 µg/ml (Mahesh and Satish 2008).

22.5.7 Antifungal Activity

Acetone extract of *S. cordifolia* had activity against *Candida albicans* strains (ATCC-9002 and ATCC 2091), *C. parapsilosis*, *C. krusei*, and *C. tropicalis* with minimum inhibitory concentration range of 8.33–12.50 µg/ml (Quedraogo et al. 2012). Nune et al. (2006) reported essential oil obtained from the leaf powder of *S. cordifolia* showed strong antibacterial and antifungal activity against *Staphylococcus epidermidis*, *S. aureus*, and *C. guilliermondii* and concluded that the assay was more satisfactory against the tested fungal species. This might be partly attributed to high alkaloid content of *S. cordifolia* which are important plant defense agents against microbial and insect herbivores. It is reported that fungi are very susceptible to alkaloids compounds as they act on mucopolysaccharide capsule of pathogenic microbes which is responsible for resistance and virulence (Quedraogo et al. 2012).

22.5.8 Hypoglycemic Activity

For decades, research studies have shown that *S. cordifolia* products help to reduce weight by reducing the fat accumulation in cell tissues and lowering blood sugar (Jain et al. 2011). The methanol extract of *S. cordifolia* aerial parts at 600 mg/kg showed the maximum hypoglycemic activity and significantly decreased (31%) blood sugar level in mice after 2 h of administration (Kanth and Diwan 1999). Ahmad et al. (2014) observed the potency of alcoholic extract of *S. cordifolia* at 400 mg/kg to decrease triglycerides, total cholesterol, low density lipids, plasma urea, plasma creatinine, lipid peroxidation, and total cholesterol and significantly increased superoxide dismutase and catalase activity in diabetic rat and also protected cell membrane damages.

22.5.9 Analgesic Activity

Pharmacological studies revealed that extract of *S. cordifolia* possesses flavonoids and alkaloids with analgesic and anti-inflammatory activity (Dinda et al. 2015). Root and aerial parts of *S. cordifolia* were previously reported to possess analgesic and anti-inflammatory activities (Siddiqui et al. 2016). Kanth and Diwan (1999) observed a dose-dependent response of mice to acetic acid writhing test for

determination of analgesic properties of *S. cordifolia* aerial extract. At 100 mg/kg dose, analgesic activity was comparable with the standard, aspirin. While, at increased dose of 600 mg/kg showed a better activity than aspirin. These results indicate its ethnobotanical importance toward pain relief and inflammation.

22.5.10 Anthelmintic Activity

A bioassay was carried out to evaluate the anthelmintic activity of aqueous and ethanol extracts of *S. cordifolia* on earthworm (*Pheretima posthuma*). The results revealed that both the extracts showed the anthelmintic properties, but aqueous extract was more promising and, hence, recommended in use in folklore medicine (Pawa et al. 2011). Similarly, Nathaniel et al. (2014) stated that methanol and aqueous extracts of *S. cordifolia* exhibit the anthelmintic effect against *P. posthuma*. A chemical isolate from root of *S. cordifolia* possessed anthelmintic, antifertility, and anti-protozoan activity against *Ascaridia galli* and *Hymenolepis nana* (Harborne et al. 1998; Khare 2004), thereby validating the efficacy and relevance of *S. cordifolia* in ethnomedicine uses.

22.5.11 Antiulcer Activity

Oral dose of 500 mg/kg methanol extract (aerial part) of *S. cordifolia* showed effective anti-ulcerogenic activity against ethanol (95%) and aspirin in Wistar albino rat (Philip et al. 2008). A study conducted by Akilandeswari et al. (2013) involving albino rats induced with peptic ulcer by administration of aspirin, ethanol, and aspirin + pylorus ligation in 0.2% agar treated with 100 mg and 200 mg/kg ethanol leaf extract of *S. cordifolia* resulted in reduced acidity and gastric secretion similar to famotidine, while at 200 mg/kg high significant potency was observed greater than famotidine, indicating that the extract possessed antiulcer property against the three ulcer-causing models.

22.6 Conclusions and Future Prospects

The traditional medical practices in India, Brazil, China, and other countries use *S. cordifolia* for treating several health problems such as asthma, bronchitis, inflammation of oral mucosa, nasal congestion, rheumatism, gonorrhea, neurological disorders, and dysentery. Modern studies have witnessed the occurrence of various bioactive principles in the plant. The major compounds included ephedrine, Ψ-ephedrine vasicine, vasicinone, β-sitosterol, and stigmasterol. Also, several flavonoids and fatty acids such as arachidic acid, linoleic acid, malvalic acid, etc. were

found in their plant parts. Studies have shown that the plant possesses anticancer, anti-inflammatory, neurodegenerative, cardioprotective, antimicrobial, hypoglycemic, analgesic, anthelmintic, and antiulcer activities. Most of these biological properties could be correlated to the occurrence of wide-ranging biologically active constituents in both plant extracts and essential oil. The available literature confirms the pharmacological significance of *S. cordifolia*, and it can be a good source of natural products. More recently, plant-based compounds are much appreciated by nutraceutical and pharmaceutical industries as they are relatively safe and cause no or negligible side effects in the treated patients. It is important to note that most of the research efforts in *S. cordifolia* are limited to only plant extracts, and only few bioactive metabolites have been isolated so far. Hence, more phytochemical and metabonomic investigations using modern analytical tools such as electron ionization (EI), electrospray ionization (ESI), matrix-assisted laser desorption/ionization (MALDI), and MALDI time-of-flight mass spectrometry (MALDI-TOF MS) methods are crucially required to identify and quantify unknown compounds with biological significance from this medicinal plant. In addition, the isolated and pure compounds need to be validated for their effectiveness using both in vitro and in vivo models. The presence of some compounds such as vasicinone, a bronchodilator, authenticates its use in the traditional medicines of Ayurveda in India. However, compounds like ephedrine and cryptolepine of *S. cordifolia* still pose a great concern about its safety and require evaluation of their toxicity. Cryptolepine and plant extracts have shown a positive response against few cancer cell lines suggesting the possible use of *S. cordifolia* against cancer. Further research efforts are required to isolate and validate many such novel anticancer lead molecules occurring in the plant extracts. To date, in vivo studies related to bioactivity and toxicity using animal models are inadequate, and there is no information on the clinical aspects of *S. cordifolia* extracts or its compounds, though widely used in different traditional medicines. Importantly, *S. cordifolia* is morphologically similar to other closely associated *Sida* species and often leads to misidentification. Therefore, correct taxonomical authentication is very critical to make use of the plant for commercial purposes. Therefore, more research studies should be encouraged so as to completely utilize this medicinal herb to treat various human illnesses including cancer.

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Chapter 23
Anticancer Properties of Natural Compounds on Prostate Cancer

Priyadarshini and Abhishek Negi

23.1 Introduction

Prostate cancer (PCa) is one of the most common types of cancer in men and the sixth leading cause of cancer deaths in men (Ferlay et al. 2010). It is primarily associated with age, with more than 80% cases occurring in men above 65 years of age. Incidence rate is low in Asian and African countries with 1–9 per 1 lakh person, but it is highest in countries like New Zealand, Western and Northern Europe, and North America. Demographic and epidemiological transitions in developing countries like India have shown an increasing trend in the burden of various cancer cases including prostate cancer (Jain et al. 2014). Few of the risk factors implicated in the cause of PCa are listed in Table 23.1.

Prostate is a part of male reproductive system and is located underneath the bladder. Unlike other cancers, PCa is slow growing and can take 10–30 years before tumor is big enough to cause symptoms or doctor to find it. Standard method of screening for PCa is digital rectal exam (DRE) and blood test for prostate-specific antigen (PSA) which above 10 ng/ml is considered dangerous (Choi et al. 2017). Androgen and androgen receptor (AR) play vital role in expression of male phenotype. AR is a ligand-dependent transcription factor that binds to its native ligands, 5-α dihydrotestosterone (DTH) and testosterone, and control expression of genes that maintain growth and survival of prostate cells (Shankar and Wu 2006). DTH binds to AR (androgen receptor) with high affinity, after displacing heat shock protein and binds to importin-α that translocate AR into nucleus. There it binds to ARE (androgen response element) and control growth of prostate (Dehm and Tindall 2006; Wang et al. 2005). So progression and development of PCa depend on androgenic stimulation. Mutation in this pathway would cause uncontrolled growth of prostate leading to PCa.

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Table 23.1 Different factors responsible for prostate cancer (PCa)

Reason	Description	References
Mutation in DNA	A number of genetic alteration cause development and progression of PCa; this includes genes that have somatic mutation in sporadic PCa like AR, ATBF1, EPHB2, P53, PTEN, and RAS; genes that have germline mutation in familial PCa like MSR1, ELAC2 (HPC2); genes with altered copy number that affects gene function like MYC, CDKN1B (P27), KLF5, NIKX3	Dong (2006)
Diet	Consumption of fruits and vegetables rich in vitamins C and D, carotenoids, zinc, flavonoid helps reduce PCa risk. Diet influences production and metabolism of androgenic hormones though exact mechanism still unclear. Further obesity has a positive relation to PCa	Boileau et al. (2003)
Inheritance	Chances of developing PCa are higher in individual with positive family history. Around 12% of cases are caused by inherited mutation in several genes like DNA repair gene – BRCA1, BRCA2, ATM, and CHEK2	Von Löw et al. (2007)
Age	About two-third of the cases are diagnosed in men above age 65 years and older	Hebert et al. (1998)
Smoking	It affects circulating hormone level like increased circulation of androsterone and testosterone or through exposure to carcinogens like N-nitroso compounds	Huncharek et al. (2010)
Calcium	High level of Ca and parathyroid hormone, which regulates Ca level in the blood, promotes PCa cell growth	Skinner and Schwartz (2008)

Advancement in screening and diagnosis of the disease in early stage have allowed better therapeutic option for cure that includes surgery, radiation, and androgen deprivation therapy (castration) with gonadotropin-releasing hormone analogs and antiandrogen (Attard et al. 2006; Huggins 1946). In the case of castration, PSA level initially decreases in about 90%, but then PCa starts to progress again after 2–3 years with all hormonal manipulation (Harris et al. 2009). In this case, PCa progresses to castration-insensitive phase of disease (castration-resistant prostate cancer-CRPC) that carries worse prognosis and translates into a survival time of 16–18 months from beginning of progression. Treatment of patients with CRPC is a significant challenge.

Systemic treatment is adopted in case of cancer being spread outside prostate gland that includes androgen deprivation therapy and chemotherapy. In case of chemotherapy, multiple drugs are administered to stop growth and division of cancer cells. These treatments have risks and side effects that may prevail over the potential benefits for some men. Intensive research is being carried out to find natural compounds to combat PCa. Medicinal herbs and their derivative phytocompounds are being progressively more documented as useful alternative treatments for prostate cancer. Various clinical studies have reported the beneficial effects of herbal medicines on prostate cancer patients. Phytocompounds derived from herbal plants are safe and cause less side effects. The aim of this chapter is to provide an overview on some of the herbal plants and their compounds, which have been associated with

cancer cases and undergone a prospective clinical investigation for identifying anticancer properties either in vivo or in vitro.

23.2 Phytocompounds Derived from Herbal Plants

23.2.1 Lycopene

An acyclic isomer of β-carotene found in high amount in tomato had been extensively studied for its medicinal properties, including prevention of PCa due to its antioxidant activity (Rackley et al. 2006). In 2014, World Cancer Research Fund International reported lycopene benefits in reducing PCa in human, where high intake of dietary lycopene, largely from tomatoes, was strongly correlated with less blood vessel formation. Chen et al. (2001) studied the antioxidative effect of lycopene on PCa patient and found that oxidative damage in lymphocytes was significantly reduced along with PSA level in the blood. They reported IC_{50} values of lycopene for DU145, PC-3, and LNCaP cell line after 96 h as 26.6 μM/l, 40.3 μmol/l, and 168.5 μmol/l, respectively. Tang et al. (2005) and Obermuller-Jevic et al. (2003) reported that lycopene induced cell cycle arrest at G0/G1 phase in the DU145 and PrEC cells. Obermuller-Jevic et al. also observed that lycopene inhibited cyclin D1 expression in G0-/G1-arrested normal prostate epithelial cells (Tang et al. 2005). Lycopene at 1 μmol inhibited cell growth by 31% while 5 μmol increased the number of cells in the G(2)/M phase of the cell cycle from 13% to 28% and decreased S-phase cells from 45% to 29% in LCNaP cells. When they were treated with 5 μmol, lycopene for 24–48 h apoptosis was observed (Hwang and Bowen 2004, 2005). Inhibition of prostate cancer was observed with tomato powder treatment alone on N-methyl-N-nitrosourea induced male rats, suggesting the anticarcinogenesis activity of lycopene (Boileau et al. 2003). Though several studies have shown effect of lycopene in controlling PCa, exact mechanism of its working is still not understood.

23.2.2 Curcumin

Curcumin is the active constituent of the rhizome of *Curcuma longa* L., has both antioxidant and anti-inflammatory properties, and has been used for centuries in Ayurvedic and Chinese medicine. Basically, a polyphenolic molecule curcumin downregulates the expression of AR as well as AR-related cofactors. Co-treatment of curcumin (20, 30, or 40 μM) with androgen on LNCaP cells had significantly reduced the AR transactivation suggesting for either direct or indirect effects on AR-mediated transcriptional activities by curcumin (Nakamura et al. 2002). Singh and Aggarwal (1995) reported the anti-inflammatory effect of curcumin whose modulation could be used as an important chemoprevention. It causes proteasomal

degradation of IκBα thereby the nuclear translocation of p65 subunit ultimately causes inhibition of NF-κB activation. Their study also suggested that curcumin completely blocked phorbol esters and H_2O_2 induced activation of NF-kB. Another study on curcumin suggested its role in downregulation of cell proliferation and antiapoptotic and metastatic gene products through suppression of I κBα kinase and Akt activation (Aggarwal et al. 2005). Expression of both Bcl-2 and Bcl-xL in LNCaP and DU145 cells was downregulated by curcumin in a time-dependent manner. It also activates procaspase-3 and procaspase-8 in both LNCaP and DU145 cells (Mukhopadhyay et al. 2001). Curcumin also suppresses cyclin-D1 by inhibiting phosphorylation of retinoblastoma protein and downregulation of its mRNA expression (Mukhopadhyay et al. 2002). The role of curcumin in metastasis of prostate cancer was studied by Dorai et al. (2014) where they suggested that curcumin modulates TGF-β signaling that causes upregulation of BMP-7 which in turn enhances E-cadherin expression and inhibits metastasis. Additionally, it downregulates expression of AR and CREB (cAMP response element binding protein) and reduces the level of activated AKT kinase (Nakamura et al. 2002).

Study by Yang et al. (2017) revealed that curcumin affected the notch signaling pathway which is involved in cell proliferation, differentiation, and apoptosis. They studied the effect of curcumin on DU145 and PC3 cell line and found that it inhibited DNA-binding ability of NICD (active product of Notch signaling pathway) in DU145 cells. Curcumin was able to inhibit the migration as well as expression of MT1-MMP and MMP2 proteins in DU145 cells (Yang et al. 2017). Sha et al. (2016) also reported protective effect of curcumin by inhibiting Notch signaling and inducing apoptosis and G0/G1 arrest in DU-145. A pilot study done by Hejazi et al. (2013) reported that curcumin can confer radioprotective effect in PCa patients by reducing the severity of urinary symptoms which are common for patients undergoing radiotherapy (Hejazi et al. 2013). A positive proinflammatory and prometastatic feedback loop exist between NFκB and CXCL1/-2 that can induce metastasis-prone phenotype due to chronic inflammation in PCa cells. Curcumin by inhibiting NFκB signaling causes disruption in this feedback loop thus leading to reduced metastasis formation in vivo (Killian et al. 2013).

23.2.3 Genistein

This small natural compound is found in soy and soy products, and for long it was thought they act as estrogen agonist or antagonist in vivo. Genistein is able to inhibit serum and EGF-stimulated growth of LNCaP and DU-145 cell lines (Peterson and Barnes 1993). Changes in cell attachment are a basic stride in the metastatic course. For a cell to move to another area, it must disconnect from the ECM, to which the essential tumor is joined. Lakshman et al. (2008) showed the effect of genistein in male athymic BALB/C mice in cell detachment where it inhibited the activation of promotility proteins. Total levels of p38 MAPK and FAK were increased, while

HSP27 represented a trend. In their previous in vitro study, they reported inhibitory activity of genistein on phosphorylation of p38 MAPK, FAK, and HSP27.

Cancer cell degrades surrounding tissue for metastatic event for which matrix metalloproteinases (MMP) are used. Studies have shown genistein ability to reduce MMP-2 production by inhibition of MMP-2 mRNA expression. Along these lines, genistein particularly diminishes a subset of proteases that degrade a major segment of the prostate ECM. With increased concentration of genistein, a decreased expression level of MMP-2 which was both dose and time dependent was observed in PC-3 and LNCaP cell lines (Kumi-Diaka et al. 2006). Genistein has also shown to upregulate miR-574-3p that decreases proliferation, invasion, and migration by reducing Bcl-xL and activating caspase-9 and caspase-3 that lead to apoptosis (Chiyomaru et al. 2013). In a different study, same authors reported that genistein inhibits PCa cell growth by downregulation of oncogenic HOTAIR that is targeted by tumor suppressor miR-34a.

Combination treatment of genistein with vitamin C was more effective in apoptosis induction in LNCap cells (Famuyiwa et al. 2016). This phytocompound along with daidzein, formononetin, and biochanin A administered to patients induces apoptosis (Jarred et al. 2002). PCa patients when treated alone with genistein extract did not show any reduction in their PSA level (DeVere White et al. 2004). Effect of synthetic genistein was verified in a phase 2 study by Lazarevic et al. (2011) on PCa patients, where 23 patients were administered with 30 mg genistein while 24 with placebo. They found 7.8% decrease in PSA level within 6 weeks. In a cross-country study, it was seen that countries with more soy consumption were significantly protective against PCa mortality (Hebert et al. 1998).

23.2.4 Green Tea

For nearly thousands of years, tea has been consumed by Chinese and other Asian countries for their herbal and medicinal properties. These are prepared from a variety of plant species containing different polyphenols like green tea containing catechins such as epigallocatechin (EGC), epicatechin (EC), epigallocatechin-3-gallate (EGCG), gallocatechin (GC), and epicatechin gallate (ECG) (Naponelli et al. 2017). Several studies have demonstrated the useful impact of catechins against PCa and have demonstrated green tea as an effective natural drink to decrease PCa possibilities. EGCG accounts for around 40% of total catechin in green tea leaves, so health benefit property of this compound is a major focus of research. EGCG has major development inhibitory properties in PCa cells, with an IC50 running from 40 to 80 μM, depending upon the cell line utilized (LNCap < PNT1a < DU145 < PC3) and the length of treatment, 24–72 h (Johnson et al. 2010). These green tea catechins (GTC) activate apoptotic pathways by generating ROS and inducing oxidative stress (Siddiqui et al. 2010). They cause cell cycle arrest at G_0/G_1 phase in human PCa cells at concentration that does not cause any toxic effect in both androgen-sensitive and androgen-insensitive PCa cells (Caporali et al. 2004). ECGC causes

inhibition of cyclin-cyclin-dependent kinase (CDK) complex involved in G_0/G_1 phase by inducing G_1-phase cyclin kinase inhibitor (Gupta et al. 2003). Moreover, Hastak et al. (2003) also reported ECGC ability to induce apoptosis in LNCaP cells by p53 stabilization through specific phosphorylation and downregulation of MDM2 protein by p14ARF tumor suppressor. Also, it downregulates NFκB activity, which results in reduced BcL2 family protein. This p53 stabilization causes overexpression of WAF1/p21 and BAX thereby shifting the balance between pro-/antiapoptotic proteins in favor of apoptosis (Hastak et al. 2003). ECGC at concentration around 10–20 μM range has shown to suppress PSA expression, AR transcription, and cell proliferation activity. The impact on PSA expression may be related to reduction of AR activity, but it should also be considered that EGCG, in vitro, can downregulate PSA by direct action on transcription and translation mechanisms (Pezzato et al. 2004).

Polyphenon E which consists of high percentage of catechins, about 56–72% as EGCG, and meager percentage of caffeine was utilized in a randomized, double-blind, placebo controlled trial in men with prostate tumor planned to undergo radical prostatectomy. This investigation found a pattern among systemic biomarkers that may propose chemopreventive action of Polyphenon E in prostate malignancy; however none of these were statistically significant (Nguyen et al. 2012). Polyphenon E when studied on PCa cell lines (Rizzi et al. 2013) revealed that cell cycle was inhibited at G0/G1 (PNT1a cells) and at G2/M (PC3) and stress was induced in ER. They further reported that p21 was upregulated and cyclinD1 was downregulated.

23.2.5 Pomegranate

Punica granatum L. has been used for several system of medicine for variety of ailments. Previously intensive examinations on the anticarcinogenic, anti-inflammatory, and antioxidant properties of pomegranate constituents have been done focusing on prevention and treatment of tumor like PCa. Some of the therapeutically beneficial components of pomegranate include ellagic acid, ellagitannins, anthocyanidins, anthocyanins, flavonoids, punicic acid, and estrogenic flavonols and flavones with ellagic acid being major component around 40% (Yilmaz 2010). The effects of pomegranate cold-pressed (oil) or supercritical CO(2)-extracted (S) seed oil, fermented juice polyphenols (W), and pericarp polyphenols (P) on DU 145, PC-3, and LNCaP cell lines exhibited proliferation inhibition (Albrecht et al. 2004).

While in a different study on androgen-insensitive PC3 cells, it was observed that pomegranate fruit juice, containing ellagic acid among others, causes apoptosis and cell growth inhibition in a dose-dependent manner. It was due to modulation of cyclin kinase inhibitor-cyclin-cdk machinery caused by induction of Bak and Bax and downregulation of Bcl-X_L and Bcl-2 (Malik and Mukhtar 2006). Further the study done by Malik et al. (2005) on mice containing androgen-sensitive cells established the involvement of cyclin kinase inhibitor cyclin-cdk network during the

antiproliferative effect of pomegranate fruit extract. Clinical trial of pomegranate juice done by Pantuck et al. (2006) and Paller et al. (2012) showed prolongation of PSA doubling time.

23.2.6 Ginger

Bioactive phenolic compounds like gingerols (GE) and shogaols (SH) present in ginger is known for its medicinal properties against cancer and angiogenesis and inflammatory effect (Katiyar et al. 1996; Surh 2002; Karna et al. 2012). Further its root contains high amount of potassium and manganese that help to maintain normal blood circulation and absorption of vitamins and minerals. Karna et al. (2012) showed gingerol-6 and gingerol-8 inhibit cell cycle progression, and its long-term exposure (72 h) at low dose (50 μg/ml) causes arrest at G2/M and apoptosis in around 50% PC3cells. They also reported that GE affect mitochondrial integrity and found cytochrome c, a pro-apoptotic factor, in the cytoplasmic fraction of LNCaP cells. They analyzed its antiproliferative activity against LNCaP, PC3, C4-2, C4-2B, and DU145 cell lines by treating them for 72 h and found that in all the cell line, cellular proliferation was inhibited.

Saha et al. (2014) conducted in vitro and in vivo study to demonstrate 6-shoganol efficacy against PCa. Using LNCaP, PC3, and DU145 cells, they reported that 6-SHO not just reduce IL-6-induced STAT3 activation and inhibit TNF-α-induced-NF-κB activity but also their target gene like cyclin-D1 survivin and cMyc. They further reported that 6-SHO modulates mRNA levels of BAX, IL-7, BCL-2, p21, and p27 that lead to cell cycle arrest and apoptosis. Though phytochemicals present in ginger have shown positive result in vitro, but since they exist in their non-active glucuronide or sulfate metabolites form, their affectivity in vivo is not similar (Karna et al. 2012). Also much more pharmacokinetic studies need to be conducted on this phytochemical before any conclusion on dose extrapolation.

23.2.7 Nimbolide

Neem has been used in Ayurvedic medicine for thousands of years, and the ancient Vedas refers neem as a tree capable of curing every illness. It has well-established benefits for skin and is commonly used in personal care products. Nimbolide (NL), a terpenoid limonoid bioactive compound of neem, has the ability to reduce prostate tumor size by decreasing Ki-67 expression and increasing caspase-3 level. As evident from Zhang et al. (2016) study it also inhibit p-STAT3 expression when given orally to TRAMP mice for 3 months and found no side effect and reduced metastasis to the lung and liver. In their in vitro study on DU145 and LNCaP cells, they found NL reduces the expression of metastasis gene, MMP-9, and ICAM1, thus decreasing invasion potential of PCa cells. They also reported role of NL in

suppressing JAK1 and JAK2 activity that in turn affects STAT3 activation. Although prostate cancer is one of the commonly diagnosed cancers worldwide, its available treatments for metastatic prostate cancer are just barely successful, and so new alternative treatments are needed to enhance patient outcomes. Raja Singh et al. (2013) in their study found that NL can act as potent anticancer agent by inducing apoptosis and inhibition of cell cycle by modulating PI3K/Akt pathways. NL suppresses IKK-induced NF-κB pathway thereby inhibiting growth in PC3 cells. Increase in mRNA and protein expression for caspase-8, caspase-9, caspase-10, and caspase-3, Fas ligand, Fas-associated death domain receptor (FADDR), Bax, Bad, and IGF-binding protein 3 due to NL treatment was also reported by them. Similar type of results was obtained in Singh et al. (2016) study where they established that NL decreases the expression level for Grb2, SODD, TNF-α, SOS mRNA, and modulated MAPK and NF-κB signaling molecules in PC-3 cells. Using neem leaves extract, Wu et al. (2014) showed its antitumor activity on LNCaP-luc-2 cell where bioactive compounds present in it suppress AR expression, PSA level, integrin β1, and calreticulin level. Upon oral administration of this extract to mice, reduced PSA level and increased AKR1C2 level were seen.

23.2.8 Quercetin

It is a naturally occurring flavonoid present abundantly in apple, onion, red wine, and tea and possesses antioxidant and antimutagenic effect. In order to check quercetin efficacy in inhibiting AR protein expression, Xing et al. (2001) administered LNCaP cells with quercetin and found reduced level of AR protein. Further, they measure PSA and hK2 amount to ascertain that quercetin blocks androgen action, which regulates growth, differentiation, and survival of normal prostate. They also reported that quercetin could downregulate NKX3.1 and ornithine decarboxylase at transcriptional level. In a separate study, quercetin has shown to inhibit proliferation in PC3 cells by downregulating level of cyclin-D and E, CDK2, and cdc25c and upregulating p21, p53, and p18. Quercetin stimulated ATF, GRP78, and GADD153 expression which are ER stress hall marker protein. Quercetin causes cell apoptosis by affecting mitochondrial function that leads to elevated production of ROS and triggering activation of caspase-8, caspase-9, and caspase-3 (Liu et al. 2012). In order to check quercetin efficacy alone and in combination with 2-methoxyestradiol (ME) for reducing tumor growth in vivo, Yang et al. (2015) xenografted LNCaP and PC3 cells in mice. They found that combination of both compounds decreases tumor growth more compared to their individual dosage and had no toxic reaction. Quercetin and 2-ME cause higher Bax/Bcl-2 and caspase-3 level that leads to apoptosis and reduce tumor growth. Quercetin was exhibited to prevent N-methyl-N-nitrosourea (MNU) and testosterone-induced prostate cancer on both ventral and dorsolateral lobes of prostate of rat model (Sharmila et al. 2013).

23.2.9 Resveratrol

Resveratrol is a naturally occurring polyphenol known for its cardioprotection and anticancer properties. It is found in red grape and is proposed as potential prostate cancer chemopreventive agent. Sirtuin 1 and phosphodiesterase are two of the direct targets for resveratrol. Taniguchi et al. (2016) conducted a study in order to identify more target of resveratrol, and they found 11 novel targets, of which one was DDX5, also called p68. DDX5 degradation suppresses mTORC1 signaling that finally results in apoptosis and reduces cancer growth. They also reported that resveratrol suppresses metastasis by downregulating mRNA expression of TGM4 and induces apoptosis by degrading PrLZ, an apoptosis-suppressor gene.

In yet another study to check resveratrol efficacy, Harper et al. (2007) administered TRAMP (transgenic adenocarcinoma mouse prostate) mice with 625 mg/kg resveratrol in diet daily and observed tumor formation in the urogenital tract of mice. They performed biopsy after 28 weeks and found mice with resveratrol diet had significantly reduced cancer grade and poorly differentiated tumors indicating its ability to slow tumor growth. In a similar study, TRAP rats were administered daily for 7 weeks with 100 μg/ml and 200 μg/ml of resveratrol. Authors reported that resveratrol downregulates AR pathway as well as androgen-responsive gene GK11. Level of AR protein was low along with neoplastic lesion showing resveratrol ability as chemopreventive and combat PCa (Seeni et al. 2008). Zinc, an essential element involved in cell signaling, is lost from the cells in PCa due to metabolic transformation of cells. In a combination study of zinc with resveratrol on typical human prostate epithelial cells, an increase in intracellular-free labile zinc and total cellular zinc status was observed along with ROS production and senescence (Zhang et al. 2009). With limited clinical evidence, resveratrol can be considered a viable option for cancer prevention but not a preferred option for treatment. Further some studies have shown evidence of its dose-dependent side effect of nausea, fatigue, and renal toxicity. Additional work is needed to be done in this area.

23.2.10 Biochanin

Biochanin is a flavonoid found in soy, peanuts, red clover, and other legumes. Biochanin along with genistein is known as potent inhibitor of 5α-reductase activity, responsible for the conversion of testosterone to DHT that has five times more affinity for prostate androgen compared to testosterone (Anderson and Liao 1968; Campbell and Kurzer 1993). Apart from that biochanin is also proven to be a moderate inhibitor of aromatase activity, enzyme found in the muscle and fat cells responsible for estrone production from androstenedione (Campbell and Kurzer 1993). In a study it was found that biochanin enhances tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis in cancer cell line DU145 and LNCaP. It causes inhibition of NF-κB (p65) activity, disrupted mitochondrial

membrane potential, and enhanced the expression of death receptor TRAIL-R2 (DR5) (Szliszka et al. 2013). Peterson and Barnes (1993) demonstrated biochanin-A effectivity as a chemopreventive agent that hinders both EGF- and serum-stimulated development in DU-145 and LNCaP cell lines. Biochanin is also able to stimulate UDP-glucuronosyltransferase activity that in turn causes accumulation of testosterone as glucuronidated metabolites in prostate cells and causes decrease of PSA, i.e., downregulation of the effect on androgen on the prostate (Sun et al. 1998; Akiyama et al. 1987). Study by Seo et al. (2011) exhibited biochanin upregulating p21 expression that causes inhibition of PLK-1 transcription and promotes apoptosis in LNCaP and PC3 cells.

23.2.11 Vitamins

These are essentially required for normal physiology and metabolic function of the body. Vitamins are thought to play a possibly critical part in the avoidance of several diseases. For instance, vitamin A may hinder tumor advancement and carotenoids and vitamins C and E might be defensive in light of cancer prevention owing to their antioxidant property. Vitamin C may prevent the development of cancer-causing agents from precursor compounds (Schuurman et al. 2002). These carotenoids and retinoids can increase the activity of several enzymes involved with antioxidant and detoxification function. They increase detoxifying enzymes by activating Nrf2 transcription factor and its binding to antioxidant response factor (ARE) of detoxifying gene. β-carotene acts on caveolin-1 and induces apoptosis in PCa cells. Several studies have demonstrated its apoptotic effect on several cell lines by disrupting mitochondrial function, inducing caspase cascade, and altering proteins involved in apoptosis such as FAS, Bcl-2, Bad, and Bcl-xL (Donkena et al. 2010).

In a study it was found that increase intake of vit-A and β-carotene in diet or as supplement could protect against development of PCa (Paganini et al. 1987). A pilot study was done on 29,133 male smokers to evaluate β-carotene (precursor of vitamin A) and α-tocopherol (vitamin E) efficacy in reducing PCa chances. Patients receiving α-tocopherol with β-carotene supplementation were less prone to cancer compared to patients receiving no medication (Heinonen et al. 1998). Studies have confirmed importance of retinoid in normal prostate growth and differentiation. Retinoids inhibit AR function, and so ALDH1A2 gene, involved in retinoid synthesis, is viewed as tumor suppressor gene (Kim et al. 2005a, b).

Hultdin et al. (2004) has associated vitamin B, which plays an important part in DNA repair, synthesis, and methylation, to increase PCa risk on high amount in plasma. While in a separate cohort study by Schuurman et al. (2002) on intake of retinol, vitamins C and E, and carotenoids against PCa risk, they found that β-cryptoxanthin intake could increase PCa risk while retinol, β-carotene, and α-carotene reduce the risk of PCa. As for vitamin D, it has been shown that it may

cause cellular dysfunction in PCa cells by altering gene expression and ultimately inhibiting cell proliferation, invasion, and angiogenesis. It increases the expression of IGFBP-3 gene which in turn increases cell cycle inhibitor protein p21. Also it inhibits prostaglandin expression by inhibiting COX-2, inducing expression of 15-PGDH, and reducing expression of FPPG receptors. Apart from inhibiting prostaglandin, vitamin D has shown to inactivate stress-activated kinase p-38, a procarcinogen and mediator of inflammation (Krishnan et al. 2007). During Selenium and Vitamin E Cancer Prevention Trial (SELECT), no significant reduction in danger of prostate tumor with either selenium or vitamin E supplements was found; however with continuous consumption of vitamin E supplement, a nonstatistically critical increment in prostate cancer risk was observed (Lippman et al. 2009). Since studies till date on vitamins have shown their entire positive, negative, and null effect in PCa, it has become complicated and problematic to consider them for prevention and cure. Research need to be continued using more advance technology and knowledge to verify their role in prostate cancer.

23.2.12 Herbal Blends

Herbal formulation consists of one or more herbs in a specified quantity to provide medicinal benefit against numerous diseases to human kind. Traditional medicinal systems like Ayurveda make use of herbs existing in nature and formulate herbal remedies making use of several herbal extracts enhancing their overall activity against the disease. These combinations are assumed to help improve immunity and strength. Cancer is a complex disease caused by several heterogenous factors and occurs in multistage. The use of single-target drug may not be effective for its treatment, while combination therapy contains multiple natural phytocompounds that can counteract different biological pathways that get activated and help cancer progression. The herbs for PCa help stop growth of cell abnormally and help regenerate normal healthy body cell (Table 23.2).

Table 23.2 Herbal formulation available for the treatment of prostate cancer

Formulation	Compounds	References
PC-SPES	Chrysanthemum, dyer's woad, liquorice, reishi, san-qi ginseng, rubescens, saw palmetto, and Baikal skullcap	Small et al. (2000)
Zyflamend	Holy basil, turmeric, ginger, green tea, rosemary, Hu Zhang, Chinese gold thread, barberry, oregano, and skullcap	Yang et al. (2007)
Equiguard	*Herba epimedii*, *Fructus rosae laevigatae*, *Fructus rubi*, *Fructus psoraleae*, *Radix morindae officinalis*, *Semen cuscutae*, *Fructus ligustri lucidi*, *Fructus schisandrae*, and *Radix astragali*	Hsieh and Wu (2007)
Pomi-T	*Pomegranate*, *green tea*, *broccoli*, and *turmeric*	Thomas et al. (2014)

23.2.13 PC-SPES

This Chinese herbal blend is a mixture of eight herbs and used worldwide against PCa since mid-1990s. In PC-SPES, PC stands for prostate cancer, and SPES is a Latin word for trust. All these eight herbs were selected based on their individual efficacy against tumor or other urinary problems. Combination of phytocompounds namely baicalein, oridonin, ginsenosides, and β-D-glucan in PC-SPES have different anticancer activities. Several studies indicate its ability to suppress PCa growth in animal models. Study by Hsien et al. (1998) confirmed that PC-SPES caused downregulation of Bcl-6 gene expression along with apoptosis in Mutu 1 cells. PC-SPES also induces apoptosis, modulates cell cycle, inhibits proliferation cells by downregulating bcl-2 and bcl-6 level, and upregulates p53, bax, and p21 protein (Chenn 2001). Baicalein, one of the major components of PC-SPES, has shown to suppress activation of caveolin-1 and AKT and mTOR by inhibiting its phosphorylation that causes apoptosis and decreases migration and invasion in DU145 and PC3 cells (Guo et al. 2015). Oridonin, another important component of PC-SPES, has shown to induce G2/M cell cycle arrest and apoptosis by upregulating p53 and 21 and downregulating cyclin-dependent kinase 1. It also increases caspase-3 and caspase-9 levels. Further Oridonin also inhibited the expression of PI3Kp85 subunit and phosphorylation of AKT (Lu et al. 2017). In an in vivo study on rat conducted by Tiwari et al. (1999), they showed that high dose of PC-SPES could reduce tumor growth and metastasis. In a clinical trial, Small et al. (2000) check PC-SPES efficacy on 70 patients and found decrease in PSA level by 80% in 32 patients (androgen-dependent) and 50% in 19 (androgen-independent) patients after 57 weeks of PC-SPES administration. In a separate study, baicalin and oridonin of PC-SPES were shown to cause antiproliferative action on CaP cell lines (Marks et al. 2002). Even though PC-SPES is a natural product extract its consumption has been associated with few side effects such as gynecomastia, nipple tenderness, loss of libido and impotency. Cases of bleeding diathesis have also been reported with use of PC-SPES.

23.2.14 Zyflamend

It's a herbal supplement consisting of ten herbal compound extracts and available in the market claiming to have anti-inflammatory and antiaging effect. In a clinical trial to check safety and efficacy of zyflamend against PCa, it was given along with dietary supplements to 23 patients for 18 months after which the PSA level was found to be reduced by 50%. Further it was noted there were no significant changes in blood chemistries and a decrease in level of NF-kappaB and serum C-reactive protein was observed (Capodice et al. 2009). Preliminary studies suggest it has

anti-inflammatory, anti-angiogenic, and antiproliferative properties. Ingredients in zyflamend modulate tumor cell eicosanoid metabolism and have property to inhibit cyclooxygenase-2 (COX-2) activity (Yang et al. 2007) which are involved in formation of prostanoids, so they inhibit inflammation and prevent phosphorylation of retinoblastoma protein.

Study related with zyflamend and prostate cancer, revealed decrease in COX-1 and COX-2 activity, cell growth suppression with elevated p21 expression, and apoptosis induction with the upregulation of caspase-3 activity (Bemis et al. 2005). Zyflamend could increase the cytotoxic effect of chemotherapeutic agent like Taxol, bicalutamide, gemcitabine, doxorubicin, etc. (Sandur et al. 2007; Kunnumakkara et al. 2011; Yan et al. 2011). Studies have shown zyflamend ability to inhibit histone deacetylase-5 and AR-dependent tumor growth in murine xenograft model, which is considered as biomarker linked with PCa growth (Huang et al. 2012). In order to demonstrate zyflamend activity in inhibiting lipid synthesis, Zhao et al. (2015) investigated its effect on AMPK activation. They reported that zyflamend increases phosphorylation of AMPK, whose activation causes inhibition of fatty acid synthesis. In addition, it also inhibited the activity of mTORC1 activity via AMPK, by phosphorylating its regulatory protein, raptor.

23.2.15 Equiguard

This Chinese herbal supplement helps in maintaining proper functioning of the kidney, by maintaining balance of the entire urological system. These nine Chinese herb supplements had shown effective growth-inhibiting property against both LNCaP and PC-3 cell lines along with decrease in PSA and AR expression level. Hsieh and Wu (2007) in order to find equiguard working mechanism conducted a study using androgen-independent CWR22Rv1 cells. They found equiguard suppressed colony formation by inhibiting cell proliferation and reducing cell cycle regulatory protein cyclin D1 level. Further it lowers PSA and AR level along with decrease in cyclooxygenase 2 which in turn causes increase in quinine reductase 1 and 2 level. In a separate study, conducted by Lu et al. (2004) on LNCaP cells, similar antitumor effect was illustrated with growth suppression and lower expression of prostate-specific genes. They performed western blot analysis and found inhibition of Rb phosphorylation along with reduction in cyclin D/E expression. They also found that cells treated with equiguard and androgen showed higher expression of Kip/p27, a cyclin-dependent kinase inhibitor and cytochrome c in cytoplasm. Equiguard is available in the market as capsule formulation under USP c-GMP guidelines and has shown a sustained increase in recent years owing to some encouraging clinical and preclinical result.

23.2.16 Pomi-T

Pomi-T is a raw dry powder herbal supplement product of four different plants (Table 23.2). It has a diverse polyphenol profile, demonstrated in their individual laboratory or phase 11 trials showing antioxidant, antiproliferative activity that's proven beneficial against cancer, and these are blended together with the idea that it would have a synergistic action against cancer. Thomas et al. (2014) evaluated Pomi-T effect on 199 men having prostate cancer by administering them with oral dose of Pomi-T. After 6 months, it was observed that there was a median increase of 14.7% in PSA level of patients. Their study relied on PSA level only, and performing other formal indicators of disease progression like biopsy could have given a better understanding of its effect and they tested the supplement for a short period of 6 month. More clinical tests need to be conducted before we can conclude its efficacy against PCa.

23.3 Conclusions and Future Prospects

For centuries, various medicinal plants have been used by folklores as medicines and disease therapeutics in most human cultures. Various phytocompounds have been studied regarding their efficacy on curative role on PCa, but small numbers of experimental studies have been offered for their development as chemopreventives. These phytocompounds can slow the progression of existing prostate cancer cells, so they may be effective as an add-on to surgery, radiation, hormone, or chemotherapy. Moreover, bioavailability of phytocompounds for the prostate and systemic and prostatic metabolism is poorly studied. For preventive treatments of PCa from promising phytocompounds, it is necessary to utilize reproducible preclinical data to validate clinical trials. Though, studies on some phytocompounds have been tested in limited population with inconclusive clinical trials, synchronized and detailed studies are required for the development of chemopreventive agents against prostate cancer.

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Chapter 24
Phytochemicals Against Cancer Stem Cells

Kok Hoong Leong, Kin Weng Kong, and Lip Yong Chung

Abbreviations

ABC	ATP-binding cassette
ATP	Adenosine triphosphate
Bax	Bcl-2-associated X
Bcl-2	B-cell lymphoma 2
Bmi-1	B-cell-specific Moloney murine leukaemia virus integration site 1
BNIP3	Adenovirus E1B 19 kDa protein-interacting protein 3
BRCA1/2	Breast cancer susceptibility gene 1/2
CSCs	Cancer stem cells
DAPK2	Death-associated kinase 2
EGCG	(-)-Epigallocatechin-3-gallate
EGF	Epidermal growth factor
EMT	Epithelial-mesenchymal transition
ER	Oestrogen receptor
Erk 1/2	Extracellular signal-regulated kinases 1/2
Ezh2	Enhancer of zeste homolog 2
FAS	Fatty acid synthase

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Gli	Nuclear glioma-associated oncogene
GSK-3α/3β	Glycogen synthase kinase-3α/3β
HBP1	HMG-box transcription factor
Hh	Hedgehog
HIF-1α	Hypoxia-inducible factor-1α
IAP	Inhibitors of apoptosis
JNK1 and 2	c-Jun N-terminal kinase 1 and 2
MAPK	Mitogen-activated protein kinase
miR	Micro-ribonucleic acid
mRNA	Messenger ribonucleic acid
Nestin	Neuroectodermal stem cell marker
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NOD/SCID	Nonobese diabetic/severe combined immunodeficiency
NSCLC	Non-small cell lung carcinomas
Oct4	Octamer-binding transcription factor 4
p53	Tumour protein 53
PBR	Peripheral benzodiazepine receptor
PDGF	Platelet-derived growth factor
PI3K	Phosphoinositide 3-kinase
PKG-1	cGMP-dependent protein kinase-1
PTCH	Patched homolog transmembrane receptor protein
PTEN	Phosphatase and tensin homologue deleted on chromosome
Rac1	Ras-related C3 botulinum toxin substrate 1
ROS	Reactive oxygen species
sFRP2	Secreted frizzled protein 2
Smo	Smoothened G-protein-coupled receptor-like protein
Snail	Zinc finger protein SNAI1
STAT3	Signal transducer and activator of transcription 3
TGF-β1	Transforming growth factor-β1
TRAIL	Tumor necrosis factor-related apoptosis-inducing ligand
Twist1	Twist-related protein 1
VASP	Vasodilator-stimulated phosphoprotein
VEGF-D	Vascular endothelial growth factor D
VEGR	Vascular endothelial growth receptor
WIF-1	Wnt inhibitory factor
XIAP	X-linked inhibitor of apoptosis
ZEB	Zinc finger E-box-binding homeobox

24.1 Introduction

In the past decade, increasing scientific evidence on cancer stem cells (CSCs) have improved our understanding of how cancer develops, resists treatment and contributes to recurrence and metastasis. Advancements in cancer biology have shed light on the complexity of cancer, where its diversity due to genetic and epigenetic heterogeneity in a tumour is just part of the overall picture (Boesch et al. 2016; Zhao

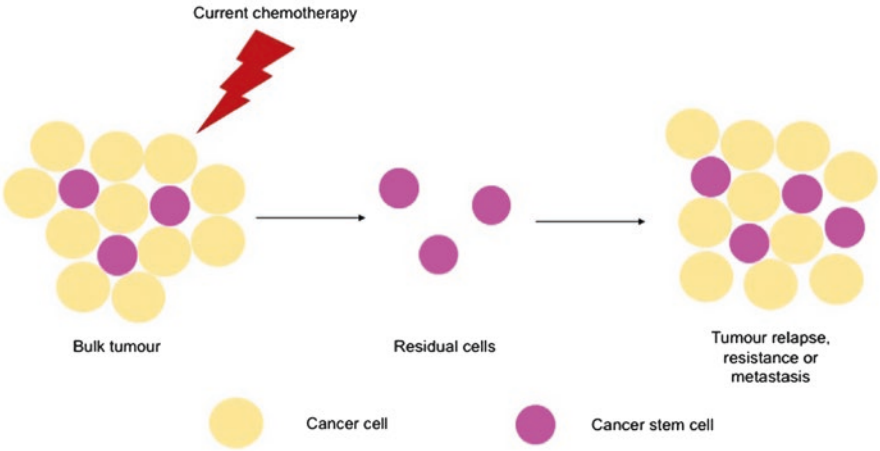

Fig. 24.1 The role of cancer stem cells in tumour relapse, resistance and metastasis

2016). In 1994, the existence of CSCs was first reported in acute myeloid leukaemia. It was initially thought to exist only in leukaemia, but the idea of CSCs gained traction a decade later as the evidence of its existence in various solid tumours emerged (Islam et al. 2015). The discovery of CSCs having unique properties such as capacity for self-renewal, differentiation and migration evokes their potential as a therapeutic target for cancer treatment (Yi et al. 2013; Wang et al. 2017). Unlike normal cancer cells that proliferate rapidly, CSCs are quiescent or divide slowly and pluripotent, giving rise to different types of cells. These traits are similar to stem cells, which suggest conventional chemotherapy that targets actively proliferating cancer cells may be inadequate to completely eradicate CSCs (Sotiropoulou et al. 2014). As a consequence, the residual CSCs can cause reconstitution of the parental tumour giving rise to relapse in cancer patient (Fig. 24.1) (Cojoc et al. 2015). In a clinical trial, it was found that acute myeloid leukaemia patients with higher fraction of leukaemia stem cells in their blood samples have a lower survival rate within a 5 years period (Eppert et al. 2011). Recapitulation of parental tumour was demonstrated in animal studies, where transplantation of a low number of CSCs (about 200 cells) can effectively cause tumour formation in mouse (Al-Hajj et al. 2003). The tumourigenic and drug-resistant properties of CSCs are of major concern to improve treatment outcome of cancer patients. Conventional anticancer therapies that treat cancer as homogenous population of malignant cells seem to be insufficient. Therefore, efforts to discover new compounds and therapeutic strategies to selectively eradicate cancer stem cells are currently of immense interest (Chen et al. 2013).

In drug discovery, natural products have been targeted as a source to develop the new drugs. According to an estimate, ~50% of the current anticancer drugs are derived from natural products or their derivatives (Newman and Cragg 2012). The paradigm shifts in targeting CSCs to improve cancer therapy have seen an increase in scientific interest on the search of novel active phytochemicals against CSCs. These phytochemicals can be broadly grouped according to the plant source: dietary

and non-dietary plants. The scientific community expands their efforts in studying phytochemicals against CSCs and understanding of the mechanisms of action against CSCs (Burnett et al. 2012; Leis et al. 2013). A better insight on the molecular mechanisms reveals potential therapeutic targets against CSCs, which broadly include modulating the self-renewal and stemness pathway, restoring defective apoptosis mechanism, regulation of drug efflux transporters, dampening survival pathways and others (Cerella et al. 2014; Sagrera et al. 2009). Thus, the aim of this chapter is to provide an overview of various phytochemicals derived from dietary and non-dietary plants to combat against the CSCs in cancer relapse and chemoresistance.

24.2 Dietary Phytochemicals Against Cancer Stem Cells

There are various dietary phytochemicals reported to be effective against various types of CSCs in both in vitro and in vivo models (Fig. 24.2; Table 24.1) (Fong and Chan 2013; Pistollato et al. 2015). The profound interest in using dietary phytochemicals stems from their wide availability, and they are generally regarded as safe as they are derived from food products. Moreover, they can be taken on a long-term basis to prevent relapse and as an adjuvant to support existing chemotherapy. The recent findings of selected dietary phytochemicals and their potential therapeutic target sites are summarized in Fig. 24.3. Importantly, mechanistic interrogation of these phytochemicals shows multi-target action against CSCs, where developing resistance toward them will be more challenging (Dhar et al. 2012; Pistollato et al. 2015).

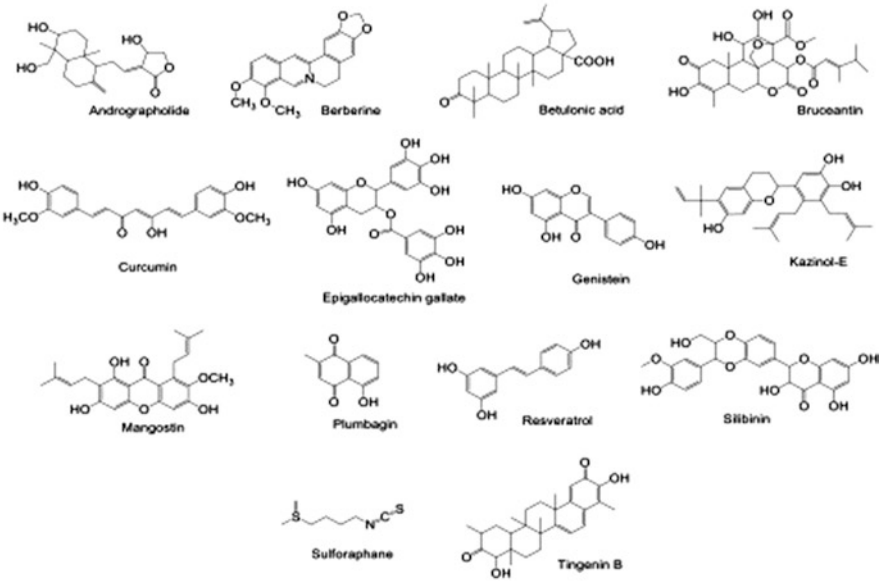

Fig. 24.2 Structures of phytochemicals against cancer stem cells

Table 24.1 Summary of various phytochemicals against CSCs in in vitro and in vivo models

Phytochemical	Family	Plants	Common name	In vitro model	In vivo model
Andrographolide	Acanthaceae	*Andrographis paniculata*	Green chirayta, creat, king of bitters, India echinacea	Human: oral carcinoma	Human oral carcinoma cells in BALB/c *nu/nu* mice
Berberine	Berberidaceae	*Berberis vulgaris*	Barberry	Mouse: neuroblastoma Human: neuroblastoma	–
Betulonic acid	Meliaceae	*Walsura pinnata*	Lantupak mata kucing	Human: acute myeloblastic leukaemia	Human acute myeloblastic leukaemia cells in zebrafish
Bruceantin	Simaroubaceae	*Brucea antidysenterica*	–	Human: multiple myeloma	Human multiple myeloma in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice
Curcumin	Zingiberaceae	*Curcuma longa*	Turmeric	Mouse: oesophageal adenocarcinoma Rat: C6 glioma Human: oesophageal squamous carcinoma, breast adenocarcinoma, colorectal carcinoma, medulloblastoma, prostate carcinoma, oesophageal and squamous carcinoma	Transgenic mouse prostate adenocarcinoma in C57BL6/J mice and human breast adenocarcinoma cells in BALB/c mice
Epigallocatechin-3-gallate	Theaceae	*Camellia sinensis*	Tea plant	Mouse: transgenic mouse prostate adenocarcinoma Human: NSCLC, colorectal carcinoma, breast adenocarcinoma, invasive breast ductal carcinoma, oral squamous carcinoma, prostate adenocarcinoma, pancreatic carcinoma, squamous carcinoma, head and neck squamous carcinoma and glioblastoma	Human invasive breast ductal carcinoma cells in NOD/SCID mice, human oral, head and neck squamous carcinoma cells in BALB/c *nu/nu* mice

(continued)

Table 24.1 (continued)

Phytochemical	Family	Plants	Common name	In vitro model	In vivo model
Genistein	Leguminosae	*Glycine max*	Soybean	Mouse: mammary epithelial, transgenic mouse prostate adenocarcinoma Human: prostate adenocarcinoma, colorectal carcinoma, prostate carcinoma, ovarian adenocarcinoma and breast adenocarcinoma	Transgenic mouse prostate adenocarcinoma in C57BL6/J mice, human mammary epithelial cells in Sprague-Dawley rat and human prostate carcinoma in BALB/c mice
Kazinol-E	Moraceae	*Broussonetia kazinoki*	Paper mulberry	Human: breast adenocarcinoma	–
Mangostin	Guttiferae	*Garcinia mangostana*	Mangosteen	Human: pancreatic epithelioid carcinoma, prostate adenocarcinoma, pancreas adenocarcinoma, NSCLC, breast adenocarcinoma, colorectal carcinoma and acute myeloblastic leukaemia	Human pancreas and breast adenocarcinoma cells in BALB/c mice, 1,2-dimethylhydrazine-induced colon cancer in F344 rats and human acute myeloblastic leukaemia cells in zebrafish
Plumbagin	Plumbaginaceae	*Plumbago zeylanica*	Ceylon leadwort, doctorbush, wild leadwort	Human: prostate adenocarcinoma	–
Resveratrol	Polygonaceae	*Polygonum cuspidatum*	Knot weed, knot grass, bistort, tearthumb, mile-a-minute and smartweed	Human: head and neck cancer, colorectal carcinoma, breast adenocarcinoma, pancreatic carcinoma and adenocarcinoma, acute myeloid leukaemia, glioma, nasopharyngeal carcinoma, acute lymphoblastic leukaemia, Waldenstrom's macroglobulinemia, medulloblastoma, atypical teratoid/rhabdoid tumour, glioblastoma, prostate adenocarcinoma, non-small cell lung cancer (NSCLC) and ovarian carcinoma	Human glioma cells in BALB/cAJcl-*nu/nu* mice, human nasopharyngeal carcinoma, glioma, atypical teratoid/rhabdoid tumour and breast adenocarcinoma cells in NOD/SCID mice, azoxymethane carcinogen induced A/J mice and human prostate adenocarcinoma cells in BALB/cAnN.Cg-Foxnlnu/CrlNarl

Silibinin	Asteraceae	*Silybum marianum*	Milk thistle	Human: NSCLC and glioblastoma	-
Sulforaphane	Brassicaceae	*Brassica* species	Broccoli, Japanese mustard spinach, cabbage, Ethiopian rape	Human: breast adenocarcinoma, cervical carcinoma, pancreatic carcinoma, prostate adenocarcinoma, ovarian adenocarcinoma and gastric carcinoma	Human breast adenocarcinoma in NOD/SCID, human pancreatic carcinoma cells in NOD/SCID and NMRI *nu/nu* mice and colorectal cancer $APC^{Min/+}$ mice
Tigenin B	Celastraceae	*Maytenus ilicifolia*	Espinheira Santa	Human: breast adenocarcinoma	–

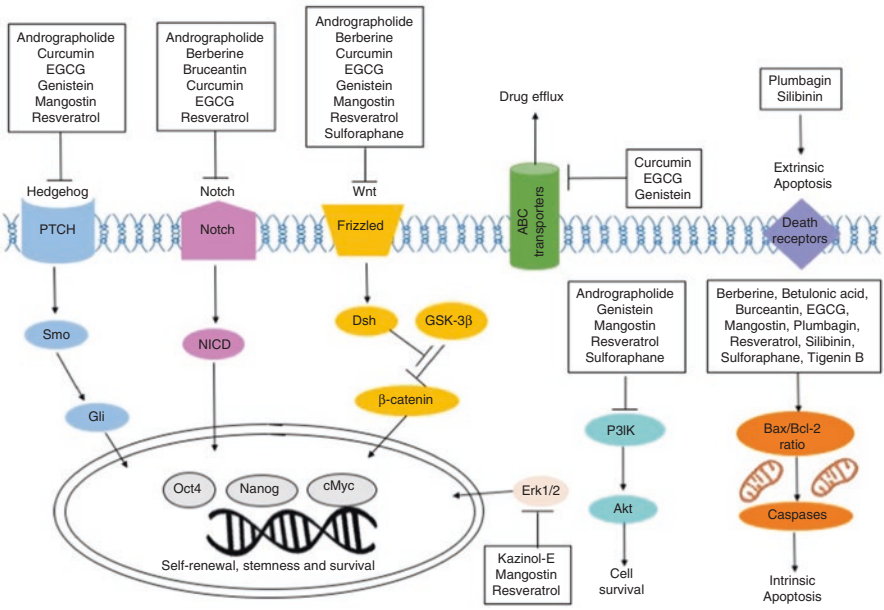

Fig. 24.3 Simplified mechanism of action of various phytochemicals against CSCs. → represents activation and —| inhibition

24.2.1 Curcumin

Curcumin is a well-studied dietary polyphenol, a flavonoid found in the rhizomes of turmeric (*Curcuma longa*) of the ginger family (Zingiberaceae), an Indian spice, commonly used in curry and preparation of mustard. It is also used in Indian folk medicine to treat dyspepsia, flatulence, infections, common colds and skin diseases (Li and Zhang 2014). Its effectiveness against various types of cancer has spurred interest on whether it is also effective against CSCs (Epstein et al. 2010). It has been demonstrated to be active against several types of cancer stem cells, for example, glioblastoma (Fong and Chan 2012) and colorectal (Ramasamy et al. 2015), oesophageal squamous (Almanaa et al. 2012), lung (Zhu et al. 2017a, b) and breast cancer (Mukherjee et al. 2014). Curcumin modulates the stemness and self-renewal mechanism in these cells through the Wnt/β-catenin, hedgehog (Hh) and Notch pathways (Li and Zhang 2014). The Wnt/β-catenin pathway, also known as the canonical Wnt pathway, is associated with self-renewal and stem cell property conferment of progenitor cells in various cancers (Clevers and Reya 2005; Holland et al. 2013). In CSCs, the Wnt/β-catenin signalling is attenuated, where Wnt-protein ligand binds to the Frizzled family receptors allowing β-catenin to translocate into the nucleus to activate transcription of Wnt-associated genes (cMyc, octamer-binding transcription factor 4 and Nanog) to control cell proliferation, angiogenesis, metastasis and inflammation (Clevers and Reya 2005; DiMeo et al. 2009). Curcumin has been

shown to reduce β-catenin levels in various types of CSCs either directly or indirectly interfering Wnt/β-catenin pathway. In colon CSCs, it blocks the binding of Wnt ligand to its receptors (Ramasamy et al. 2015) and reduces the transcriptional co-activator p300, a positive regulator of Wnt/β-catenin (Ryu et al. 2008) and caspase-3-mediated cleavage of β-catenin (Jaiswal et al. 2002). The inhibitory effect of Wnt/β-catenin is also observed in breast CSCs (Kakarala et al. 2010; Dandawate et al. 2016) through a different mechanism where E-cadherin sequesters free β-catenin in the cytoplasm and prevents epithelial-mesenchymal transition (EMT) (Mukherjee et al. 2014) and suppresses the activation of Wnt/β-catenin pathway in lung CSCs (Zhu et al. 2017a, b).

Hh signalling is another aberrant pathway in CSCs giving rise to drug resistance, relapse and metastasis. It is important for embryonic development, but relatively quiescent in adult cells, being activated only during tissue maintenance and repair. The pathway is activated when Hh ligands (Sonic Hh, Indian Hh, Desert Hh) bind to patched homolog transmembrane receptor protein (PTCH) releasing smoothened G-protein-coupled receptor-like protein (Smo) resulting in nuclear glioma-associated oncogene (Gli) transcription factors activation (Justilien and Fields 2015). Curcumin is able to block Sonic Hh signalling in medulloblastoma cells, an aggressive primary brain tumour (Elamin et al. 2010), and colon (Zhu et al. 2017a, b) and prostate cancer (Slusarz et al. 2010). However, in breast cancer, the inhibition of Hh-Gli signalling is through upregulation of p21, also known as cyclin-dependent kinase inhibitor 1, which has a function in cell cycle regulation (Mohapatra et al. 2015). Another major pathway in embryonic development is the Notch signalling. It is important for cell-cell communication and controls multiple cell differentiation. The Notch signalling is reported to be highly upregulated in CSCs and implicated in EMT to cause metastasis. Notch receptors, which is a single-pass transmembrane receptor, are activated when its ligands (Delta-like 1, 3 and 4, and Jagged-1 and Jagged-2) from a neighbouring cell bind to it. This cell-cell activation causes truncation of the intracellular Notch domain, which migrates into the nucleus causing transcription of genes associated with EMT and CSCs maintenance (Espinoza et al. 2013). It was found that curcumin downregulated the Notch signalling through suppressed expression of its ligand (Jagged-1) in oesophageal cancer cells (Subramaniam et al. 2012).

Curcumin also regulates the expression of epithelial growth factor receptor (EGFR) in colon CSCs, which is known to be highly aberrant in cancer cells (Yu et al. 2009). In oesophageal squamous cancer, it subdued the inflammation signalling through NF-kB by reducing CSC population (Almanaa et al. 2012). In addition, curcumin has been reported to inhibit various targets such as ABC transporters in glioma cancer (Fong and Chan 2012), hypoxia-inducible factor-1α (HIF-1α) in hepatoma, zinc finger protein SNAI1 (Snail) in breast cancer and zinc finger E-box-binding homeobox (ZEB) in colon cancer (Zhang et al. 2015a, b). It also decreased the proliferation of glioblastoma CSCs via the induction of reactive oxygen species (ROS), prevention of mitogen-activated protein kinase (MAPK) pathway activation and downregulation of signal transducer and activator of transcription 3 (STAT3) activity and inhibitors of apoptosis (IAP) family members (Gersey et al. 2017).

Despite the promising results in cellular environment, curcumin faces challenges in clinical setting, where its lipophilicity and metabolic instability yield low bioavailability (Kidd 2009; Bar-Sela et al. 2010).

24.2.2 Epigallocatechin-3-Gallate

The content of tea catechins, particularly (-)-epigallocatechin-3-gallate (EGCG), is highest in green tea (*Camellia sinensis*) leaves of the flowering plants of the family Theaceae (Bushman 2009). EGCG is reported to be effective against various cancers through the Wnt and Hh signalling. The modulation of EGCG on β-catenin expression is through restoration of the Wnt inhibitory factor (WIF-1) expression in lung cancer (Gao et al. 2009; Zhu et al. 2017a, b), GSK-3α and GSK-3β signalling in colon cancer (Pahlke et al. 2006) and HMG-box transcription factor (HBP1), a transcriptional repressor of Wnt-targeted genes in suppressing metastasis in breast cancer (Kim et al. 2006). Noteworthy, the inhibition of breast cancer metastasis also occurred via other pathways through the suppression of vasodilator-stimulated phosphoprotein (VASP) expression, Ras-related C3 botulinum toxin substrate 1 (Rac1) (Zhang et al. 2009) and vascular endothelial growth factor D (VEGF-D) (Mineva et al. 2013). On top of that, EGCG also inhibited the growth of oestrogen receptor negative breast CSCs via downregulation of ER-α36 and EGFR expression (Pan et al. 2016). In preventing EMT that leads to metastasis, EGCG drives the cells toward epithelial propensity through E-cadherin expression and reduces Snail and vimentin expressions that control mesenchymal characteristics (Chang et al. 2012). However, inhibition of EMT by EGCG in nasopharyngeal CSCs is through inactivation of NF-κB and reduction of Twist1 (Li et al. 2015).

The effect of EGCG on Hh signalling in downregulation of Gli-1 mRNA expression in prostate CSCs was reported by Slusarz et al. (2010), while, the downregulation of PTCH and Gli-1 expression in human chondrosarcoma (a type of soft tissue cancer) and Sonic Hh in pancreatic CSCs was also demonstrated (Tang et al. 2010a, b, 2012). Additionally, it triggers apoptosis, a natural mechanism of cell death, by activating caspase-3/caspase-7 and inhibits the expression of antiapoptotic signals such as Bcl-2, survivin and XIAP (Tang et al. 2010a, b). In squamous carcinoma cells, EGCG reduces the B-cell-specific Moloney murine leukaemia virus integration site 1 (Bmi-1) level and enhancer of zeste homolog 2 (Ezh2); both are associated with cell survival (Balasubramaniam et al. 2010). EGCG also suppressed Notch1, Bmi1 and Ezh2 and upregulated self-renewal suppressive miRNAs in drug-resistant colorectal CSCs (Toden et al. 2016). The AMP-activated protein kinase is also shown to be activated by EGCG in breast cancer cells leading to cell growth arrest (Chen et al. 2012). In head and neck squamous carcinoma CSCs, EGCG shows multi-target effects. It suppresses stem cell Notch signalling and downregulates the ABC transporter genes in amplifying cisplatin-mediated cell death (Lee et al. 2013). Similarly, it is also observed in glioblastoma CSCs where it intensifies temozolomide toxicity (Zhang et al. 2015a, b).

24.2.3 Genistein

Genistein is a natural isoflavone mainly found in the members of the Leguminosae family, which includes soy, lentil, bean and chickpea. The content of genistein is highest in soybeans, seeds from the legume plant *Glycine max*. There is established scientific evidence on its anticancer and antiangiogenic potentials (Li et al. 2011). Genistein was found to upregulate the expression of GSK-3β that sequesters β-catenin and downregulate the Wnt signalling in prostate cancer cells (Sarkar et al. 2010). It increases the expression of E-cadherin in blocking Wnt/β-catenin signalling in breast cancer (Su and Simmen 2009) and upregulate the expression of secreted frizzled protein 2 (sFRP2) gene, which is a Wnt pathway antagonist in colon cancer (Zhang and Chen 2011) Additionally, Hedgehog signalling is downregulated through reduction of Gli mRNA expression and translation in these cancer cells (Zhang et al. 2012). Genistein is a known phytoestrogen and proven to suppress the EMT potential in ovarian cancer cells through inhibition of the oestrogen receptor and restoration of TGF-β1 signalling (Kim et al. 2015). In a rather unique experiment conducted by Montales et al. (2012) investigating the effects of diet rich genistein, sera of mice fed with genistein was incorporated into culture media of breast CSC. Their findings indicated that genistein inhibited mammosphere formation through the inhibition of Akt and enhanced tumour suppressor phosphatase and tensin homologue deleted on chromosome 10 (PTEN) expression. CSCs are able to survive chemotherapy due to its ability to maintain low intracellular drug concentration by overdriving its ABC transporter for drug efflux. In breast cancer, genistein competitively inhibits the drug efflux, which may suggest the possible combination therapy with antitumor agents in targeting chemoresistant cancer cells (Imai et al. 2004). Additionally, it caused breast CSCs to differentiate and lose its stem cell property (Liu et al. 2016).

24.2.4 Mangostin

Mangostin are xanthones naturally found in mangosteen, the fruits of *Garcinia mangostana* of the Guttiferae family. The plant is abundant in the Asian region, and there are three major types of mangostin (α, β and γ), which differ in the number of hydroxyl and methoxy substituents (Ibrahim et al. 2016). α-Mangostin is shown to ameliorate the production of ROS in hypoxic pancreatic cancer cells with concomitant reduction in HIF-1α in preventing EMT and Gli1 expression in moderating stemness (Lei et al. 2014). In anti-metastatic effect, it was shown to inhibit prostate cancer through downregulation of c-Jun N-terminal kinase 1 and 2 (JNK1 and 2) (Hung et al. 2009), inhibition of PI3K/Akt pathway in pancreatic cancer (Xu et al. 2014) and extracellular signal-regulated kinase 1/2 (Erk 1/2) in lung and breast cancer (Lee et al. 2010; Shih et al. 2010). Moreover, it subdued the NF-κB-mediated inflammatory response (Hung et al. 2009; Lee et al. 2010; Shih et al. 2010). An

animal study using BALB/c mice shows α-mangostin was able to induce apoptosis in metastatic breast cancer by decreasing the levels of phosphorylated Akt, which plays a central role in cell growth, antiapoptosis, angiogenesis and metastasis (Shibata et al. 2011). The Wnt/β-catenin pathway is shown to be inhibited by α-mangostin in a separate in vivo study, investigating its effect in colon cancer (Nabandith et al. 2004). In cell culture study, it is found that α- and γ-mangostin attenuated Wnt/β-catenin through activation of cGMP-dependent protein kinase-1 (PKG-1), an upstream repressor of β-catenin (Yoo et al. 2011). Besides solid tumours, our research group found that α-mangostin is effective against leukaemia stem cells by inducing caspase-dependent apoptosis, which tipped the cells toward Bcl-2-associated X (Bax) against Bcl-2 in causing mitochondrial membrane depolarization (Hashim and Leong 2016).

24.2.5 Resveratrol

Resveratrol is another popular polyphenol, a stilbenoid with established evidence on its antitumour properties. It is a natural phytoalexin to defend a plant against fungal infection (*Botrytis cinerea*) and ultraviolet irradiation damage. It was initially found in the roots of the medicinal plant *Polygonum cuspidatum* used in Chinese and Japanese medicine and is also present in grapes, wine, peanuts and soy (Alamolhodaei et al. 2017). It is demonstrated that resveratrol can impede self-renewal capacity of CSCs; suppress EMT through downregulation of Slug, ZEB and E-cadherin expressions; reverse stemness by attenuating Oct4, Nanog and neuroectodermal stem cell marker (Nestin) signals; and induce apoptosis through B-cell lymphoma 2 (Bcl-2) and X-linked inhibitor of apoptosis (XIAP) modulators in head and neck cancer (Hu et al. 2012) and pancreatic cancer (Shankar et al. 2011). It was also reported that resveratrol inhibits proliferation and migration in glioblastoma CSCs, via the modulation of Wnt signalling pathway-related genes, decreasing of β-catenin levels and downregulation of twist-related protein 1 (Twist1) and Snail, causing in the suppression of EMT (Cilibrasi et al. 2017). Interestingly, in breast CSCs, lipogenesis through suppression of fatty acid synthase (FAS) is involved in increasing pro-apoptotic signals, death-associated kinase 2 (DAPK2) and Bcl2/adenovirus E1B 19 kDa protein-interacting protein 3 (BNIP3) to induce apoptotic death (Pandey et al. 2011, 2013).

The Hh pathway is tamed in pancreatic cancer through suppression of PTCH, Smo and Gli expressions (Mo et al. 2011) and suppression of interleukin-6-stimulated Sonic Hh activation in leukaemia (Su et al. 2013), whereas, abrogation of stemness by proteasome degradation of Nanog via tumour protein 53 (p53) activation is observed in glioma CSCs (Sato et al. 2013) and in nasopharyngeal CSCs, where p53 is further linked to EMT and metabolic shift (Shen et al. 2013). Perhaps resveratrol is one of the natural biomodulator of multi-pathways, and its involvement in Notch signalling is also shown through the activation of glycogen synthase kinase-3β (GSK-3β) in leukaemia (Cecchinato et al. 2007). Canonical Wnt signal-

ling is suppressed in low concentrations of resveratrol in colon cancer (Hope et al. 2008; Reddivari et al. 2016), Waldenstrom's macroglobulinemia (Roccaro et al. 2008) and breast cancer (Fu et al. 2014). To enhance the efficacy of radiotherapy, resveratrol was used to sensitize medulloblastoma (Lu et al. 2009), teratoid/rhabdoid (Kao et al. 2009), primary glioblastoma (Yang et al. 2012) and prostate CSCs (Chen et al. 2017). EMT transition in metastasis is also prevented through transforming growth factor-β1 (TGF-β1) inhibition in lung cancer (Wang et al. 2013), restriction of PI3K/Akt/NFκB signalling in pancreatic cancer (Li et al. 2013a, b), Akt and p53 in glioblastoma (Clark et al. 2017), epidermal growth factor (EGF) attenuation (Vergara et al. 2011), preventing Erk pathway activation in ovarian cancer (Baribeau et al. 2014) and HIF-1α suppression of hypoxia-induced metastasis in colon cancer (Wu et al. 2008). However, resveratrol faces the same fate as curcumin in human bioavailability studies of having poor bioavailability (Kidd 2009).

24.2.6 Silibinin

Milk thistle (*Silybum marianum*) is a well-known flowering plant of the Asteraceae family used for centuries as liver tonic and originally found in regions spanning from Southern Europe to Asia. Commercial product of milk thistle, termed silymarin, contains seven major flavonolignans and a flavonoid. Silibinin is one of the flavonolignans, which exists as a mixture of two diastereoisomers, silybin A and silybin B (Kroll et al. 2007). It is reported to have antitumour properties and found to downregulate Snail, zinger finger E-box-binding homeobox (ZEB) and N-cadherin levels associated with mesenchymal characteristic in non-small cell lung carcinomas (NSCLC). Notably, simultaneous treatment with silibinin reversed the erlotinib (EGFR tyrosine kinase inhibitor)-resistant cells, which was used to enrich CSCs in the culture (Corominas-Faja et al. 2013). Noteworthy, combination with another natural flavonoid, luteolin, found in green vegetables such as broccoli, celery and artichokes, can induce intrinsic and extrinsic apoptosis in glioblastoma CSCs (Chakrabarti and Ray 2015).

24.2.7 Sulforaphane

It has long been recognized that the consumption of cruciferous vegetables reduces the risk of developing cancer. One of the phytochemicals attributed to its chemopreventive effect is sulforaphane, a widely studied isothiocyanate present in vegetables and particularly abundant in broccoli. Broccoli belongs to the mustard family (Brassicaceae). There are a variety of *Brassica* species such as *B. rapa* (broccoli raab and Japanese mustard spinach), *B. oleracea* (cabbage, Chinese broccoli and romanesco broccoli), *B. carinata* (Ethiopian rape), *Brassica fruticulosa* (Mediterranean cabbage) and others (Juge et al. 2007). Sulforaphane has been

shown to suppress breast CSCs self-renewal capacity through downregulation of the Wnt/β-catenin pathway (Li et al. 2010), chronic leukaemia CSCs (Lin et al. 2012), and in both cervical carcinoma and hepatocarcinoma (Park et al. 2007). In pancreatic CSCs, self-renewal is blocked through interference of the SHh-Gli signalling (Li et al. 2013a, b; Rodova et al. 2012). Additionally, it suppresses the stemness factors such as Nanog and Oct-4 (Rodova et al. 2012), induces caspase-driven apoptosis by reducing BcL-2 levels, overcame the tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) resistance cells (Shankar et al. 2008) and prevented NFκB nuclear translocation (Kallifatidis et al. 2009; Rausch et al. 2010). Interestingly, synergistic inhibition of pancreatic CSCs by sulforaphane with other natural compounds such as quercetin and catechins is also proven to be effective (Appari et al. 2014; Srivastava et al. 2011). In breast cancer, sulforaphane suppresses EMT by downregulating peripheral benzodiazepine receptor (PBR) and vimentin expressions and prevented angiogenesis by reducing vascular endothelial growth receptor (VEGR) and platelet-derived growth factor (PDGF) markers (Hunakova et al. 2009). On the other hand, Ezh2 is inhibited by sulforaphane in suppressing aggressive melanoma CSCs (Fisher et al. 2016). The PI3K/Akt pathway is linked to cellular quiescence, proliferation and differentiation of colon (Shen et al. 2007) and ovarian (Chaudhuri et al. 2007) CSCs. Modulation of epigenetic such as miR-124 occurs in gastric CSCs which silenced the cytokine interleukin-6 that is responsible for the downstream STAT3 activation, involved in self-renewal and apoptosis processes (Wang et al. 2016).

24.3 Non-dietary Phytochemicals Against Cancer Stem Cells

It is known that plants secrete natural toxins or metabolites to protect themselves against predators or competitors, which can be exploited in battling cancer. In contrast to phytochemicals derived from food, interest in investigating wild plants for antitumour properties is driven by ethnopharmacological use or folk medicine, historical archive or scientific curiosity of the investigators (Newman and Cragg 2012). Owning to geographic speciation of wild plants, the discovery of active compounds is frequently dependent on its accessibility. Therefore, the antitumour effect of these plants against CSCs is likely to be less widely studied (Table 24.1). Nonetheless, discoveries from wild plants are worth mentioning owning to their diverse unique chemical structures (Fig. 24.2) and different modes of actions (Fig. 24.3). In this section, we highlight the most recent reports on non-dietary phytochemicals against CSCs.

Andrographis paniculata (Burm. f) Ness is a herbal plant used as traditional medicine, which belongs to the Acanthaceae family, and mainly found in India, Thailand and China. The leaves and stem of the plant contain a diterpene lactone, and andrographolide that exhibits antitumour activity. The compound is able to abrogate stemness in oral carcinoma through upregulation of epigenetic modulator, miR-218 that suppresses Bmi1, which interacts with several pathways such as Wnt, Akt, Notch and hedgehog (Yang et al. 2017).

Berberine is a plant alkaloid initially found in *Berberis* species in the Berberidaceae family. Commonly known species as the European barberry, *B. vulgaris* is found in Europe, North Africa, the Middle East and Central Asia. It has been used to treat diarrhoea, intestinal ulcers, blood, vomiting and renal calculi in folk medicine (Imanshahidi and Hosseinzadeh 2008). Berberine seems to elicit multiple pathways in suppressing neuroblastoma cells. Cell stemness was suppressed through β-catenin and Notch, induced apoptotic death by increasing Bax/Bcl-2 ratio and prevented EMT by normalizing E-cadherin levels (Naveen et al. 2016).

Bruceantin, a triterpene quassinoid, was first extracted from *Brucea antidysenterica* from the Simaroubaceae family. It is a small tree growing up to 7 m found in tropical part of Africa, where all the parts of the tree are traditionally used as anthelmintic and to treat fever, diarrhoea, indigestion, stomach ache, asthma and skin complications. Bruceantin has been shown to be effective against various cancers such as melanoma, colon, breast cancer and leukaemia (Cuendet et al. 2004; Cuendet and Pezzuto 2004). Additionally, it has been shown to be effective in multiple myeloma CSCs by modulating the Notch signalling and induces mitochondria-dependent apoptosis in in vitro and in vivo setting (Issa et al. 2016).

The flavonoid, Kazinol-E, is found in *Broussonetia kazinoki*, under the Moraceae family and commonly known as paper mulberry. The leaves of the plant are used in oriental medicine to treat burns and acne. It was initially reported to have anti-inflammatory and cytoprotective properties. However, recently report shows Kazinol-E inhibited breast CSCs through competitive binding of the Erk1 at the adenosine triphosphate (ATP) binding pocket (Jung et al. 2015, 2016).

The plant metabolite plumbagin is found in the roots of the *Plumbago zeylanica* herb in the Plumbaginaceae family, which is also known as Ceylon leadwort, doctorbush or wild leadwort. The compound is reported to be able to inhibit various types of cancers via multiple pathways that regulate proliferation, survival, invasion and metastasis (Lee et al. 2012). Recently, this naphthoquinone derivative is demonstrated to be effective against prostate CSCs as well as breast cancer susceptibility gene 1/2 (BRCA1/2) mutated prostate cells by triggering both intrinsic and extrinsic apoptosis (Reshma et al. 2016).

In the Celastraceae family, a triterpenoid isolated from the root bark of the plant *Maytenus ilicifolia*, tigenin B shows cytotoxicity against breast CSCs (Cevatemre et al. 2016). The popular name of the plant is Espinheira Santa, which is traditionally used for stomach ailment such as gastritis, ulcers and nausea in Brazilian folk medicine. It has been shown to exhibit antitumour properties against a variety of cancers (Araújo et al. 2013). In breast CSCs, the compound elicited endoplasmic reticulum stress and mitochondrial-mediated apoptotic death (Cevatemre et al. 2016). Our research group have discovered another triterpenoid, betulonic acid is active toward leukaemia CSCs. The compound is a natural analogue of the well-known betulinic acid, where it differs by having a ketone instead of hydroxyl in its chemical structure (Fig. 24.2) (Leong et al. 2017). We have been focusing on plants of the Meliaceae family because of their interesting bioactivities such as antimalarial, antihypertensive and antitumour (Leong et al. 2016). The betulonic acid was isolated from the bark of *Walsura pinnata* Hassk, found in tropical countries of

Southeast Asia and locally known as “Lantupak mata kucing” in Malaysia. We demonstrated that betulonic acid induced mitochondrial-mediated apoptosis through modulation of the Bax/Bcl-2 ratio in leukaemia CSCs and zebrafish model. More importantly, CSCs was found to be 4–12-folds more resistant to current chemotherapy, cytarabine and idarubicine through antiapoptotic modulator, survivin. However, betulonic acid can effectively suppress survivin levels to prevent acquired resistance (Leong et al. 2017).

24.4 Conclusions and Future Prospects

The attribution of cancer stem cells in chemoresistance, relapse and metastasis has added a new dimension of complexity in cancer treatment. Properties of cancer stem cells that differ from the bulk tumour render current chemotherapy ineffective, as proven by scientists. Therefore, there is a growing need to mine new anticancer agents that specifically target CSC population. Since plants have been a reliable source of drug discovery, natural product scientists have started to explore potential phytochemicals toward CSCs in various solid tumours and leukaemia. In this regard, discoveries of active phytochemicals can be divided based on its source, either from dietary or non-dietary plants. Phytochemicals from the dietary source such as curcumin, resveratrol, genistein, EGCG, mangostin and sulforaphane are more widely studied on various types of CSCs. Discoveries show that these compounds can modulate multiple pathways in abrogating CSCs. A multiple target compound may offer an advantage toward preventing multi-resistance development in CSCs. It is interesting to observe these food phytochemicals’ ability to control stemness, block efflux transporters, inhibit metastasis and restore apoptosis and self-renewal capacity in CSCs. Importantly, these effects were replicable in animal models despite some reports on poor bioavailability. Nonetheless, the structures of these phytochemicals can be an inspiration for medicinal chemist to improve their drug-like properties and develop formulations to improve bioavailability. Moreover, the mechanism of action of these bioactive compounds will give insights into cellular targets against CSCs. On the other side of the spectrum, phytochemicals from wild plants could offer structurally diverse compounds. There are many discoveries of phytochemicals based on ethnopharmacological uses, but the huge biodiversity means there is still largely uncharted discoveries awaiting. The probability of discovering novel structures is higher in wild plants, which may in essence contribute to the development of a new family of chemotherapeutic agents.

However, there are some challenges toward discovering potent phytochemicals against CSCs. Phytochemicals that are not easily accessible may suffer from insufficient evidence of efficacy and through understanding of mechanism of action, which may lose out in the selection process of a development pipeline. At the in vitro level, it is often laborious and time-consuming to enrich sufficient CSCs for testing, particularly cancers with low CSCs. The lack of facilities and expertise to conduct in vivo studies using immune-deficient models will also hamper the development of

a potential chemotherapeutic agent as proof of efficacy in animals is a prerequisite to clinical studies. In clinical studies, it is difficult to ascertain whether a potential chemotherapeutic agent is effective against CSCs as CSCs levels in patient's tumour is unknown. Moreover, clinical study is often conducted in a period shorter than the time needed to observe tumour relapse. Albeit such challenges, collaborative efforts among scientists across the globe to complement each other's research strength can solve these challenges toward improving chemotherapy.

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