# Chapter 2
# Plant miRNAs and Phytomolecules As Anticancer Therapeutics


**Nikita Shukla, Virendra Shukla, and Sangeeta Saxena**

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

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

N. Shukla · V. Shukla · S. Saxena (✉)
Department of Biotechnology, Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, India
e-mail: dr_sangeeta_saxena@yahoo.com



M. S. Akhtar, M. K. Swamy (eds.), *Anticancer Plants: Mechanisms and Molecular Interactions*, https://doi.org/10.1007/978-981-10-8417-1_2

## 2.1 Introduction

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

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

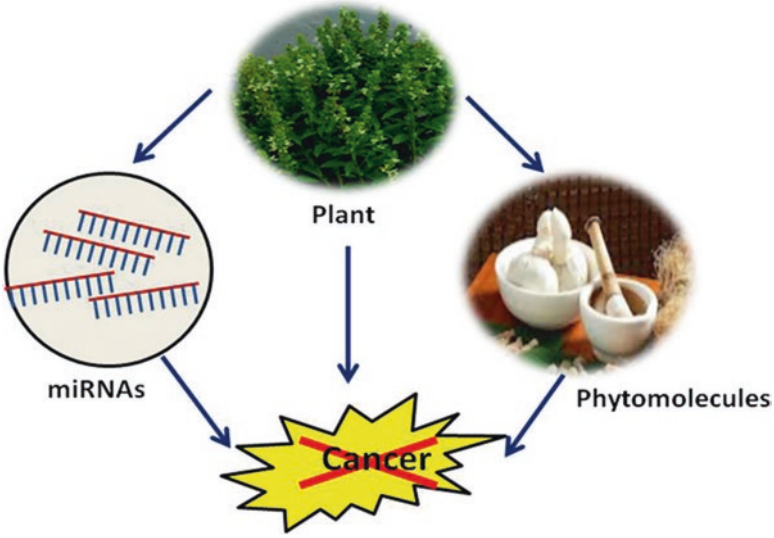


**Fig. 2.1** Schematic diagram shows plant molecules/miRNAs inhibit cancer progression

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

## 2.2 Anticancer Activities of Plants and Their Derived Compounds

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

## 2.3 Therapeutic Potential of miRNAs

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

**Table 2.1** Some important medicinal plants having anticancer activity

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

(continued)

**Table 2.1** (continued)

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

### 2.3.1 miRNA Biogenesis

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

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

### 2.3.2 Plant-Based miRNAs in Therapeutics Development

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

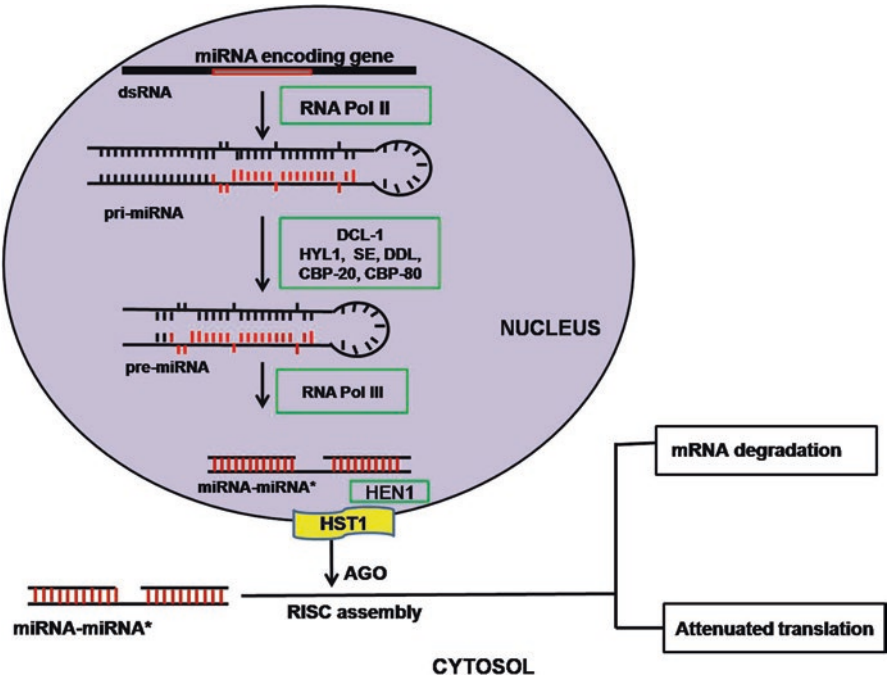


**Fig. 2.2** miRNA biogenesis in plants

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

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

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

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

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

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

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

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

### *2.3.3 Plant Extracts/Phytomolecules Regulating miRNA*

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

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

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

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

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

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

## 2.4 Conclusions and Future Prospects

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

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

## References

Aggarwal BB, Shishodia S (2006) Molecular targets of dietary agents for prevention and therapy of cancer. Biochem Pharmacol 71:1397–1421

Ahmad A, Sarkar SH, Bitar B, Ali S, Aboukameel A, Sethi S, Li Y, Bao B, Kong D, Banerjee S, Padhye SB, Sarkar FH (2012) Garcinol regulates EMT and Wnt signaling pathways *in vitro* and *in vivo* leading to anticancer activity against breast cancer cells. Mol Cancer Ther 11:2193–2201

Akhtar MS, Birhanu G, Demisse S (2014a) Antimicrobial activity of *Piper nigrum* L. and *Cassia didymobotyra* L. leaf extract on selected food borne pathogens. Asian Pac J Trop Dis 4:S911–S919

Akhtar MS, Degaga B, Azam T (2014b) Antimicrobial activity of essential oils extracted from medicinal plants against the pathogenic microorganisms: a review. Issues Biol Sci Pharm Res 2:1–7

Alvarez-Garcia I, Miska EA (2005) MicroRNA functions in animal development and human disease. Development 132:4653–4662

Aryal R, Yang X, Yu Q, Sunkar R, Li L, Ming R (2012) Asymmetric purine-pyrimidine distribution in cellular small RNA population of papaya. BMC Genomics 13:682. https://doi.org/10.1186/1471-2164-13-682

Banerjee N, Talcott S, Safe S, Martens-Talcott SU (2012) Cytotoxicity of pomegranate polyphenolics in breast cancer cells *in vitro* and *in vivo*: potential role of miRNA-27a and miRNA-155 in cell survival and inflammation. Breast Cancer Res Treat 136:21–34

Bushati N, Cohen SM (2007) MicroRNA functions. Annu Rev Cell Dev Biol 23:175–205

Chin AR, Fong MY, Somlo G, Wu J, Swiderski P, Wu X, Wang SE (2016) Cross-kingdom inhibition of breast cancer growth by plant miR159. Cell Res 26:217–228

Coseri S (2009) Natural products and their analogues as efficient anticancer drugs. Med Chem 9:560–571

Cragg GM, Newman DJ (2005) Plants as a source of anticancer agents. J Ethanopharmacol 100:72–79

Del Follo-Martinez A, Banerjee N, Li X, Safe S, Martens-Talcott S (2013) Resveratrol and quercetin in combination have anticancer activity in colon cancer cells and repress oncogenic microRNA-27a. Nutr Cancer 65:494–504

Dhar S, Hicks C, Levenson AS (2011) Resveratrol and prostate cancer: promising role for microRNAs. Mol Nutr Food Res 55:1219–1229

Dong Z, Han MH, Fedoroff N (2008) The RNA-binding proteins HYL1 and SE promote accurate *in vitro* processing of pri-miRNA by DCL1. Proc Natl Acad Sci U S A 105:9970–9975

Dugas DV, Bartel B (2004) MicroRNA regulation of gene expression in plants. Curr Opin Plant Biol 7:512–520

Duursma AM, Kedde M, Schrier M, Ie Sage C, Agami R (2008) miR 148 targets human DNMT3b protein coding region. RNA 14:872–877

Friedman RC, Farh KK, Burge CB, Bartel DP (2009) Most mammalian mRNAs are conserved targets of microRNAs. Genome Res 19:92–105

Goldie JH (2001) Drug resistance in cancer: a perspective. Cancer Metastasis Rev 20:63–68
Gupta SC, Kim JH, Prasad S, Aggarwal BB (2010) Regulation of survival, proliferation, invasion angiogenesis and metastasis of tumor cells through modulation of inflammatory pathways by nutraceuticals. Cancer Metastais Rev 29:405–434
Haggar FA, Boushey RP (2009) Colorectal cancer epidemiology: incidence, mortality, survival and risk factors. Clin Colon Rectal Surg 22:191–197
Itokawa H, Ibraheim ZZ, Ya FQ, Takeya K (1993) Anthraquinones, naphthohydroquinone dimmers from *Rubia cordifolia* and their cytotoxic activity. Chem Pharm Bull 41:1869–1872
Jia L, Zhang D, Xiang Z, He N (2015) Nonfunctional ingestion of plant miRNAs in silkworm revealed by digital droplet PCR and transcriptome analysis. Sci Rep 5:12290. https://doi.org/10.1038/srep12290
Karunagaran D, Joseph J, Santhosh Kumar TR (2007) Cell growth regulation. In: Aggarwal BB, Surh YJ, Sishodia S (eds) The molecular targets and therapeutic uses of Curcumin in health and disease. Adv Exp Med Biol 595:245–268
Kharb M, Jat RK, Gupta A (2012) A review on medicinal plants used as a source of anticancer agents. Int J Drug Res Technol 2:177–183
Kim S, Yang JY, Xu J (2008) Two Cap-Binding Proteins CBP20 and CBP80 are involved in processing primary microRNAs. Plant Cell Physiol 49:1634–1644
Kingston DGI (2005) Taxol and its analogs. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer agents from natural products. Brunner-Routledge Psycology Press/Taylor and Francis Group, Boca Raton
Kosaka N, Izumi H, Sekine K, Ochiya T (2010) microRNA as a new immune-regulatory agent in breast milk. Silence 1:7. https://doi.org/10.1186/1758-907X-1-7
Kruszka K, Pieczynski M, Windels D, Bielewicz D, Jarmolowski A, Szweykowska-Kulinska Z, Vazquez F (2012) Role of microRNAs and other sRNAs of plants in their changing environments. J Plant Physiol 169:1664–1672
Lam TK, Shao S, Zhao Y, Marincola FM, Pesatori AC, Bertazzi PA, Caporaso NE, Wang E, Landi MT (2012) Influence of quercetin-rich food intake on microRNA expression in lung cancer tissues. Cancer Epidemiol Biomark Prev 21:2176–2184
Lam JK, Chow MY, Zhang Y, Leung SW (2015) siRNA versus miRNA as therapeutics for gene silencing. Mol Ther Nucleic Acids 4:e252
Lee KH, Xiao Z (2005) Podophyllotoxins and analogs. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer agents from natural products. Brunner-Routledge Psychology Press/Taylor and Francis Group, Boca Raton
Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854
Li Y, Kong D, Wang Z, Sarkar FH (2010) Regulation of microRNAs by natural agents: an emerging field in chemoprevention and chemotherapy research. Pharm Res 27:1027–1041
Liang G, Zhu Y, Sun B, Shao Y (2014) Assessing the survival of exogenous plant microRNA in mice. Food Sci Nutr 2:380–388
Liang H, Zhang S, Fu Z, Wang Y (2015) Effective detection and quantification of dietetically absorbed plant microRNAs in human plasma. J Nutr Biochem 26:505–512
Liu C, Axtell MJ, Fedoroff NV (2012) The helicase and RNAseIIIa domains of Arabidopsis Dicer-Like 1 modulate catalytic parameters during microRNA biogenesis. Plant Physiol 159:748–758
Lobbes D, Rallapalli G, Schmidt DD, Martin C, Clarke J (2006) SERRATE: a new player on the plant microRNA scene. EMBO Rep 7:1052–1058
Luo Y, Wang P, Wang X, Wang Y, Mu Z, Li Q, Fu Y, Xiao J, Li G, Ma Y, Gu Y, Jin L, Ma J, Tang Q, Jiang A, Li X, Li M (2017) Detection of dietetically absorbed maize-derived microRNAs in pigs. Sci Rep 7:645. https://doi.org/10.1038/s41598-017-00488-y
Mahima RA, Deb R, Latheef SK, Abdul Samad H, Tiwari R, Verma AK, Kumar A, Dhama K (2012) Immunomodulatory and therapeutic potentials of herbal, traditional/indigenous and ethnoveterinary medicines. Pak J Biol Sci 15:754–774

Martens-Talcott SU, Noratto GD, Li X, Angel-Morales G, Bertoldi MC, Safe S (2013) Betulinic acid decreases ER-negative breast cancer cell growth *in vitro* and *in vivo*: role of sp transcription factors and microRNA-27a:ZBTB10. Mol Carcinog 52:591–602

Miska EA (2005) How microRNAs control cell division, differentiation and death. Curr Opin Genet Dev 15:563–568

Pandey G, Sharma M (2006) Autochthonous herbal products in the treatment of cancer. Phytomedica 7:99–104

Prakash OM, Amit K, Pawan K, Ajeet A (2013) Anticancer potential of plants and natural products: a review. Am J Pharm Sci 1:104–115

Rahier NJ, Thomas CJ, Hecht SM (2005) Camptothecin and its analogs. In: Cragg GM, Kingston DGI, Newman DJ (eds) Anticancer agents from natural products. Brunner-Routledge Psycology Press/Taylor and Francis Group, Boca Raton

Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP (2002) MicroRNAs in plants. Genes Dev 16:1616–1626

Rocha A, Wang L, Penichet M, Martins-Green M (2012) Pomegranate juice and specific components inhibit cell and molecular processes critical for metastasis of breast cancer. Breast Cancer Res Treat 136:647–658

Rooij EV, Kauppinen S (2014) Development of microRNA therapeutics is coming of age. EMBO Mol Med 6:851–864

Sarangi MK, Padhi S (2014) Plants with potential anticancer activities- a review. Int J Phytomed 6:1–15

Saxena S, Kumar A, Babu SG (2014) PLANT miRNAs: key players in inter-kingdom and intra-kingdom gene regulation. Int J Pharma Bio Sci 5:374–389

Schmidt MF (2014) Drug target miRNAs: changes and challenges. Trends Biotechnol 32:578–585

Siegel RL, Miller KD, Jemal A (2016) Cancer statistics. CA Cancer J Clin 66:7–30

Sivalokanathan S, Ilayaraja M, Balasubramanian MP (2005) Efficacy of *Terminalia arjuna* (Roxb.) on N-nitrosodiethylamine induced hepatocellular carcinoma in rats. Indian J Exp Biol 43:264–267

Sunkar R, Li YF, Jagadeeswaran G (2012) Functions of microRNAs in plant stress responses. Trends Plant Sci 17:196–203

Swamy MK, Akhtar MS, Sinniah UR (2016) Antimicrobial properties of plant essential oils against human pathogens and their mode of action: an updated review. Evidence-Based Compl Altern Med 2016:3012462. https://doi.org/10.1155/2016/3012462

Tili E, Michaille JJ, Alder H, Volinia S, Delmas D, Latruffe N, Croce CM (2010) Resveratrol modulates the levels of microRNAs targeting genes encoding tumor-suppressors and effectors of TGFβ in signaling pathway in SW480 cells. Biochem Pharmacol 80:2057–2065

Tsang WP, Kwok TT (2010) Epigallocatechin gallate up-regulation of miR-16 and induction of apoptosis in human cancer cells. J Nutr Biochem 21:140–146

Wang J, Li Y, Wang X, Jiang C (2012a) Ursolic acid inhibits proliferation and induces apoptosis in human Glioblastoma cell lines U251 by suppressing TGF-β1/miR-21/PDCD4 pathway. Basic Clin Pharmacol Toxicol 111:106–112

Wang L, Ho J, Glackin C, Martins-Green M (2012b) Specific pomegranate juice components as potential inhibitors of prostate cancer metastasis. Transl Oncol 5:344–355

Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75:855–862

Witwer KW (2012) XenomiRs and miRNA homeostasis in health and disease: evidence that diet and dietary miRNAs directly and indirectly influence circulating miRNA profiles. RNA Biol 9:1147–1154

Witwer KW, McAlexander MA, Queen SE, Adams RJ (2013) Real-time quantitative PCR and droplet digital PCR for plant miRNAs in mammalian blood provide little evidence for general uptake of dietary miRNAs. RNA Biol 10:1080–1086

Xiang S, Fruehauf J, Li CJ (2006) Short hairpin RNA-expressing bacteria elicit RNA interference in mammals. Nat Biotechnol 24:697–702

Xie K, Khanna K, Ruan S (2010) Expression of microRNAs and its regulation in plants. Semin Cell Dev Biol 21:790–797

Yu B, Bi L, Zheng B, Ji L, Chevalier D, Agarwal M, Ramachandran V, Li W, Lagrange T, Walker JC, Chen X (2008) The FHA domain proteins DAWDLE in Arabidopsis and SNIP1 in humans act in small RNA biogenesis. Proc Natl Acad Sci U S A 105:10073–10078

Zhang B, Wang Q, Pan X (2007) MicroRNAs and their regulatory roles in animals and plants. J Cell Physiol 210:279–289

Zhang L, Hou D, Chen X, Li D, Zhu L, Zhang Y, Li J, Bian Z, Liang X, Cai X, Yin Y, Wang C, Zhang T, Zhu D, Zhang D, Xu J, Chen Q, Ba Y, Liu J, Wang Q, Chen J, Wang J, Wang M, Zhang Q, Zhang J, Zen K, Zhang C (2012) Exogenous plant MiR168a specifically targets mammalian DLRAP1: evidence of cross-kingdom regulation by microRNA. Cell Res 22:107–126

Zhou DH, Wang X, Feng Q (2014) EGCG enhances the efficacy of cisplatin by downregulating hsa-miR-98-5p in NSCLC A549 cells. Nutr Cancer 66:636–644