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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