

Hardeep Singh Tuli *Editor*

# Current Aspects of Flavonoids: Their Role in Cancer Treatment

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*“This Book is Dedicated to Beloved Parents”*

# Preface

Cancer is a major cause of death worldwide and becomes the biggest killer in the twenty-first century. It has been ranked second in mortality rate following cardiovascular diseases in most of the countries. Every year, the number of people being diagnosed with cancer is increasing very fast. Due to the lack of significant improvement in diagnosis, treatment, and prevention, cancer has or will soon become the number one killer in most parts of the world. Induced side effects and acquired resistance against anticancer drugs create the hassle for the treatment of cancer and enthusiasm for the development of new approaches.

Epidemiological studies show a cancer-protective effect of diets rich in fruits and vegetables and develop a possibility of curing cancer with biologically active plant secondary metabolites. There are huge groups of such compounds, collectively called “phytochemicals,” which provide flavour and colour to edible plants. Flavonoids are one among such kind of compounds that exert anticarcinogenic effects in various animal models of cancer. A great progress has also been made in exploring pharmacological mechanisms of actions. Such mechanisms include the detoxification and enhanced excretion of carcinogens; suppression of inflammatory processes; inhibition of mitosis, angiogenesis, and metastasis; and induction of apoptosis at various stages in the progression.

This book describes the complete information of such bioactive (flavonoids) molecules, including general introduction, chemistry, absorption and metabolism, mechanisms of action, toxicology, and future perspectives at a single platform. Therefore, in-depth knowledge of flavonoid chemistry and their anticancer mechanisms of actions, along with the latest nanotechnology-based implementations in drug delivery, will help the scientific community to understand the biology of cancer as well as to design novel anticancer strategies.

Mullana-Ambala, India

Hardeep Singh Tuli

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## About the Author

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# Abbreviations

$\mu\text{M}$	Micro-molar
4CL	4-Coumaroyl
ACC	Acetyl-CoA carboxylase
AChE	Acetylcholinesterase
ADME	Absorption, distribution, metabolism, and excretion
AhR	Aryl hydrocarbon receptor
AKT	Protein kinase B
ALT	Alanine aminotransferase
AMPK	AMP-activated protein kinase
ANP	Atrial natriuretic peptide
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUC	Area under the curve
AUS	Auresidin synthase
Bcl-2	B-cell lymphoma 2
BCRP	Breast cancer resistance protein
BMI	Body mass index
BMP2	Bone morphogenetic protein 2
BUN	Blood urea nitrogen
C/EBPa	CCAAT element-binding protein a
C/EBPb	CCAAT element-binding protein b
C3H	p-Coumarate 3-hydroxylase
C4H	Cinnamate-4-hydroxylase
CaM kinase II	Ca <sup>2+</sup> /calmodulin-dependent protein kinase II
CDK	Cyclin-dependent kinases
CHI	Chalcone isomerase
CHO	Chinese hamster ovary cell
CHR	Chalcone reductase
CHS	Chalcone synthase
CoQ10	Coenzyme Q10
COX-2	Cyclooxygenase-2

CPT1	Carnitine palmitoyltransferase 1
CXCR 4	C-X-C chemokine receptor type 4
CYP	Cytochromes P450
CYP2E1	Cytochrome P450 2E1
Cyt. c	Cytochrome c
DFR	Dihydroflavonol 4-reductase
DMBA	7,12-Dimethylbenz[a]anthracene
DNA	Deoxyribonucleic acid
EGCG	Epigallocatechin gallate
EGF	Epidermal growth factor
EGFR	Endothelial growth factor receptor
EPO	European Patent Office
ER	Endoplasmic reticulum
ERG1	Early growth response protein 1
ERK1/2	Extracellular signal-regulated kinases 1/2
EROD	Ethoxyresorufin <i>O</i> -dealkylase
F3'5'H	Flavonoid 3'5'hydroxylase
F3'H	Flavonoid 3'hydroxylase
F3H	Flavanone 3-hydroxylase
FABP4	Fatty acid-binding protein 4
FAS	Fatty acid synthase
FGFR2	Fibroblast growth factor receptor 2
G6P	Glucose-6-phosphatase
GAGAA	$\gamma$ -aminobutyric acid a
GLUT	Glucose transporter
GLUT2	Glucose transporter 2
GREB1	Growth regulation by estrogen in breast cancer 1
GSH-PX	Glutathione peroxidase
HCT	p-hydroxycinnamoyl-CoA shikimate/quinate p-hydroxycinnamoyl transferase
HER2	Human epidermal growth factor receptor
HIF-1 $\alpha$	Hypoxia-inducible factor 1 $\alpha$
HMGB1	High-mobility group box protein 1
HO	Heme oxygenase
HSCs	Hepatic stellate cells
HuBChE	Human butyrylcholinesterase
IC50	Half maximal inhibitory concentration
ICAM-1	Intercellular adhesion molecule 1
IFS	Isoflavone synthase
IgE	Immunoglobulin E
IGF	Insulin-like growth factor
IGFBP-3	Insulin-like growth factor-binding protein 3
IKK	I $\kappa$ B kinase
IL-1 $\beta$	Interleukin 1-beta
IL-1b	Interleukin-1b

IL-6	Interleukin-6
iNOS	Inducible nitric oxide synthase
ITZ	Itraconazole
JNK	c-Jun N-terminal kinase
Kg	Kilogram
Krt-14	Keratin 14
LCR	Leucoanthocyanidin reductase
LDH	Lactate dehydrogenase
LDOX	Leucoanthocyanidin dioxygenase
LXR- $\beta$	Liver X receptor- $\beta$
m	Meter
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte Chemoattractant Protein-1
MCTs	Monocarboxylate transporter
MDA	Malondialdehyde
MEK-1/2	Mitogen-activated protein/extracellular signal-regulated kinase-1/kinase-2
MG	Myasthenia gravis
Min	Minutes
MMP-9	Matrix metalloproteinase 9
Mol	Mole
MPO	Myeloperoxidase
MRP1 & MRP2	Multidrug resistance protein
mTORC1	Mammalian target of rapamycin complex 1
NADPH	Nicotinamide adenine dinucleotide phosphate
NF- $\kappa$ B	Nuclear factor kappa-light chain enhancer of activated B
NFATc1	Nuclear factor of activated T-cells 1
NHDF	Normal human dermal fibroblasts
NHEK	Normal human epidermal keratinocytes
Nrf 2	Nuclear factor erythroid 2-related factor 2
OAT	Organic anion transporter
OATP	Organic anion-transporting polypeptide
OSCC	Oral squamous cell carcinoma
OVX	Ovariectomized
P-gp	P-glycoprotein
PAL	Phenylalanine ammonia lyase
PDGF	Platelet-derived growth factor
PEP	Prolyl endopeptidase
PGC-1 $\alpha$	Peroxisome proliferator-activated receptor- $\gamma$ coactivator
PGDF	Platelet-derived growth factor
PGE2	Prostaglandin E2
PI3K	Phosphatidylinositol 3-kinase
PL	Phospholipid
PLC 1	Phospholipase C 1
PLGA	Poly lactic-co-glycolic acid

PPAP- $\alpha$	Peroxisome proliferator-activated receptor alpha
PPAR $\gamma$	Peroxisome proliferator-activated receptor
PPARc	Peroxisome proliferator-activated receptor c
PTK	Protein tyrosine kinase
PTP1B	Protein tyrosine phosphatase 1B
RAGE	Receptor for advanced glycation end products
RMP <sub>r</sub>	Rifampicin resistance
ROS	Reactive oxygen species
RSK	Ribosomal S6 kinase
SAKP	Serum alkaline phosphatase
SAR	Structure-activity relationship
SBIL	Serum bilirubin
SD	Sprague-Dawley
SDF 1	Stromal cell-derived factor-1
SDH	Sorbitol dehydrogenase
SEDDS	Self-emulsifying drug delivery system
SEPW1	Seleno protein W1
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamate-pyruvate transaminase
SIPO	State Intellectual Property Office
SIRT 1	Sirtuin 1
Sirt1	Silent mating type information regulation 2, homolog 1
SNEDDS	Self-nanoemulsifying drug delivery system
Sp1	Specificity protein 1
SREBP-1	Sterol regulatory element-binding proteins
STAT1/3	Signal transducer and activator of transcription 3
STAT6	Signal transducer and activator of transcription 6
TC	Total cholesterol
TCDD	Tetrachlorodibenzo-p-dioxin
TG	Triglycerides
TGF- $\beta$ 1	Transforming growth factor-beta 1
Th2	T-helper 1 cell
TLR4	Toll-like receptor 4
T <sub>max</sub>	Time at which C <sub>max</sub> is observed
TNF- $\alpha$	Tumor necrosis factor- $\alpha$
Tph-1	Tryptophan hydroxylase 1
TRAP	Tartrate-resistant acid phosphatase
UCP3	Uncoupling protein 3
UDP	Uridine diphosphate
UGT	UDP-glucuronosyltransferase
USPTO	United States Patent and Trademark Office
VCAM-1	Vascular cell adhesion protein 1
VGEF	Vascular endothelial growth factor
WIPO	World Intellectual Property Organization

# Chapter 1

## General Introduction and Sources of Flavonoids



Robinka Khajuria, Shalini Singh, and Amrit Bahl

### 1 Introduction

Flavonoids are a diverse group of polyphenolic plant metabolites that are present in fruits, grains, vegetables, tea, and wine (Panche et al. 2016). They were first isolated in 1930 from oranges and considered to be a member of a new class of vitamins, namely, as vitamin P. It was much later established that the new isolate was a flavonoid (Kumar and Pandey 2013). So far, over 10,000 flavonoids have been identified, and these natural products have become an essential component of various nutraceutical, medicinal, cosmetic, and pharmaceutical applications (Kozłowska and Szostak-Węgierek 2014). This increased use of flavonoids can be attributed to their anti-oxidative, antimutagenic, anticarcinogenic, anti-inflammatory properties and their ability to modulate major cellular enzyme function (Kumar and Pandey 2013). Flavonoids are hydroxylated phenolic compounds located in the nucleus of mesophyll cells and ROS generation centers synthesized by plants in response to microbial infection. The chemical nature of flavonoids depends on their structural class, degree of hydroxylation, other substitutions and conjugations, and degree of polymerization (Kelly et al. 2002; Agati et al. 2012). Based on their chemical structure, flavonoids are categorized into six subclasses known as flavonols, flavones, flavanols, flavanones, isoflavones, and anthocyanins. The activities of each subclass depend upon their chemical structure (Katyal et al. 2014).

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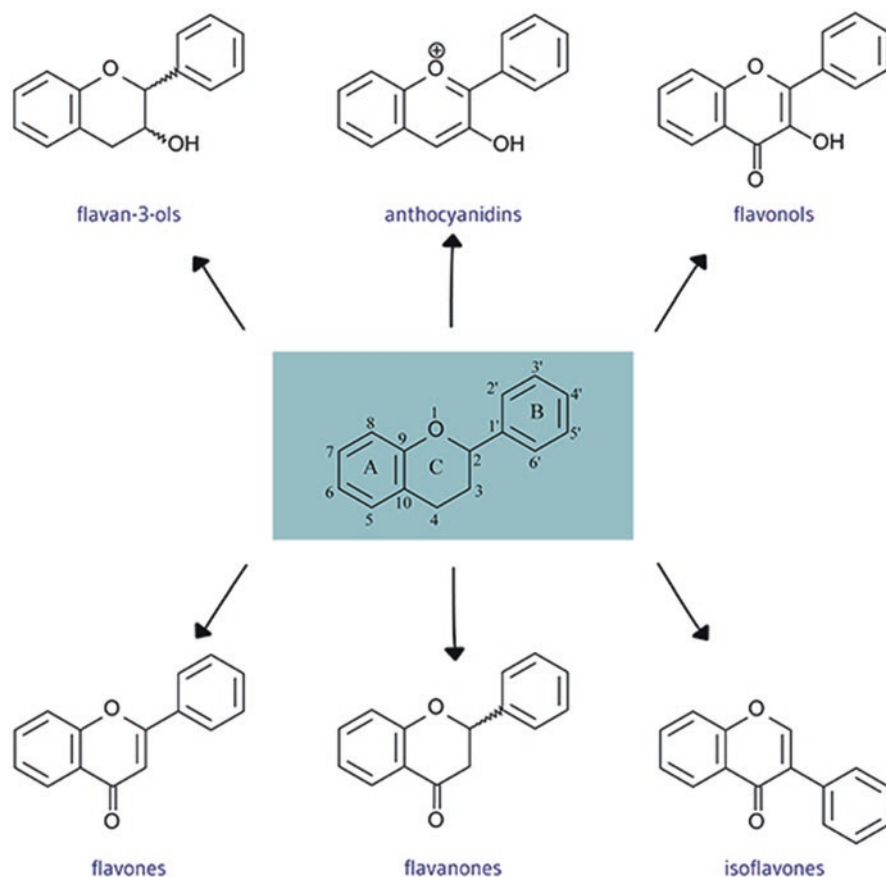
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## 2 Structure and Classification

As stated above, the chemical nature of flavonoids depends on their structural class and degree of substitutions and conjugations. Although they differ in the structure around the heterocyclic oxygen ring, all of them have the characteristic C6-C3-C6 carbon skeleton as shown in Fig. 1.1 (Yao et al. 2004). Their structure comprises of two benzene rings (A and B) linked by an oxygen-containing pyrene ring (C). Structurally, flavonoids are classified into two families, viz., 3-hydroxyflavonoids and 3-desoxyflavonoids. The former comprises of a hydroxyl group at C-3 positions of the C ring and includes flavonoids such as flavonols, leucoanthocyanidins, anthocyanidins, and catechins, while the latter comprising flavanones and flavones lack a hydroxyl group at C-3. Within these two families, the classification is based on the pattern in which additional hydroxyl or methyl groups have been introduced at the



**Fig. 1.1** Basic Structure of flavonoid subclasses. (Adapted from <https://pi.oregonstate.edu/mic/dietary-factors/phytochemicals/flavonoids>)



different positions of the molecule. For instance, in isoflavonoids, the B ring is bound to C-3 of ring C, while in anthocyanidins and catechins, carbonyl group on C-4 is absent (Williams and Harborne 1994). Flavonoids mainly exist in plants as glycosides, while those without a sugar moiety known as aglycones occur less frequently. At least eight different monosaccharaides or combinations of can attach to the different hydroxyl groups of the aglycones. The large number of flavonoids can be attributed to the different combinations of aglycones and these sugars. Among these sugars, glucose and l-rhamnose are the most commonly found ones. The glycosides are usually O-glycosides, with the sugar moiety attached to the hydroxyl group at the C-3 or C-7 position (Erlund 2004).

### 3 Mechanism of Flavonoid Action

In vitro and animal studies have revealed that food and beverages rich in flavonoids are associated with a decreased risk of age-related diseases (Khan et al. 2014). The benefits of flavonoids are due to their ability to scavenge a wide range of reactive oxygen, nitrogen, and chlorine radicals such as such as hydroxyl, peroxy, superoxide, and peroxyntrous acid. They also chelate ions which causes a decrease in the metal ion prooxidant activity (Silva et al. 2002). They have also been reported to inhibit free radical-mediated cytotoxicity and lipid peroxidation, inhibit tumor growth, and modulate endogenous hormone activity. Therefore, flavonoids may provide protection against chronic diseases such as atherosclerosis and cancer and assist in the management of menopausal symptoms. It is because of all these benefits that flavonoids are referred to as semi-essential food components (Yao et al. 2004). There are reports that show that flavonoids have better than commonly used natural and synthetic antioxidants such as ascorbic acid and  $\alpha$ -tocopherol and trolox, butylated hydroxyanisole, and butylated hydroxytoluene, respectively (Tsimogiannis and Oreopoulou 2004; Soobrattee et al. 2005).

### 4 Natural Sources of Flavonoids

Flavonoids are the most widely distributed phenolic compounds found in plants, especially those capable of carrying out photosynthesis. They are responsible for taste, color, prevention of fat oxidation, and protection of vitamins and enzymes. The distribution of flavonoids in plants depends on various factors such as variation and the degree of light exposure. For example, light intensity accelerates the formation of the higher oxidized flavonoids.

Flavonoids are also found in human and animals, but they are provided by the plant-based diet of the organism rather than being synthesized in situ (Clifford and Cuppett 2000; Yao et al. 2004). Plant-derived flavonoids are classified into ten chemical groups among which flavanones, flavones, flavans (flavanols),

**Table 1.1** Food sources of flavonoids

Subgroup	Representative flavonoids	Food sources
Flavanols	Catechins, gallic acid, epicatechin, epigallocatechin gallate, procyanidin	Fruits and flowers, apples, hops, tea, beer, wine, fruit juice
Flavanones	Hesperidin, naringenin, eriodictyol, neohesperidin	Citrus fruits, cumin, oranges, grapefruits, peppermint
Flavones	Apigenin, chrysin, luteolin, diosmetin, luteolin	Herbs, cereals, fruits, parsley, thyme, vegetables, flowers
Flavonols	Isorhamnetin, kaempferol, quercetin, myricetin, rutin	Onions, cherries, apples, broccoli, kale, tomatoes, berries, tea, red wine, Tartary buckwheat
Anthocyanin	Pelargonidin, cyanidin, delphinidin, and malvidin	Teas, honey, fruits, vegetables, nuts, olive oil, cocoa, and cereals
Isoflavonoids	Daidzein, genistein, glycitein, formononetin	Legumes (e.g., soybeans)

isoflavonoids, flavonols, and anthocyanins commonly occur in the diet. Flavonols constitute the most abundant flavonoids in foods, while flavanones occur in citrus fruits and flavones in herbs. Strawberries and other berries are rich in anthocyanins, while isoflavones are found in high amounts in soy foods (Aherne and O'Brien 2002). Anthocyanins and catechins are found in tea, fruits, and vegetables (Yao et al. 2004). The following section discusses the occurrence of different flavonoids in food (Table 1.1).

## 5 Occurrence of Flavonoids in Food

1. **Flavonols (3-hydroxyflavones):** The antioxidant properties of flavonols make them one of the most analyzed subgroups of flavonoids. These phytochemicals are found mainly in vegetables, fruits, and plant-based beverages such as green tea, black tea, and red wine. Apple, grape berries, tomato, onion, broccoli, and red lettuce are the major sources of flavonols (Brodowska 2017). Among the different dietary flavonols, quercetin is the most common flavonol present in food. It is present in various fruits and vegetables, but it is present in highest concentrations in onions. The food source of quercetin varies from country to country, depending on the availability of the food. For example, in Japan and the Netherlands, the main source of quercetin is tea, while in Italy wine is the major source of quercetin. In Finland, Greece, and the United States, onion and apples are the main dietary sources of quercetin. Quercetin exists in plants in different glycosidic forms, among which quercetin-3-rutinoside (quercetin-3-rhamnoglucoside or rutin) is the most widespread form. Quercetin in onions is bound to one

or two glucose molecules and is known as quercetin-4V-glucoside and quercetin-3,4V-glucoside, respectively. Other known dietary quercetin glycosides include quercetin galactosides from apple and quercetin arabinosides, present in berries. Other flavonols such as kaempferol (broccoli), myricetin (berries), and isorhamnetin (onion) are also present in the diet (Erlund 2004).

2. **Flavanones:** Are found extensively in citrus fruits. Although their concentration is highest in the solid tissues, but juices have also been reported to contain several hundred milligrams per liter of flavanones. Among the different flavanones found in citrus fruits, hesperidin (hesperetin-7-rutinoside) and narirutin (naringenin-7-rutinoside) from oranges and mandarins, respectively, are the major ones. Grapefruits are also known to contain flavanones known as naringin and narirutin (20%). Tomatoes and tomato-based products are also known to contain another flavanone known as naringenin. Tomato skin, especially from fresh tomatoes, contains naringenin chalcone that is converted to naringenin during processing to tomato ketchup (Erlund 2004).
3. **Flavones:** Are structurally similar to flavonol compounds with an extra substitution of hydroxyl group at the carbon 3-position. The two major dietary flavones are Apigenin and luteolin. The former is found in wheat sprouts, onions, parsley, oranges, chamomile, and tea, while the latter occurs in broccoli, onion leaves, celery, carrots, parsley, cabbages, peppers, chrysanthemum flowers, and apple skins (Lin et al. 2008).
4. **Anthocyanidins:** Are a group of natural pigments which are responsible for imparting color. They are responsible for the blue, purple, red, and orange color of fruits and vegetables. Till date, more than 500 different anthocyanidins have been reported in literature. Major dietary sources of anthocyanidins include fruits, vegetables, nuts, tea, honey, olive oil, cocoa, and berries such as black currant and blueberries. Some of the other dietary anthocyanidins include cyanidin, delphinidin, pelargonidin, and malvidin (Brodowska 2017).
5. **Isoflavones:** Are a very distinctive subclass of flavonoid compounds that consist of a 3-phenylchromen skeleton, derived chemically from the 2-phenylchromen skeleton by an aryl migration mechanism. They are commonly found in legumes, especially in soy. They have also been reported in green split peas, chickpeas, lima beans, black beans, clover sprouts, and sunflower seeds. Genistein and daidzein are the major isoflavones present in human diet (Clavel et al. 2005).
6. **Flavanols:** Are a complex group of polyphenols that range from the monomeric flavan-3-ols (e.g., catechin, epicatechin) to polymeric procyanidins called condensed tannins. They are found in fruits and fruit-derived products. They are also found in tea, red wine, cocoa, apples, kiwi, and cereals. However, they almost do not exist in vegetables and legumes except lentils and broad beans. Flavanols have also been reported in peels or seeds of fruits and vegetables as well (Brodowska 2017).

## 6 Conclusion

Flavonoids have gained a lot of interest in the last few decades with a number of beneficial effects being reported every year. The fact that this useful group of phytochemicals is a normal dietary component found in different fruits and vegetables that is consumed all over the world has generated immense interest not only in the scientific community but general population as well. Though a variety of benefits of flavonoids such as anticancer properties, antioxidant properties, anti-inflammatory properties, and cardiovascular and nervous system well-being have been reported, still a lot of research is needed to completely understand the usefulness of flavonoids in the diet to improve human health. The study of flavonoids is complex because of the wide variation in the chemical structures of the different subclasses. There is also a great scarcity of data in the long-term effects of chronic flavonoid ingestion. There is not only a need to deepen our understanding of these beneficial phytochemicals, but directing research toward the discovery of new flavonoids is also of utmost importance. In this context there is a need of continuing detailed *in vivo* and *in vitro* studies that will provide a hopeful and safe picture for the future.

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# Chapter 2

## Analytical Techniques for the Identification and Quantification of Flavonoids



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### 1 Introduction

The ability to provide timely, accurate, and reliable data is central to the role of analytical chemistry and is especially true in the discovery, development, and manufacturing of important flavonoids. From ancient times plant-based foods have been known for their healthy effects and for their actions in the prevention of illnesses, these effects being due to certain secondary metabolites (flavonoids) and their free radical scavenging activity. Flavonoids have potential for removing harmful chemicals from the body by acting as antioxidants and they demonstrate strong activity in healing, as do vitamins and tetraterpenoids. Polyphenolics are among the main biological molecules that are attractive because of their considerable protective chemical activity in the body. The flavonoids are a group of organic compounds formed after the reduction of cinnamic acid through three malonyl-CoA molecules. All flavonoids arise from this initial reaction, which is catalyzed by the enzyme chalcone synthase. Plant-based foods such as vegetables provide a considerable supply of dietary antioxidants, in addition to bioactive dietary components (flavonols) and organic sulfur compounds (Chaudhary et al. 2014). Compounds in foods that aid in the prevention and reduction of diseases are termed nutraceuticals, and foods such

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as vegetables and fruits that are a rich source of nutritional supplements have been used because of their diverse properties (Chaudhary et al. 2018). In the medical field, flavonoids have been recognized to have a broad range of pharmacological activities, e.g., antioxidant, antitumor, fungicidal, anthelmintic, anti-inflammatory, anti-allergenic, antimicrobial, and antiviral. Chalcones, a subclass of flavonoids, are usually converted rapidly into phenylbenzopyrans, and with additional modification, flavones, isoflavones, and anthocyanins are formed. Flavonoids are divided into various subclasses (Table 2.1), such as flavonols, e.g., quercetin; flavones, e.g., luteolin; flavanones, e.g., naringenin; flavans, e.g., catechin; anthocyanidins, e.g., cyanidin; and chalcones, e.g., tetrahydroxychalcone (Lee et al. 2005). Additional structural elaboration, mainly through glycosylation but also via acylation or alkylation, gives us the huge variety of flavonoid structures seen throughout the plant kingdom. Another particular advantage the analyst has in flavone analysis is the distinctive ultraviolet (UV) (or UV-vis) spectra of the six flavonoids quercetin, luteolin, genistein, cyanidin, naringenin, and epigallocatechin gallate (EGCG). Recently, we have reported that beta-ionone obtained from chalcones is a strong antiproliferative molecule (Sharma et al. 2013) and beta-ionone is derived from endoperoxides in chalcones by apoptosis (Sharma et al. 2014). Biosynthesis and genetic manipulation of plant pathways will improve the nutritional aspects of fruits and vegetables. Various techniques are used for flavonoid identification, and examples of these techniques that use online spectroscopic detection technology are: gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), LC-Fourier transform infrared (FTIR), LC-nuclear magnetic resonance (NMR), LC-NMR-MS, LC-photodiode array (PDA), and capillary electrophoresis (CE)-MS (Sarker and Nahar 2012). The application of these analytical instruments has significantly broadened the analysis of flavonoids from different sources, taking less time than traditional methods.

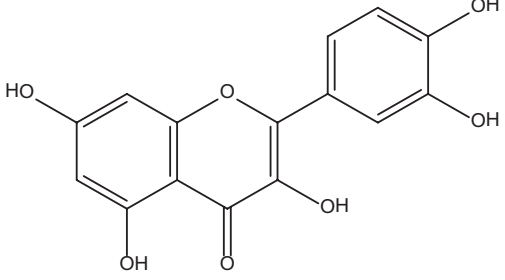
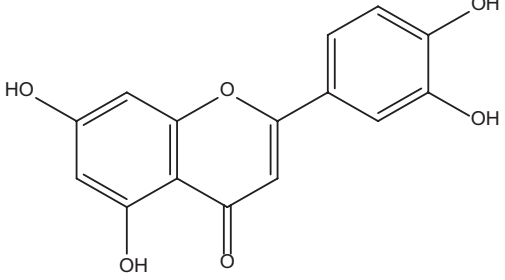
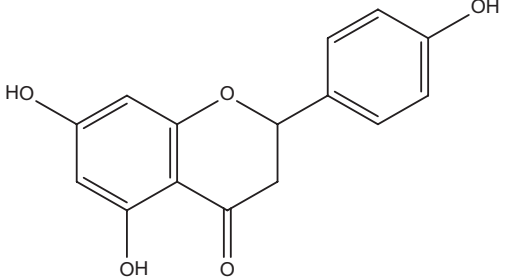
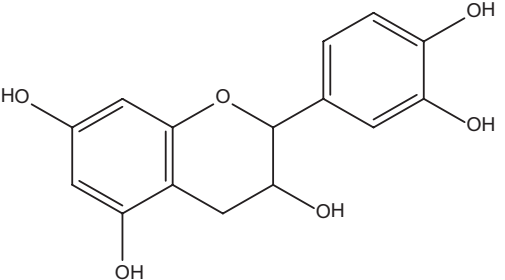
The present chapter focuses on the application of analytical techniques such as spectroscopic, chromatographic, tandem, and time-of-flight (TOF) to the isolation, separation, characterization, and online partial identification of molecules; chemical fingerprinting; and quality control of plant-based drugs, as well as metabolomic studies, with specific examples.

## 2 Tests for Detection (Preliminary Identification)

There are various tests for the preliminary identification of flavonoids, e.g.:

- Shinoda test
- Sodium hydroxide test
- p-Dimethylaminocinnamaldehyde test

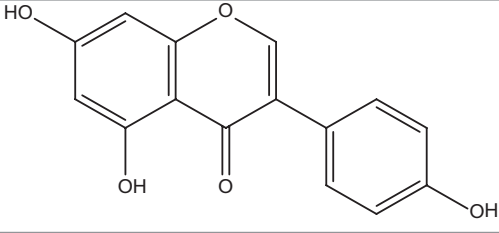
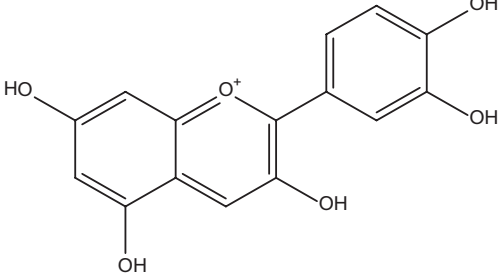
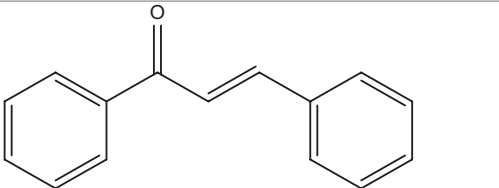
**Table 2.1** Different subclasses of flavonoids

Subclass no.	Subclass	Example	Structure
1	Flavonols	Quercetin	 <p>The structure of Quercetin is a flavonol. It consists of a central chromone ring system. The A-ring (left) has hydroxyl groups at positions 5 and 7. The C-ring (right) has a hydroxyl group at position 3 and a 3,4,5-trihydroxyphenyl group at position 2.</p>
2	Flavones	Luteolin	 <p>The structure of Luteolin is a flavone. It consists of a central chromone ring system. The A-ring (left) has hydroxyl groups at positions 5 and 7. The C-ring (right) has a 3,4,5-trihydroxyphenyl group at position 2.</p>
3	Flavanones	Naringenin	 <p>The structure of Naringenin is a flavanone. It consists of a central chromane ring system. The A-ring (left) has hydroxyl groups at positions 5 and 7. The C-ring (right) has a 4-hydroxyphenyl group at position 2.</p>
4	Flavans	Catechin	 <p>The structure of Catechin is a flavan. It consists of a central chromane ring system. The A-ring (left) has hydroxyl groups at positions 5 and 7. The C-ring (right) has a 3,4,5-trihydroxyphenyl group at position 2 and a hydroxyl group at position 3.</p>

(continued)



**Table 2.1** (continued)

Subclass no.	Subclass	Example	Structure
5	Isoflavones	Genistein	
6	Anthocyanidins	Cyanidin	
7	Chalcones	Chalcone	

## 2.1 Estimation of Total Flavonoid Content (TFC)

The flavonoid content of various plant extracts was monitored by the  $\text{AlCl}_3$  method. This assay is colorimetric, in which aluminum chloride utilizes four positions of the carbon ketone group and the third and fifth OH groups of flavonoids. The content of flavonoids was determined and represented in terms of milligrams of quercetin per gram of dry extract (Madaan et al. 2011).

## 2.2 Tandem Techniques

### 2.2.1 Gas Chromatography-Mass Spectrometry (GC-MS)

The analytical GC-MS techniques utilize gas chromatography and mass spectrometry together. The GC works on the principle that a mixture will be broken down into individual substances upon heating. The heated gases will pass through a column

opening along with an inert gas (helium). Mass spectroscopy identifies the compounds by the mass of the individual substance. The mass is compared with a library database stored in a computer with a fragment pattern. The advantage of GC is its high sensitivity for the volatile and non-volatile compounds that are frequently derivatives. Combined silica capillary columns are used in GC to attain more resolution. Flavonoids are transformed to the corresponding trimethylsilyl (TMS) derivatives, which are usually introduced on the nonpolar column. Further, the break-up of the molecules is carried out at temperatures programmed up to 300 °C with a linear gradient of 30–90 min. The mass by charge ratio of ion and fragments produced by the loss of methyl group or CO and retro Diels–Alder reactions are generally used.

Different investigators have used GC-MS in some plants for flavonoid detection (Canini et al. 2007). Canini et al. (2007) identified quercetin in the leaf of *Carica papaya* by GC-MS examination. The GC-MS investigation was performed in the selective ion monitoring (SIM) mode. The detection of each peak was attained by determining the retention time (RT) and the relative mass spectra of the compounds in the leaf extract, corresponding to the area ratio of characteristic ions with individual standards. For quantitative analysis, a series of solutions of internal standards with known concentrations was used. Genistein was used as the internal standard for flavonoid detection.

Schmidt et al. (1994) identified 51 flavonoid aglycones by GC. Flavonoid-rich extracts were prepared from the flowers of *Arnica alpha* subspecies *attenuata* by employing Sephadex LH-20, a column with methanol from the methanol-soluble part of a methylene dichloride extract. Füzfai and Molnár-Perl (2007) demonstrated that anthocyanidins and flavanones produced oximes, while flavonol and flavones produced a TMS derivative. The fragmentation patterns and quantization potential of three anthocyanidins, one flavonol, two flavones, and two flavanones were reported as indicating TMS and oxime derivatives. Farag et al. (2007) reported the extensive detection of flavonoids in *Medicago truncatula* by GC-MS.

### 2.2.2 Liquid Chromatography-Mass Spectroscopy (LC-MS)

In LC, liquid in the columns passes between immiscible, stationary, and movable phases. The compound of concern in the movable phase is isolated on the basis of its varying physicochemical exchanges through the fixed and movable phases. The principle of the phytochemical screening of flavonoids by LC with MS depends on the isolation and identification of natural flavonoid moieties in negative or positive ionic form to elucidate their mass behavior.

Fabre et al. (2001) identified 11 naturally occurring flavonoids, representing flavones, flavonol, and flavanone, by using high-performance (HP) LC coupled with negative ion electrospray ionization (ESI)-MS/MS. Each molecule was further investigated through loop insertion on trap MS. The negative ion ESI-MS/MS

behavior of the different aglycones examined and a study shows remarkable variation if matched with the previously reported patterns obtained using ionization techniques in positive ion mode. Sulaiman and Balachandran (2016) reported that *Tragia involucrata* extract contained flavonoids including iridin, dihexosylquercetin, and quercetin-3-O-rutinoside. The analysis was carried out by LC-ESI-MS Agilent 6520 accurate mass Q-TOF LC/MS. Duan et al. (2011) reported a method with high accuracy, sensitive for LC-MS/MS synchronized identification of different flavonoids after oral administration of *Verbena officinalis* L. extract for their pharmacokinetic studies with 5-min runtime.

### 2.2.3 Liquid Chromatography-Diode Array Detector (LC-DAD)

A DAD is a non-destructive detector that measures UV absorption at single or multiple wavelengths of the column eluent. LC coupled with a DAD is capable of detecting the complete UV-Vis range of increased molecular spectral signature.

Zehl et al. (2011) assessed approximate and measurable amounts of flavonoids in four therapeutically used *Drosera* species, for quality control purposes. They used a reliable, cheap, and consistent reverse phase-LC-DAD technique for the simultaneous quantization of flavonoids and ellagic acid derivatives and this method was thoroughly validated.

### 2.2.4 Liquid Chromatography-Nuclear Magnetic Resonance (LC-NMR)

The combination of LC with NMR spectroscopy has been at the frontline of the latest and best technologies to deal with structural issues in flavonoid compounds (Gonnella 2013). Braunberger et al. (2013) reported that the structure of 13 compounds was revealed by LC-MS, LC-NMR, and offline NMR experiments after the isolation of quercetin and kaempferol and their glycone conjugates. Zehl et al. (2011) reported 13 compounds isolated from *Drosera* species, using LC-MS and LC-NMR tests after focused separation by offline heteronuclear two-dimensional NMR.

### 2.2.5 Capillary Electrophoresis (CE)

Capillary electrophoresis is a novel technique for the large-scale investigation of pharmaceuticals, and it has been used for flavonoid analysis for the past decade. In contrast to conventional chromatographic methods, it has admirable separation, resolution, and quick runtime; it is simple to automate, and has less solvent and sample consumption. It is a potent method for the approximate and measurable investigation of flavonoids and it also enhances detection when various detectors are attached to the CE equipment. Different types of CE separation are used, e.g.,

capillary zone electrophoresis (CZE), micellar electrokinetic chromatography (MEKC), and capillary electrochromatography (CEC). Fonseca et al. (2007) reported 11 phenolic compounds, including seven flavonoids, isolated from *Chamomilla recutita* separated by CEC coupled with UV with eluent phosphate buffers. Sun et al. (2008) reported that the flavonoids catechin and quercetin were isolated from different types of wines, separated by MEKC coupled with UV with an eluent borate buffer. Chen et al. (2008) reported hesperidin and naringin in grapefruit peel and juice, separated by CE coupled with an electrochemical detector having an eluent borate buffer. Zhang et al. (2008) determined kaempferol, quercetin, and catechin isolated from *Chrysanthemum* separated by CZE coupled with an amperometric detector having an eluent mixture of methyl and ethyl alcohol modifiers. Segura-Carretero et al. (2008), using CE-TOF-MS, reported delphinidin and the cyanidin-3 derivative sambubioside as major compounds in *Hibiscuss abdariffa* L, while other minor compounds were also determined by the same method.

### 2.2.6 Infrared (IR) Spectroscopy

Infrared spectroscopy is a technique in which IR radiation utilizes the same fundamentals as FTIR, requiring a mathematical process that converts the raw data into an actual spectrum. Wulandari et al. (2016) reported a simple, selective, and eco-friendly method for determining flavonoids in medicinal plant extracts by chemometrics, using near-infrared (NIR) spectroscopy. FTIR testing exposed the existence of phenols and flavonoids in propolis from Brazil and the United Kingdom, pomegranate, and dragon's blood. The Brazilian propolis showed the greatest percentage of phenolic content compared with pomegranate and propolis from the United Kingdom. The maximum flavonoid content was reported in propolis from the United Kingdom, followed by Brazilian propolis, pomegranate, and sage, all of which had similar flavonoid contents, while dragon's blood had the minimum flavonoid content (Oliveira et al. 2016).

### 2.2.7 Matrix-Assisted Laser Desorption/Ionization-Time-of-Flight Mass Spectroscopy (MALDI-TOF-MS)

MALDI is a laser striking matrix ionization technique that creates ions from small molecules to transform analyte molecules into the gas phase with minimal fragmentation and without decomposing them. A mass analyzer is used to analyse TOF.

Monagas et al. (2010) attempted to determine the molecular weight distribution of proanthocyanidin through MALDI-TOF. Frison-Norrie and Sporns (2002) determined flavonoids in the seed coats of almonds using MALDI-TOF-MS; further, the results were verified by HPLC.

### 2.2.8 Ultra-High-Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry (UHPLC/Q-TOF-MS)

The UHPLC/Q-TOF-MS method is a new approach in chromatographic separation and has been successfully employed for fast, high-resolution separation with the required sensitivity. The TOF-MS method is helpful for elucidation of the structure of the separated compounds and for identification of their fragmentation patterns. Wu et al. (2017) reported UHPLC/Q-TOF-MS information on phenolics isolated from *Canarium pimela* leaves and noted their vasorelaxant and body-protective chemical activity; 16 molecules were tentatively detected, of which 9 were flavonoids. Bhatt et al. (2017) identified and characterized 12 different compounds isolated from the foliage of *Zanthoxylum armatum* via ultra pressure liquid chromatography (UPLC) ESI-Q-TOF-MS in positive ion mode. Further, they developed a rapid and simple UPLC-DAD method for determining these compounds. Overall, 18 different compounds were identified by evaluating RT values, UV findings, and MS/MS fragments by tandem mass spectrometry.

### 2.2.9 Nuclear Magnetic Resonance (NMR)

NMR is a physical property in which nuclei in a magnetic field absorb and reemit electromagnetic radiation. The chemical shift of a particular nucleus can be correlated with its chemical environment. The scalar coupling (or J-coupling) indicates an indirect interaction between individual nuclei, mediated by electrons in a chemical bond under suitable conditions. The area of a resonance is related to the number of nuclei giving rise to it. The most common type of NMR is  $^1\text{H}$  NMR (proton) and another common NMR type is  $^{13}\text{C}$  carbon. Apart from these common types of NMR there are various homonuclear through-bond correlation methods such as correlation spectroscopy (COSY) and total correlation spectroscopy (TOCSY). Furthermore, heteronuclear through-bond correlation methods such as heteronuclear single quantum correlation (HSQC) and heteronuclear multiple bond correlation (HMBC) are also used, with other through-space correlation methods such as nuclear overhauser effect spectroscopy (NOESY) also being employed.

#### 2.2.10 $^1\text{H}$ NMR

The  $^1\text{H}$  NMR technique is a boon for organic chemists, as it differentiates various kinds of protons present in any scaffolds. In India, the available  $^1\text{H}$  NMR instruments range from 60 to 1200 MHz. The method provides information about the various protons attached to different entities. A spectral line suitable to a particular proton splits up into two or more spectral lines after interacting with the magnetic field of the nonequivalent proton on the adjacent carbon. This splitting up of signals is known as spin-spin coupling, and the number of signals on splitting depends upon the number of protons that affect this signal. The number of signals after coupling

will be equal to the number of protons. The distance between the centers of two adjacent peaks in a multiplet, obtained by spin-spin coupling, is called the coupling constant ( $J$ ). It is expressed in hertz and its value lies between 0 and 20 Hz; the value of  $J$  does not depend upon the magnitude of the external field. The  $^1\text{H}$  center is the main experimental basis of this spectroscopy.

### 2.2.11 $^{13}\text{C}$ NMR

The  $^{13}\text{C}$  spectrum offers further characterization of a molecule as it relates directly to the carbon skeleton. Carbon-12 has no nuclear spin; hence, we are forced to observe the carbon-13 nucleus. So far  $^{13}\text{C}$  NMR is an important tool in chemical structure elucidation in organic chemistry (Fig. 2.1).

## 2.3 Chromatographic Techniques

### 2.3.1 High-Performance Thin-Layer Chromatography (HPTLC)

HPTLC is the most sophisticated version of TLC. The HPTLC method enables the maximum separation of the analyte band; it utilizes state-of-the-art instrumentation, and is routinely used in analytical laboratories. HPTLC methods have been described by various authors (Avula et al. 2012; Baghel et al. 2017; Bhandari et al. 2007; Bilu et al. 2005; Cui et al. 2011) and these methods have been found to be optimal depending upon the plant species and other factors. Cui et al. (2011) describe the HPTLC-mediated detection and evaluation of flavonoids from eight species of *Indocalamus*.

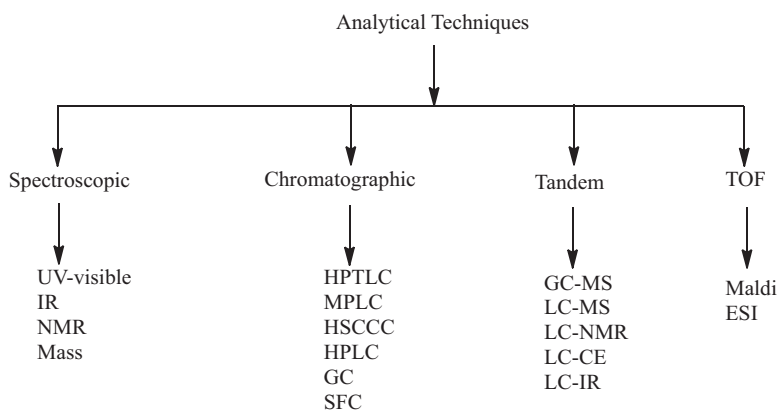


Fig. 2.1 Schematic of different analytical techniques

### 2.3.2 Medium Pressure Liquid Chromatography (MPLC)

MPLC refers to column chromatography with the utilization of pressure for the separation of metabolites (5–20 bars). Introduced in the 1970s, MPLC overcame one of the major problems of chromatography, i.e., limited sample loading. Compared with open column chromatography, MPLC provides both better separation and shorter duration of the separation. With increasing pressure on the column, the particle size is known to be reduced and the solvent range is found to be increased. The smaller the particle size, the better is the resolution of separation (Hostettmann and Terreaux 2000). MPLC can accommodate a large sample and therefore is ideal for flavonoid separation. MPLC is generally used in conjunction with other purification techniques for higher purity. Wang et al. (2010) describe, in some detail, a method of separation of flavonoids from *Belamcanda* using MPLC.

### 2.3.3 High-Speed Counter Current Chromatography (HSCCC)

Yoichiro Ito developed HSCCC (Berthod et al. 2009) in the 1980s. The separation refers to the differential distribution of solute particles between two non-miscible liquid phases and an immobile stationary liquid phase, which is held in place by a centrifugal force field. The distribution of the amount of solute between two liquid phases is represented by the ratio of the solute in the upper and lower phases, also known as the distribution coefficient ( $K_D$ ). For good separation, it is recommended to have a distribution ratio of between 0.5 and 2. At present, HSCCC is not close to HPLC in regard to resolution and efficiency because of its lower power of separation. However, the amount and the volume that can be loaded on the HSCCC equipment, and its cheaper cost, make it a suitable analytical method. HSCCC-MS equipment can be complementary to HPLC-MS. HSCCC has been used in conjunction with other chromatographic methods for better results (Chen et al. 2003, 2005; He et al. 2010; Li et al. 2014). Chen et al. (2005) have used this method for the separation of flavonoids, describing the splitting of flavonoids from the seeds of *Oroxylum indicum*. HSCCC can be coupled with a suitable mass spectrometer without any additional pump for MS analysis. Using this method, Chen et al. (2005) have been able to show the separation of a mixture of standard flavonoids such as baicalein and chrysin, using extracts obtained from the seeds of *Oroxylum indicum*. Others have used this method in conjunction with HPLC to separate flavonoids (Li et al. 2014), and He et al. (2010) used HSCCC in conjunction with HPLC to separate flavonoids, including quercetin, from “blackcurrant” leaves

### 2.3.4 High-Performance Liquid Chromatography (HPLC)

HPLC is one of the best and most efficient techniques employed extensively for the separation of compounds in analytical chemistry. Any compound miscible with solvents compatible with HPLC can be injected, split, identified, and quantified using

HPLC (Marston 2007). The method developed by Engida et al. (2013) describes the extraction of flavonoids from the plant *Sarang semut* quite nicely. The plant sample was extracted at an optimum condition for HPLC analysis using the method of Weisz et al. (2009). Using this method, Engida et al. (2013) were able to identify and quantify five flavonoids (kaempferol, luteolin, rutin, quercetin, and apigenin) by matching the retention time and the spectral characteristics against the standards, and the amount was subsequently deduced from the calibration curves. There are a few other reports (Baghel et al. 2017; Lee et al. 2011; Mattila et al. 2000; Pereira et al. 2004; Weisz et al. 2009) that describe the usage of HPLC alone or with some other chromatographic technique for the separation of flavonoids.

### 2.3.5 Supercritical Fluid Chromatography (SFC)

In SFC a supercritical fluid is the movable phase, which typically is a very low-viscosity compressible fluid, allowing for rapid mass transfer. This liquid facilitates the utilization of higher velocities in the method, resulting in much shorter examination times while maintaining efficiency. The movable phase is usually supercritical CO<sub>2</sub> with a low quantity of unprocessed modifier (usually methanol). The columns can be conventionally filled HPLC columns. Because of the non-polar nature of supercritical CO<sub>2</sub>, SFC works like normal-phase HPLC and methanol enhances the separation efficiency. SFC has an advantage over HPLC in that retention times are shorter and compound peaks are sharper and this provides better sensitivity of detection. Since CO<sub>2</sub> is transparent in the IR detection range, FTIR detectors can also be used in addition to the utilization of UV detection. SFC can be used with both flame ionization detection and mass spectrometry without any requirement for sample derivatization for gas liquid chromatography (GLC). This is a considerable advantage over GLC (Lafont et al. 2012). SFC was utilized by Huang et al. (2017) for the separation of flavonoids from *Chrysanthemum morifolium*. Briefly, 5 g of sample powder was extracted with ethanol: water (7:3) at 50 °C for 2 h. The samples were dried and dissolved in ethanol:water (7:3), followed by filtration through 0.22- $\mu$ m membrane filters. Mobile phase A was CO<sub>2</sub> (supercritical) and mobile phase B was 0.1% phosphoric acid in MeOH. Huang et al. (2017) documented various conditions for the different columns they used in their study.

## 3 Conclusion

This chapter has outlined various analytical techniques, including chromatographic, spectroscopic, electrophoretic, and electrochemical methods, and their respective protocols; these techniques are beneficial for the pharmaceutical, food, and biotechnological industries. The methods outlined in this chapter are quick, cheap, reliable, and useful and are validated methods for phytochemical screening and for the identification of markers; the methods can be developed on an industrial scale and their use can enhance human welfare.



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# Chapter 3

## Chemistry and Synthetic Overview of Flavonoids



Ajay Sharma, Hardeep Singh Tuli, and Anil K. Sharma

### 1 Introduction

Natural products have been used as food products and for therapeutic benefits for thousands of years. Flavonoids are the class of natural polyphenolic compounds derived as secondary metabolites from plants and fungus, and their direct association has been reportedly found with the human health. They have been known to play multiple roles in plants including UV filtration, detoxifying agents, symbiotic nitrogen fixation, self-healing agents, and floral pigmentation. Besides, they also act as chemical messengers, antimicrobial defensive agents, auxin transport inhibitors, physiological regulators, photoreceptors, and cell cycle inhibitors. They are being used as health-benefited and disease-averting dietary supplements because they possess a wide range of biochemical and pharmacological activities in the containment of various diseases including oxidative damage, chronic diseases, cardiovascular diseases, cancer, neurodegenerative diseases, gastrointestinal disorders, and others. Flavonoids are supposed to interact with receptive sites or receptors of the cells. Molecular structures, physical and chemical properties of the receptor largely determine what moieties are essential for affinity with the receptors (de la Rosa et al. 2010; Andersen and Markham 2006). The goal of this chapter is to highlight the structural features, classification, and their common food sources along with brief chemical and biosynthetic methods of flavonoids. In addition to this, the structure-activity relationship facilitates the relationship between their molecular structure and biological or physicochemical activities. It will be helpful in the

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improvement of the effect or the potency of flavonoids by altering its chemical structural functionalities and development of the new flavonoid derivatives of therapeutic values.

## 2 Structural Features and Classification

The word “flavonoid” originates from Latin word flavus which means “yellow.” Flavonoids are widely distributed in plant kingdom especially in fruits, flowers, vegetables, and herbs included as pigment color from yellow to red to blue. They are responsible for vivid coloration, taste properties, prevention of fat oxidation, and vitamin as well as enzyme protection in foods and plants. More than 8000 flavonoids of which several hundred are found in edible plants have been reported and characterized (Bone and Mills 2013). Chemically, flavonoids have 2-phenylchromane nucleus (C6-C3-C6) which consists of a heterocyclic pyrane ring (C) fused with the ring (A) and linked to the benzene ring (B). The various groupings of multiple hydroxyls (-OH), methoxyl (-OCH<sub>3</sub>), and glycoside group substituents along with oxo group at position 4 of ring C are present on the basic skeleton of flavonoids. They can be categorized into a variety of subclasses on the basis of the different oxidation level, unsaturation and substitution pattern of the C ring, as well as the bonding position of ring B to either C2/C3/C4 carbon of ring C. Among the subclasses of flavonoids, each compound differs in the substituent’s position on the rings A and B from others. The flavonoids further classified into other subclasses by the account of the ring B bonding position either at C2/C3/C4 position of ring C as well as the structural features of ring C. In addition of the ring B position at C2 position, flavones have C2–C3 double bond along with oxo group (=O) at C4 position of ring C while flavonols have a hydroxyl group (OH) at C3 position of ring C as well as a double bond between C2 and C3 along with oxo group (=O) at C4 position of ring C. Besides these subclasses, flavanones are also known as dihydroflavones because they have saturated C rings, i.e., lacking C2–C3 double bond. In a similar way, flavanonols have also saturated C rings, i.e., lacking C2–C3 double bond, but the hydroxyl group is present at position C3 of the same ring. Therefore they are also known as dihydroflavonols or 3-hydroxy derivatives of flavanones. In case of flavan-3-ol, the hydroxyl group (-OH) presents at position C3 of ring C with two chiral centers at positions C2 and C3 of ring C and absence of oxo group (=O) at position C4 of the same ring. The presence of chiral centers may result in the possibility of four diastereoisomers. They have the ability to form polymers, resulting in the formation of proanthocyanidins which undergo acid-catalyzed cleavage, and to form the anthocyanidins. When the ring B is attached to the C3 position of the ring C in flavonoids, they are called as isoflavones, whereas the same ring presents at the C4 position of ring C in the case of neoflavonoids. The next subclass of flavonoids is the plant pigments which are commonly called as anthocyanidins responsible for plant color. These are flavylium cations while counterions are mostly chloride. Chalcones and dihydrochalcones also belong to flavonoids because of

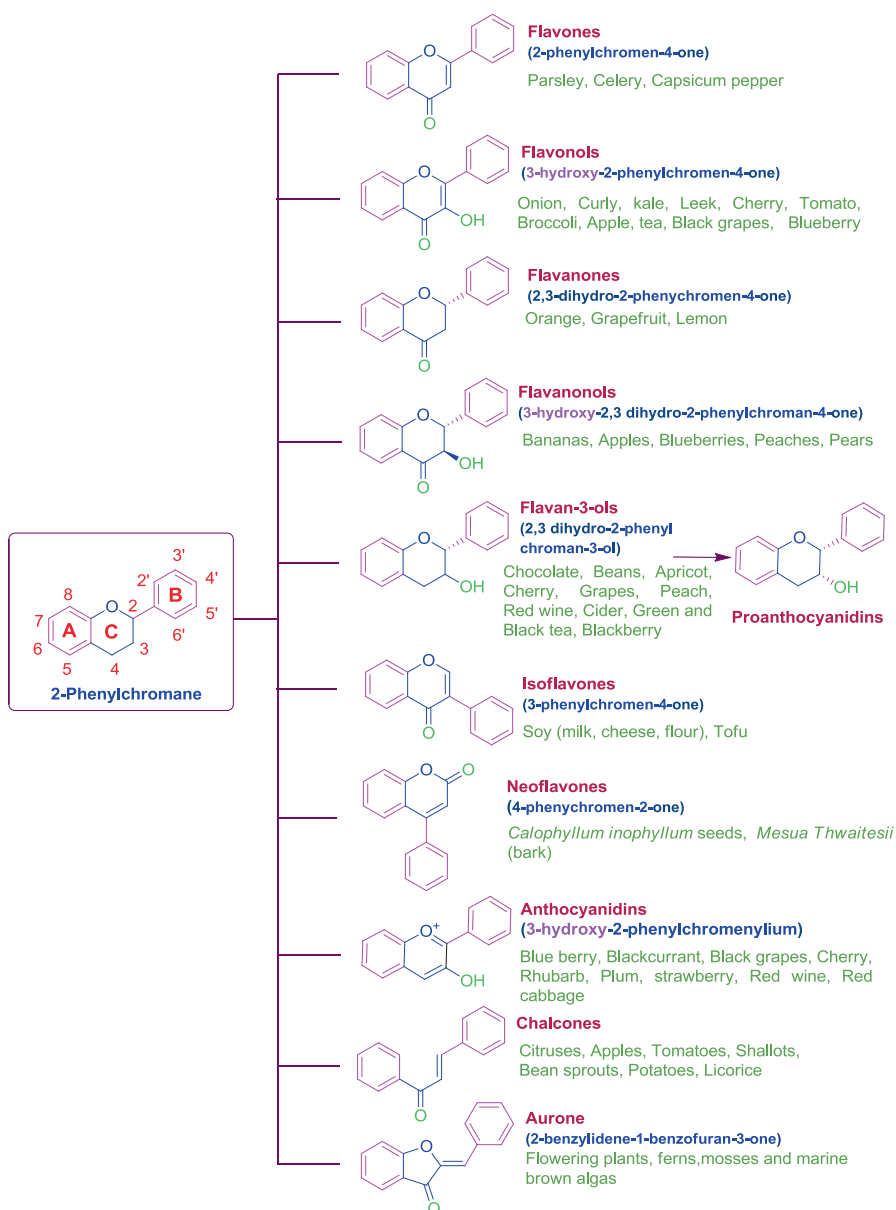
their synthetic pathways being similar to that of flavonoids despite having an open structure. Aurones is a rarely occurring subclass of flavonoids in nature and contains a benzofuran ring system which is linked at position 2 of benzylidene. They exist in two isomeric forms, i.e., (E) and (Z) configurations. The information about different subclasses of flavonoids and their derivatives along with common sources is summarized in Figs. 3.1 and 3.2 (Bhagwat et al. 2013; Kozłowska and Szostak-Węgierek 2014; Kumar and Pandey 2013; Erdman et al. 2007; de la Rosa et al. 2010; Andersen and Markham 2006). These flavonoids may exist as aglycones which are basic structures of these compounds and their methylated, acetylated, sulfonated congener and glycoside derivatives. All the structural features or configurations of flavonoids such as the number of hydroxyl (OH) functional groups and substitution pattern of functional groups in nucleus edifice determines the bioavailability, metabolism, biochemical, and pharmacological activities (Kumar and Pandey 2013; Erdman et al. 2007; Teles et al. 2018; Correia-da-Silva et al. 2013; Wen et al. 2017; Bone and Mills 2013; Sharma et al. 2018a, b).

### 3 Structure-Activity Relationship

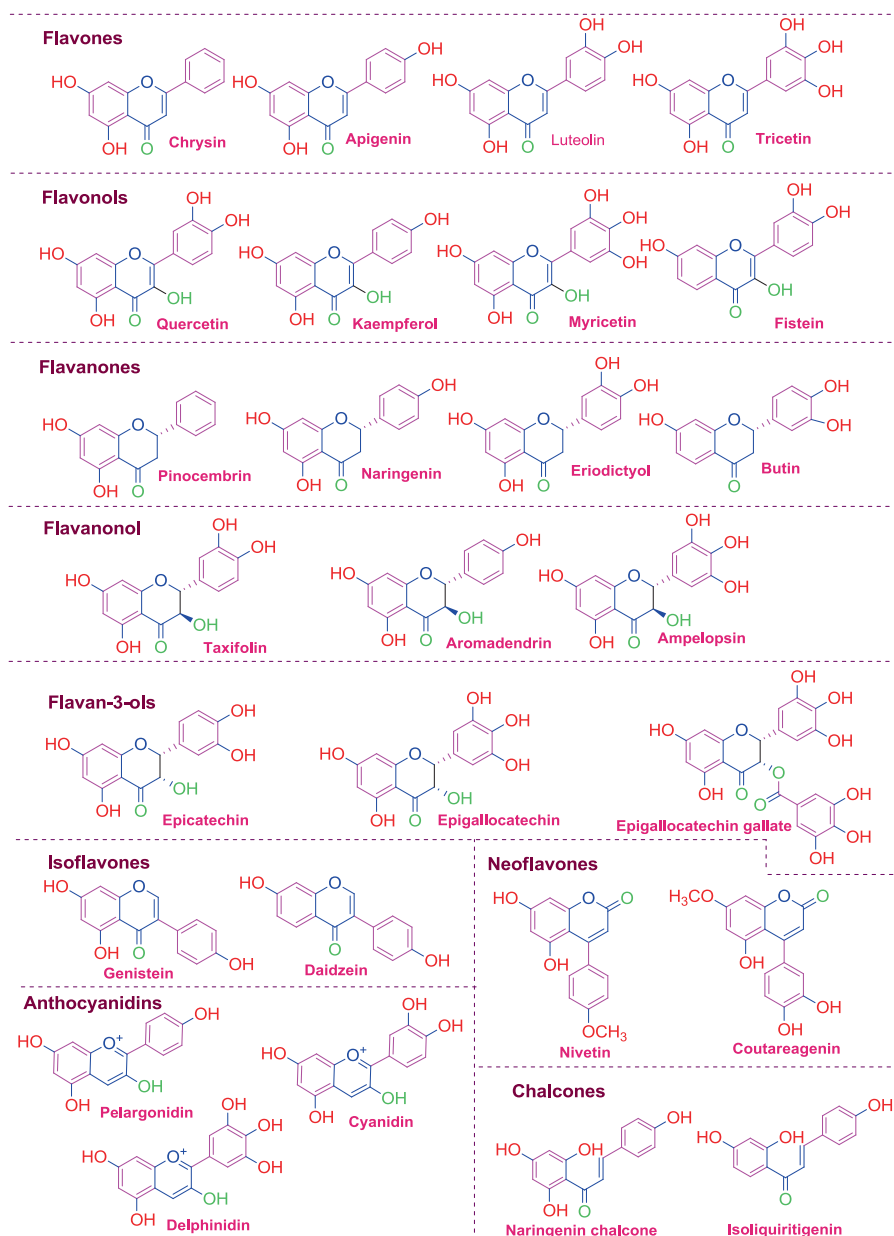
The chemical structure along with substituent's nature and their positions affects the apparent potency of the bioactive compound. It facilitates the determination of chemical groups which is accountable for evoking a remarkable biological effect in the organism. The chemical structure and functionalities of flavonoids, that is, the presence and positions of hydroxyl groups and substitution pattern of functional groups and C2–C3 double bond, are responsible to interact with receptive sites or receptors in the tissue that are accountable for their biochemical and pharmacological properties. In general, the structure-activity relationship of flavonoids among various therapeutic applications has been summarized as below:

The favorable health effects of flavonoids have been reported due to various proposed mechanisms such as antioxidant effects, enzyme inhibition, gene regulation, and metal chelation (Erlejmán et al. 2004). The free radicals cause various injuries that can be prevented by flavonoids through the following mechanisms: (a) direct reactive oxygen species (ROS) scavenging; (b) antioxidant enzyme activation; (c) metal chelation property; (d)  $\alpha$ -tocopheryl radical reduction; (e) oxidase inhibition; (f) mitigation of oxidative stress instigated by nitric oxide; (g) rise in uric acid levels; and (h) rise of antioxidant activities of low molecular antioxidants (Prochazkova et al. 2011). The major structural features of flavonoids for antioxidant activity are given below:

- (i) The number of hydroxyl groups and their substitution patterns on B ring affects the antioxidant activity. These parameters confer the formation of phenoxyl radical after the hydrogen atom donation and result in the high stability of the flavonoid due to the electron delocalization. The following parameters about a number of hydroxyl groups and their substitution pattern on B ring are:



**Fig. 3.1** The basic skeleton of flavonoids and their subclasses with common food sources



**Fig. 3.2** Chemical structures of some commonly occurring derivatives of natural flavonoid's subclasses with different substitution patterns have been represented



- (ia) 1,2-Benzenediol (catechol moiety)
  - (ib) 1,4-Benzenediol (hydroquinone moiety)
  - (ic) 1,2,3-Benzenetriol (galloyl moiety)
- (ii) The presence of a 4-oxo group with C2–C3 double bond on the C ring causes the movement of the electron to the C ring from phenoxyl radicals of the B ring.
  - (iii) The 3-OH group present in combination with C2–C3 double bond in flavonoids increases the resonance stabilization for electron movement across the molecule.
  - (iv) The presence of 3- and 5-OH groups in ring A with the 4-oxo functionality in C rings is a crucial factor for maximum radical scavenging ability.
  - (v) The presence of hydroxyl group at C3 position of ring C is also vital for anti-oxidant activity because it enhances the stability of the flavonoid radical.

Due to the occurrence of 3-OH, flavonols and flavan-3-ols are planar, whereas the flavones and dihydroflavones are slightly twisted. The planarity factor is responsible for conjugation and electron dislocation which are further responsive to increase flavonoid phenoxyl radical stability. Removal of the 3-OH group abolishes the planarity and conjugation which lower down the desired antioxidant properties. The glycosylation at 3-OH group also decreases their activity in comparison with their corresponding aglycones because of the steric effect having pronounced effect on activity (Sharma et al. 2018a; Dai and Mumper 2010; Heim et al. 2002).

The prooxidant activity of flavonoids, as well as their electrophilic coupling reactions with biological molecules, has also been proposed for their anticancer and anti-inflammatory effects. This includes the oxidation of flavonoids into electrophilic quinones (o-quinones or p-quinones), and these quinones are very reactive toward nucleophilic natured thiols and amino groups of proteins and glutathione. These reactions lead to the formation of different addition products that are responsible for their valuable biological effects. The presence of functionalities on B ring like either catechol moiety, hydroquinone moiety, or galloyl moiety in flavonoids is significant, and, on oxidation, these lead the formation of electrophilic quinones while resorcinol (1,3-benzenediol) cannot readily undergo oxidization. The presence of a C2–C3 double bond and hydroxyl groups at 5 and 7 positions of ring A with 4' position at ring B are the requisite basic structural features for anti-inflammatory activity. The presence of hydroxyl group at either 2' or 3'-position of ring B reduced the activity, while the 5'-OH group or 4'-OCH<sub>3</sub> on ring B abolished the activity. The hydroxy derivatives have more potency than their corresponding methoxy derivatives. The glycosides also possess lower potency than their corresponding aglycones (Sharma et al. 2018a; Nambi et al. 1996; Ravishankar et al. 2013, Batra and Sharma 2013; Chen et al. 2016; Lopez-Lazaro 2002). The cytotoxicity and apoptosis induction effect of flavonoids on human leukemia cells were reported by Chang et al. The structure-activity analysis displayed that the presence of the C2–C3 double bond may be crucial for effective cytotoxicity. In addition to this, the hydroxyl group at positions 3 (ring C) and 6 (ring A), as well as the catechol moiety in ring B, may

enhance the cytotoxic activity, while 5-OH and resorcinol moiety in ring B may reduce the cytotoxic activity (Chang et al. 2010).

Flavonoids may show a defensive role in the fight against cancer, cardiovascular diseases, and age-linked degenerative diseases. They could interact with several effluxes like P-gp (P-glycoprotein), MRP1 and MRP2 (multidrug resistance proteins), BCRP (breast cancer resistance protein), and uptake transporters including OATP (organic anion-transporting polypeptide), OAT (organic anion transporter), and MCTs (monocarboxylate transporters) (Wang and Morris 2014).

P-gp is supposed to act as an energy-dependent pump to effluence the anticancer agents from the tumor cells. The flavonoids have the ability to inhibit P-gp activity and act as the possible candidates to modulate multidrug resistance revealed by Kitagawa 2006. The structure-activity relationship studies suggested (1) the presence of C2–C3 double bond as well as the linkage of ring B at C2 position of ring C; (2) the number of double-bond (planar structure), i.e., 2–3; (3) the number of hydroxyl groups (at positions 3 and 5); and (4) the substitution of either 6-, 7-, 8-, or 4'-hydroxyl group of the A or B rings with hydrophobic groups. These features are responsible for high P-gp-modulating activities, while the glycosylation would dramatically decrease the activity of flavonoids (Kitagawa 2006; Zandena et al. 2005; Wang and Morris 2014).

Flavonoids have modulating activity toward the efflux transport protein MRP1 in the treatment of infectious diseases and cancer. The SAR studies specified that flavones and flavonols possessed more potency than flavanols, flavanonols, flavanones, and isoflavones. The inhibitory action of flavonoids decreases in case of glycosylation. For high MRP1 inhibitory activity, the following structural features are responsible: (1) the presence of two to three double bonds for planar molecular structure, (2) the presence of hydroxyl at both 3' and 4' positions on the B ring, and (3) the substitution of 4'-hydroxyl group on the B ring with hydrophobic group. The pyrogallol group on the B ring is a vital structural characteristic for inhibition of MRP2 by the flavonoids (Wang and Morris 2014).

The structural traits of flavonoids for maximal inhibitory BCRP activity (breast cancer resistance protein) include the following: (1) the number of double bonds, i.e., two to three for the planar molecular structure; (2) the presence of 5-OH group and absence of 3-OH group; (3) the position of ring B at C2 carbon of ring C; and (4) the substitution of hydroxyl group at 6-, 7-, 8-, or 4'-positions with hydrophobic substituents. The lower BCRP-inhibiting activities are observed in glycosides (Wang and Morris 2014).

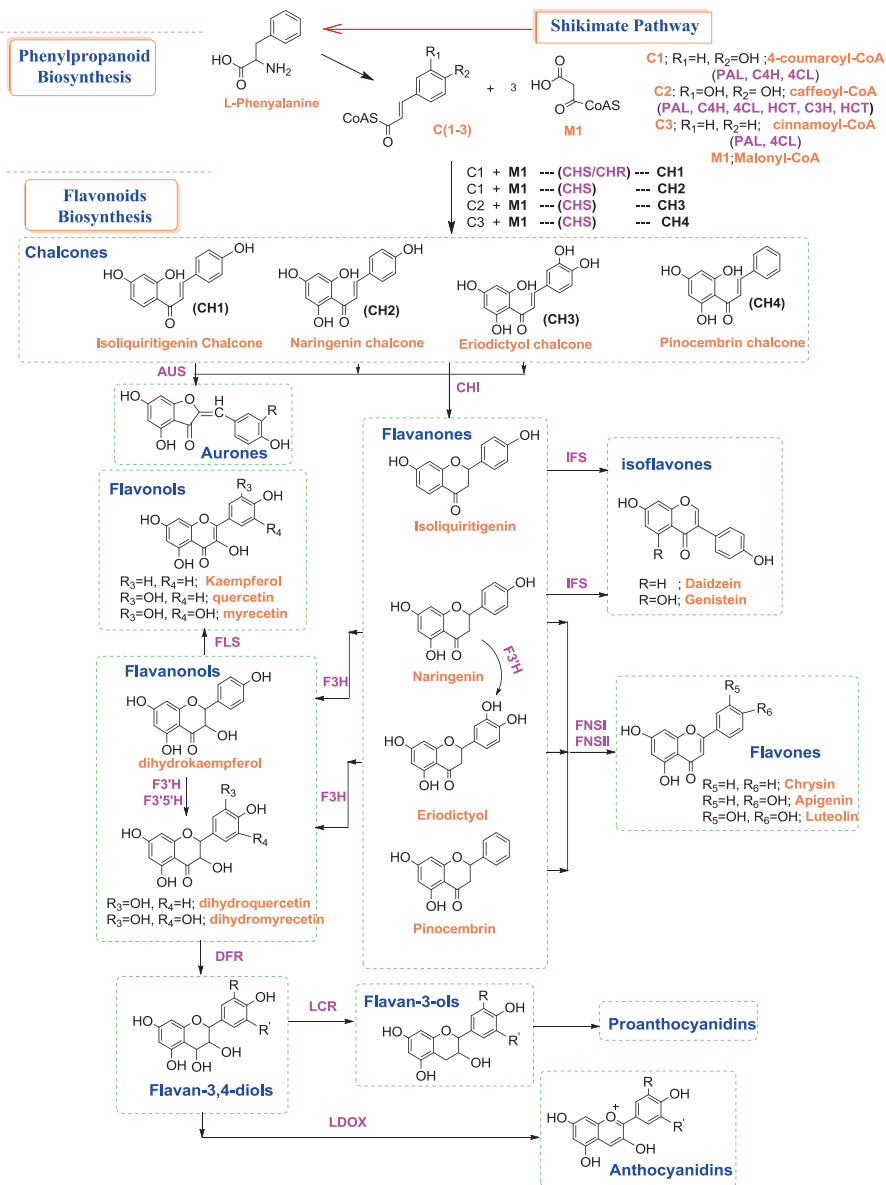
## 4 Biosynthesis of Flavonoids

Flavonoids are one of the categories of products from plant aromatic pathway. The biosynthesis pathway of plant aromatics generally consists of three sections, i.e., the shikimate, phenylpropanoid, and flavonoid route. The shikimate pathway produces phenylalanine aromatic amino acids, while phenylpropanoid segment produces the

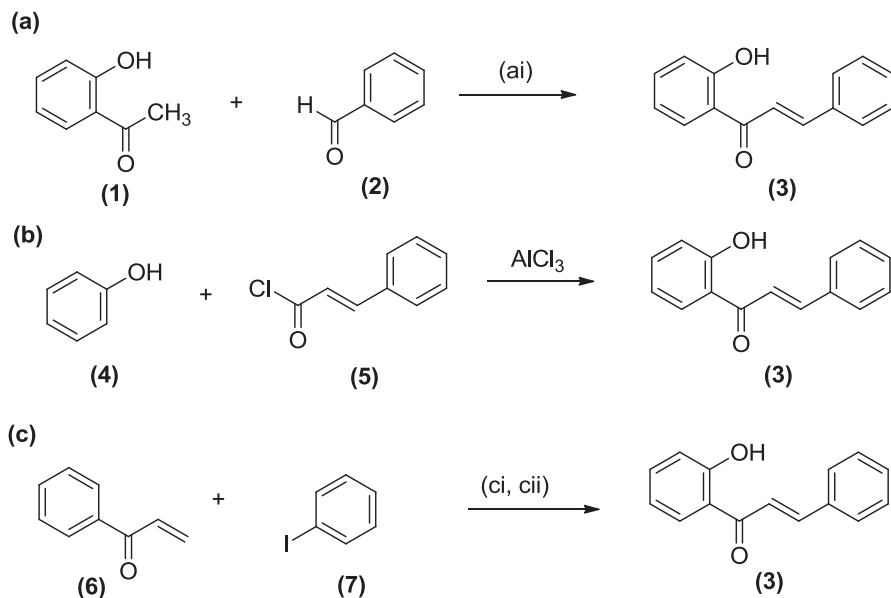
cinnamic acid derivatives such as 4-coumaroyl-CoA, caffeoyl-CoA, and cinnamoyl-CoA, building blocks of flavonoids. In the flavonoid pathway, various flavonoid compounds are produced by the action of a variety of enzymes (Hrazdina 1992). The CHS (chalcone synthase) is a key enzyme which carried out the condensation of cinnamic acid derivatives with three molecules of malonyl-CoA, resulting in the formation of chalcone intermediates such as naringenin chalcone, eriodictyol chalcone, and pinocembrin chalcone. In some cases, the CHR (chalcone reductase) with CHS results in the formation of isoliquiritigenin chalcone. These chalcones are common intermediates which stereospecifically cyclized into respective flavanones by the action of CHI (chalcone isomerase). The chalcones undergo an oxidative cyclization by the action of AUS (aureusidin synthase) to form a five-member heterocycle fused to the A ring of the aurone. The isoflavone synthase (IFS) carried out the conversion of flavanones into isoflavones through the 1,2-aryl migration of ring B, while the conversion of flavanones to flavones is carried out through the sequential removal of the vicinal hydrogen atoms from C2 and C3 and generation of C2–C3 double bond in the ring C by the action of FNS (flavone synthase). The hydroxylation of flavanones at C3 position of ring C is carried out by F3H (flavanone 3-hydroxylase) which results the formation of flavanonols. The flavanonols are intermediate in the biosynthesis of flavonols, catechins, and anthocyanidins. FLS (flavonol synthase) is responsible for the conversion of flavanonols into flavonols by introducing C2–C3 double bond between ring C. The hydroxyl group is generated at 4 position in place of oxo group of flavanonols by the action of DFR (dihydroflavonol 4-reductase). The flavan-3,4-diols are substrates for formation of flavan-3-ols and anthocyanidins by the action of leucoanthocyanidin reductase (LCR) and leucoanthocyanidin dioxygenase (LDOX), respectively (Fig. 3.3) (Morreel et al. 2006; Miranda et al. 2012; Ferreyra et al. 2012).

## 5 Chemical Synthetic Methods of Flavonoids

A large group of natural products is known to contain usually a heterocyclic ring which can also be prepared by chemical synthesis through semi-synthesis and total synthesis approaches. These approaches play a central role in the field of organic chemistry by resolving even challenging synthetic targets in the easy and cost-effective way (Sharma et al. 2014, 2015; Khare et al. 2016). However, there is still a wide scope of research to achieve the desired structural features from a biological point of vision such as the arrangements of the functional groups, rings with respect to one another and the number of carbon atoms along with other atoms including their stereochemical elements, etc. In the last few decades, several attractive developments of methods and approaches related to the synthesis of flavonoids have been reported in the literature (Wagner and Farkas 1975; Kshatriya et al. 2018, Sharma et al. 2018a, b). In this instance, the various reports regarding chalcones' synthesis, which belong to the flavonoid family, have been elucidated previously (Cazarolli et al. 2013; Zhuang et al. 2017; Gomes et al. 2017). More specifically, 2'-hydroxy



**Fig. 3.3** The plausible schematic pathway of flavonoid biosynthesis in plants, starting with general phenylpropanoid metabolism and illustrating the major subclasses such as chalcones, aurones, isoflavonoids, flavones, flavonols, flavandiols, proanthocyanidins, and anthocyanidins. The names of common enzymes involved have been abbreviated as follows: aureusidin synthase (AUS); cinnamate-4-hydroxylase (C4H), chalcone isomerase (CHI), chalcone reductase (CHR), chalcone synthase (CHS), 4-coumaroyl:CoA-ligase (4CL), *p*-coumarate 3-hydroxylase (C3H); dihydroflavonol 4-reductase (DFR), flavanone 3-hydroxylase (F3H), flavone synthase (FNSI and FNSII), flavonoid 3' hydroxylase (F3'H); flavonoid 3'5' hydroxylase (F3'5'H); *p*-hydroxycinnamoyl-CoA:nshikimate/quinate *p*-hydroxycinnamoyltransferase (HCT); isoflavone synthase (IFS), leucoanthocyanidin dioxygenase (LDOX); leucoanthocyanidin reductase (LCR); phenylalanine ammonia-lyase (PAL)



**Fig. 3.4** The plausible approaches toward the synthesis of 2'-hydroxy chalcones; (a) Claisen-Schmidt reaction of substituted acetophenones and aromatic aldehyde; (b) Friedel-Crafts condensation of phenols and cinnamoyl chloride; (c) Heck coupling reaction of aryl vinyl ketone and iodobenzene [(ai) NaOH/KOH, EtOH, or acid catalyst/bronsted acidic ionic liquid, MW or grindstone method or ultrasound accelerated method:(ci) Pd(OAc)<sub>2</sub>, Ph<sub>3</sub>P, CH<sub>3</sub>CN, Et<sub>3</sub>N; (cii) MeONa, THF/MeOH; (ciii) Et<sub>3</sub>Sn, DMF]

chalcones are valuable synthon for the synthesis of other flavonoid subclasses. The synthesis of 2'-hydroxy chalcones (**3**) is generally achieved either by (a) Claisen-Schmidt reaction which involves a base-catalyzed reaction of substituted acetophenones (**1**) and aromatic aldehyde (**2**) through conventional methods and greener methods or (b) condensation of phenols (**4**) with cinnamoyl chloride (**5**) through Friedel-Crafts reaction and (c) Heck coupling reaction which involves the action of iodobenzene (**7**) on aryl vinyl ketone (**6**) (Fig. 3.4) (Sharma et al. 2018a, b; Kakati and Sarma 2011; Stoyanov et al. 2002; Kumar et al. 2008; Qian et al. 2013; Bianco et al. 2003, 2004). The most commonly used synthetic approaches are (a) Algar-Flynn-Oyamada approach, (b) Allan-Robinson approach, (c) Baker-Venkataraman approach, (d) Claisen-Schmidt approach, (e) Karl von Auwers approach, (f) Kostanecki approach, (g) Mentzer Pyrone approach, and (h) Suzuki-Miyaura approach (Fig. 3.5).

### 5.1 Algar-Flynn-Oyamada Approach

Algar-Flynn-Oyamada synthetic approach of flavonoid synthesis involved the oxidative cyclization of 2'-hydroxychalcones (**3**) with methoxy groups at different positions in the two aromatic nuclei in the presence of hydrogen peroxide under

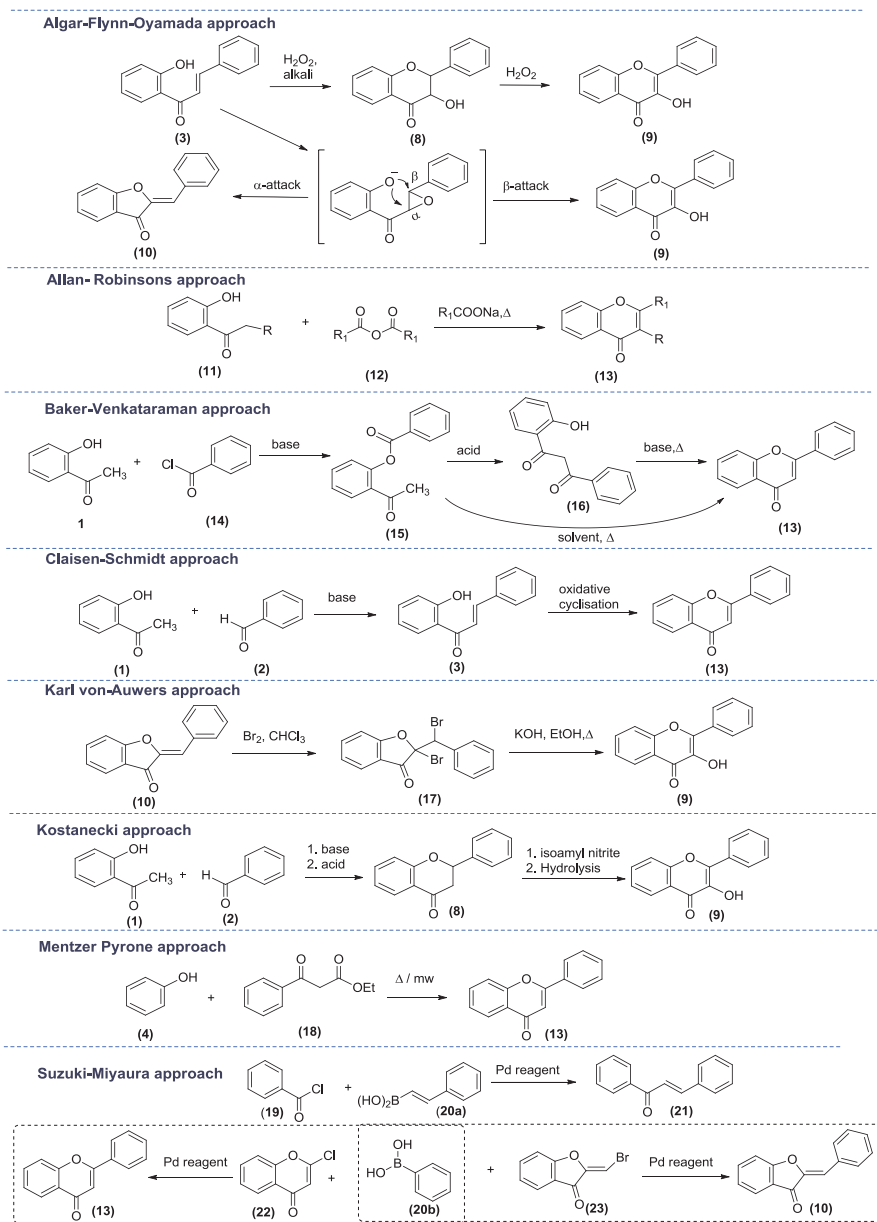


Fig. 3.5 The different effectual methods of flavonoid's chemical synthesis

alkaline conditions. If a methoxy group is present at 6' position in 2'-hydroxychalcone, the aurone (**10**) will be the main product rather than flavonol (scheme 1). This reaction has wide application for the preparation of flavanonols (**8**) and flavonols (**9**) (Wang 2010a; Li 2009a).

## 5.2 *Allan-Robinson Approach*

This approach established the synthesis of flavone or isoflavone derivatives (**13**) by means of the condensation between 2-hydroxyacetophenones (**11**) and aromatic acid anhydride (**12**) using the sodium salt of corresponding aryl carboxylic acid anhydride. An aryloxy or alkoxy group is present at  $\omega$  position of the acetophenone which is a favorable condition for the reaction (Wang 2010b; Li 2009b; Kshatriya et al. 2018; Kashyap et al. 2017, 2018; Sharma et al. 2018a, b).

## 5.3 *Baker-Venkataraman Approach*

Baker-Venkataraman approach was reported for the synthesis of flavone by rearranging the o-acyloxyketones (**15**) into  $\beta$ -diketones (**16**) under basic conditions via intramolecular acyl transfer. The ring closure of the dibenzoylmethane (**16**) is effected by the treatment with the acid catalyst so as to form flavone whereas direct conversion of o-acyloxyketones (**15**) into flavone by heating in the solvent (Wang 2010c; Li 2009c; Kshatriya et al. 2018).

## 5.4 *Claisen-Schmidt Approach*

Claisen-Schmidt approach is one of the well-known methods for the production of chalcones (**3**). The oxidative cyclization of chalcones using various acidic or basic catalysts (Lewis and Bronsted acid/base) resulted in the flavones (**13**) (Kshatriya et al. 2018; Kashyap et al. 2017, 2018; Sharma et al. 2018a, b).

## 5.5 *Karl von Auwers Approach*

Karl von Auwers approach involves the formation of 1,2-dibromo addition product (**17**) by the reaction of aurone (**10**) with bromine. In alkaline conditions, the attack of hydroxyl ion on 1,2-dibromo adducts that undergo dehydrohalogenation and result in the formation of flavonols (**9**) (Li 2005).

### 5.6 *Kostanecki Approach*

This synthetic approach utilizes Claisen condensation between benzaldehydes (**1**) and 2-hydroxy acetophenones (**2**). The flavanones (**8**) are obtained in acidic conditions as a condensation product, which reacts with isoamyl nitrite and subsequent upon hydrolysis gives flavonols (**9**) (Kashyap et al. 2017, 2018; Sharma et al. 2018a, b).

### 5.7 *Mentzer Pyrone Approach*

This approach involves the synthesis of flavone derivatives (**13**) by the reaction between phenols (**4**) and  $\beta$ -ketoesters (**18**) at high temperature for a longer period or in a microwave irradiation (Wang 2010d).

### 5.8 *Suzuki-Miyaura Approach*

The Suzuki-Miyaura approach utilizes the reaction of compounds (**19**, **22**, **23**) containing  $sp^2$ -hybridized carbon and halogen bond with boronic acids/esters (**20a**, **b**) in the presence of palladium compounds (Selepe and Heerden 2013).

## 6 Conclusion

Flavonoids have been abundantly present in human diet such as in fruits, vegetables, and beverages (tea, wine) owing to their wide spread in the plant kingdom. It is a wide class of polyphenolic compounds with 2-phenylchromane nucleus. This class of compounds is being intensively investigated because of their health-associated therapeutic, biochemical, and pharmacological benefits. The structural features, their classification, and structure-activity relationships are extremely helpful to understand the relations between their molecular structures and biological and physicochemical activities. The existence of hydroxyl groups on ring A, double bonds, and oxo group with ring B hydroxyl group substitution pattern are the requisite structural feature for their activity toward the health benefits. Chemically, they are synthetically accessed by various methods. Among these, the Baker-Venkataraman approach ( $\beta$ -diketones formation) or the Claisen-Schmidt condensation (chalcones formation) and their successive cyclisation pathways to 2-phenylchromane heterocycles are mostly adopted by different studies. The development of the new flavonoid derivatives with improved therapeutic values would be useful for chemists, biologist, and biochemist to understand, design, and insert new functionalities into these biomedical compounds and further test the modified compounds for their biological effects.



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# Chapter 4

## Metal Complexation and Patent Studies of Flavonoid



Valentina Uivarosi, Alexandra Cristina Munteanu, Ajay Sharma, and Hardeep Singh Tuli

### 1 Introduction

Flavonoids comprise a large group of polyphenolic compounds, which are ubiquitous in nature. To date, more than 8000 flavonoids are found in fruits, vegetables, and plant-derived beverages (Babu and Liu 2009). Over the years, flavonoids have been given considerable attention in the search for new biologically active molecules because of their traditional use as antiviral, antiallergic, anti-inflammatory, and antitumor agents (Panche et al. 2016). Flavonoids also possess immune-enhancing properties (Vajdy 2011). One epidemiological study shows that flavonoid intake from certain foods was associated with risk reduction of death due to coronary heart disease and heart attacks (Mink et al. 2007). Interestingly, epidemiological data on consumption of foods with a rich content of flavonoids points to the notion that some of these natural polyphenols may favor healthy ageing and could be associated with prolonged life span (Pallauf et al. 2016).

Moreover, there is an extensive body of evidence in the literature regarding the antioxidant properties of flavonoids, which depend largely on both the number and the position of hydroxyl groups attached to the flavonoid backbone (Abbas et al. 2017). While most of the biological activities are correlated with the antioxidant properties, other properties of flavonoids like signaling molecules, enzyme inhibitors, and DNA intercalators and chelators of metal ions must be also taken into account.

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From a chemical point of view, flavonoids possess as central unit a 2-phenylchromen-4-one (2-phenyl-1-benzopyran-4-one) backbone (Table 4.1). Thus, the diphenyl-propane (C6–C3–C6) or benzo- $\gamma$ -pyrone (chromone) core structure of flavonoids comprises two benzene rings, denoted as A and B, usually connected from the pyran ring, denoted as C (Uivarosi and Munteanu 2017). Hydroxyl groups, sugar moieties, or methyl groups may be attached to this base structure. Taking into account the oxidation degree of the C-ring, the hydroxylation pattern of the nucleus and the C3 substituent, eight different flavonoids were described: flavonols, flavanones, flavones, isoflavones, catechins (flavan-3-ols or flavanonols), anthocyanidins, dihydroflavonols, and chalcones (Table 4.1) (Malešev and Kuntić 2007). Flavonoids are widespread in nature mostly as glycosides, since the sugar group improves the hydrophilicity of the flavonoid molecules (Kasprzak et al. 2015). Table 4.1 lists the main six subclasses of flavonoids and some selected representatives.

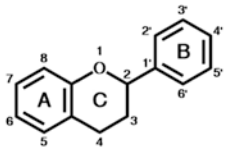
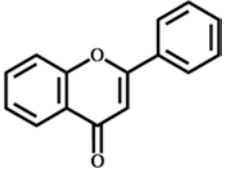
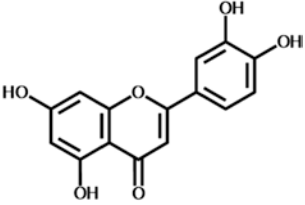
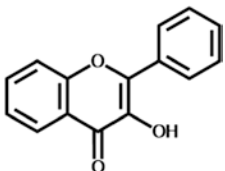
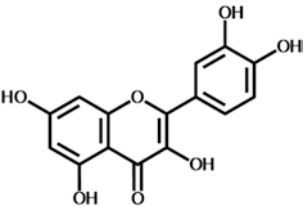
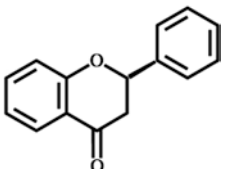
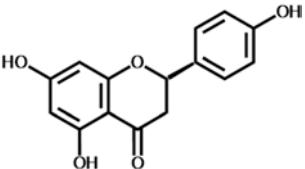
By involving the oxo and hydroxyl groups, flavonoids can bind various metal ions, thus resulting in metal-flavonoid chelate complexes. In the following, the complexation site, the stoichiometry and stability of these complexes, as well as the role of pH in complex formation will be thoroughly discussed. Also, complexation of metal ions with oxidizing properties, e.g., Fe<sup>III</sup>, Ru<sup>IV</sup>, Ru<sup>III</sup>, and Au<sup>III</sup>, is particularly relevant; reactions between flavonoids and mentioned metal ions may involve electron transfer (redox reactions), since most flavonoids have good reducing capacity (Abbas et al. 2017). This chapter will further include relevant information on the most recent papers published regarding the biological properties of flavonoids, with an emphasis on the anticancer and chemopreventive activities. The aim of this paper is to summarize the knowledge gathered in regard to the metal complexation ability and anticancer and chemoprotective effects of six natural flavonoids (luteolin, quercetin, naringenin, EGCG, genistein, cyanidin) with a special attention given to recent studies and suggest directions for future research.

## 2 Metal Complexation Sites in Flavonoids and Recent Literature

### 2.1 General Considerations

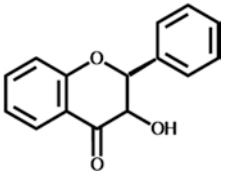
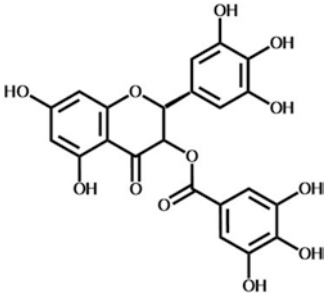
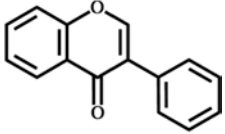
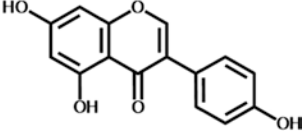
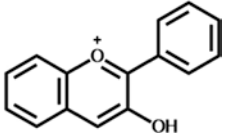
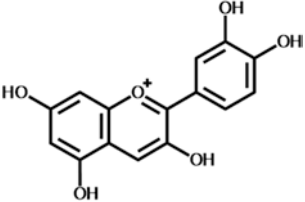
The literature cites numerous physicochemical studies that aimed at revealing the ability of flavonoids to interact with metal ions, at identifying the metal chelation sites, the metal/ligand ratio, and the structure of the resulting complexes. The aforementioned aspects regarding the interaction of various metal ions and flavonoids will be further addressed in this chapter.

**Table 4.1** Main flavonoid subclasses, chemical structure, and nomenclature of the selected representatives

Flavonoid subclass	General structure	Chemical structure and nomenclature of the selected representatives
	 <p data-bbox="318 469 465 522"><b>Flavonoid base structure</b></p>	
<b>Flavones</b>		 <p data-bbox="747 769 836 786"><b>Luteolin</b></p> <p data-bbox="553 795 965 839">2-(3,4-Dihydroxyphenyl)-5,7-dihydroxy-4<i>H</i>-chromen-4-one</p>
<b>Flavonols</b>		 <p data-bbox="747 1113 842 1130"><b>Quercetin</b></p> <p data-bbox="553 1139 983 1183">2-(3,4-Dihydroxyphenyl)-3,5,7-trihydroxy-4<i>H</i>-chromen-4-one</p>
<b>Flavanones</b>		 <p data-bbox="736 1427 847 1444"><b>Naringenin</b></p> <p data-bbox="553 1453 918 1497">(S)-5,7-dihydroxy-2-(4-hydroxyphenyl)chroman-4-one</p>

(continued)

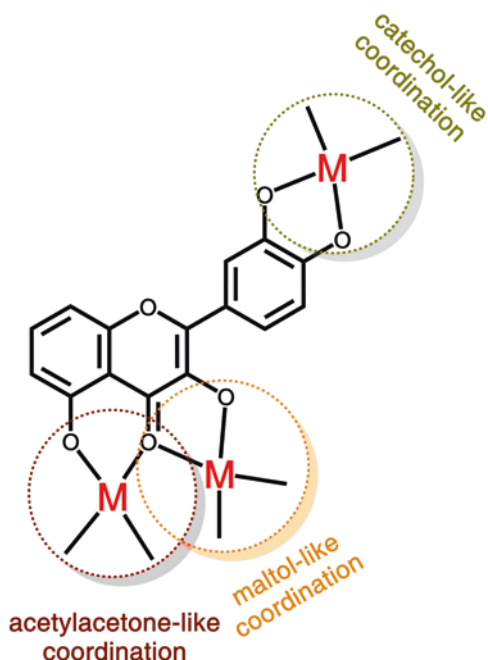
**Table 4.1** (continued)

Flavonoid subclass	General structure	Chemical structure and nomenclature of the selected representatives
<b>Flavan-3-ols (flavanonols)</b>		 <p data-bbox="675 575 910 601"><b>Epigallocatechin gallate</b></p> <p data-bbox="553 605 898 680">[(2<i>R</i>,3<i>R</i>)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)chroman-3-yl]3,4,5-trihydroxybenzoate</p>
<b>Isoflavones</b>		 <p data-bbox="745 857 839 883"><b>Genistein</b></p> <p data-bbox="553 887 1018 931">5,7-Dihydroxy-3-(4-hydroxyphenyl)-4<i>H</i>-chromen-4-one</p>
<b>Anthocyanidins</b>		 <p data-bbox="745 1174 839 1201"><b>Cyanidin</b></p> <p data-bbox="553 1204 1018 1231">2-(3,4-Dihydroxyphenyl)chromenylium-3,5,7-triol</p>

### 2.1.1 Flavonoids and Their Ability to Form Chelate Complexes

Interactions of flavonoids with metal ions can result in chelate formation. Flavonoids possess three possible metal-chelating sites that can be involved in binding of metal ions: (1) the 3-hydroxy-4-ketone groups in the C-ring (denoted as the “3–4 site”), resulting in a maltol-like coordination mode; (2) the 5-hydroxy group in the A-ring and 4-carbonyl group in the C-ring (denoted as the “4–5 site”), resulting in an acetylaceton-like coordination mode; and (3) 3',4'-dihydroxy groups located on

**Fig. 4.1** The possible coordination modes of the flavonoid molecules. (Sanna et al. 2014)



the B-ring (denoted as the “3′-4′ site”), resulting in a catechol-like coordination mode (Fig. 4.1). The preferred binding site is correlated with the flavonoid structure, the metal ion, the pH value, and the solvent or solvent system where the reaction takes place.

### 2.1.2 The Preferred Site of the Central Ion

The hydroxyl group in the positions 3 or 5 and the adjacent carbonyl (C = O) group are the main groups involved in the formation of complexes with metal ions. Also, the hydroxyl groups eventually present in the B-ring could form chelate complexes. Secondary, in the case of flavonoid glycosides, the hydroxyl groups belonging to the sugar moiety can also participate in metal binding (Selvaraj et al. 2014).

We will present further the case of iron, the most abundant trace metal in the body, and the complexation features flavonoids bearing different structural motifs (quercetin, luteolin, galangin, kaempferol, and chrysin). Electronic structure calculations revealed that the preferred chelation site for Fe is the 3–4 site; for complexes containing one Fe, the Fe-flavonoid binding strength at different sites decreased in the order 3–4 > 4–5 > 3′-4′ (if present) for all of the flavonoid molecules studied. The binding of the Fe atom to the 3–4 chelating site causes the breakage of the 4-C=O double bond and the deprotonation of the 3-hydroxyl group, resulting in the formation of two new Fe-O bonds. The binding of Fe atom at the 4–5 site is both thermodynamically and kinetically less favorable, since deprotonation of the 5-OH



group requires more energy than deprotonation of the 3-OH group. For complexes containing two Fe atoms, after Fe binding at the 3–4 site, steric repulsion prevents chelation of another metal ion at the deprotonated 4–5 site. However, the 3'–4' site is still available for metal binding, although the resulting complex is considerably less stable than the one Fe complex with Fe at the 3–4 chelating site (Ren et al. 2008).

### 2.1.3 The Role of pH in Complex Formation

Flavonoids are weak polybasic acids, based on the manifold hydroxyl groups present in their structure. A very interesting study concluded that (1) the acidity of the 5-OH or 3-OH group decreases as a consequence of the formation of an intramolecular hydrogen bond (OH group is a hydrogen bond (HB) donor), (2) the acidity of the OH group in the catechol group (HB acceptor) does not significantly increase in comparison to that of a non-H-bonded group (e.g., the acidity of 7,8-dihydroxyflavone is almost the same as the acidity of 7-hydroxyflavone), and (3) with the exception of morin, the 7-OH group is the most acidic site, and the presence of other hydroxyls in positions 3, 5, and 6 does not considerably change the acidity of the 7-OH group. The acidity of the investigated flavonoids have been found to vary in the following order: 3-hydroxyflavone < 3,6-dihydroxyflavone  $\approx$  6-hydroxyflavone < 3,5,7,3',4'-pentahydroxyflavone (quercetin) < 5,7-dihydroxyflavone (chrysin) < 7-hydroxyflavone  $\approx$  3,5,7-trihydroxyflavone (galangin) < 7,8-dihydroxyflavone < 5,7,4'-trihydroxyflavone (naringenin) < 3,5,7,2',4'-pentahydroxyflavone (morin) (Musialik et al. 2009).

Therefore, pH considerably influences complex formation. In this regard, at pH values lower than 3.0, flavonoids are predominantly present in their undissociated form, and, therefore, complex formation is unlikely to occur. An exception to this rule has been reported for the  $\text{Al}^{3+}$  1:1 complex with quercetin, which is formed at pH = 2.0. High pH values favor deprotonation of flavonoids, but, in this case, more complex species are formed, hydrolysis of the metal ions also occurs, and, usually, hydroxo-complexes are formed. Flavonoid metal complexes are typically formed in slightly acidic or neutral pH, rarely in basic medium. The optimal pH for complex formation is around 6. Several studies have reported that in acidic medium, the 3–4 or the 5–4 sites are involved in coordination, whereas in basic medium, the 3',4' chelating site is more likely involved. Thus, at pH > 5, the deprotonation of the 3',4'-dihydroxy group on B-ring leads to a high delocalization of the oxygen electrons, which in turn facilitates the delocalization of the  $\pi$  electrons and, in consequence, the formation of a stable 5-membered chelate ring with the metal ion (Shi et al. 2011).

Acidic pH may also favor electron transfer processes, such as the reduction of  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$  by fisetin. At higher pH values,  $\text{Fe}^{3+}$  and  $\text{Fe}^{2+}$  complexes have been reported to coexist (Cornard and Merlin 2003).

### 2.1.4 Stoichiometry of the Complexes

The most common stoichiometries of the flavonoid metal ion complexes are 1:1, 1:2, and 2:1; however, other metal/ligand ratios including 1:3, 2:2, 2:3, and 3:1 are also possible. For steric reasons, complexes with more than two flavonoid ligands are very rare, being characteristic for rare earth metal ions. Also for steric repulsions, the simultaneous binding of two metal ions to the 3–4 and 4–5 sites is unfavorable. For instance, mass spectrometry experiments evidenced that the possible stoichiometries for iron-quercetin complexes range from 1:1 to 1:3. Although three quercetin molecules are required to saturate the bonds of the Fe ion, the binding energy per molecule is highest for complexes with 1:2 molar ratio (Ren et al. 2008).

### 2.1.5 Stability of the Complexes

Flavonoids and their metal complexes are poorly soluble in water, so that the complex stabilities were determined in other solvents or solvent mixtures (e.g., in alcoholic solution), which renders the data difficult to use in a direct comparison. It should be noted that the ability of the solvent to form hydrogen bonds and its polarity have an impact on the metal ion-ligand interactions. For instance, Al<sup>3+</sup> complexation by 3-hydroxyflavone, 5-hydroxyflavone, and 3'4'-dihydroxyflavone in methanol is significantly decreased in the presence of water. Other factors that should be considered include the choice of buffer (e.g., phosphate buffer). However, as a general conclusion, most 1:1 flavonoid metal complexes have moderate ( $5 < \log\beta < 10$ ) or high ( $\log\beta > 10$ ) stabilities, while, generally speaking, the 1:2 correspondents are more stable ( $\log\beta > 10$ ) (Table 4.2).

In some cases, highly stable complexes could be formed with an anion as in the case of titanyl oxalate  $\text{TiO}(\text{C}_2\text{O}_4)_2^{2-}$  with rutin (Kuntić et al. 2000), although this is in disagreement with the crystal field theory (Kasprzak et al. 2015). In this case, bonding in metal-flavonoid complexes probably occurs by electron transfer from the d orbital of the metal ion to the  $\pi^*$  orbital of the flavonoid, through M(d)-O(p) hybridization (Ren et al. 2008).

**Table 4.2** Stability constants for quercetin metal ion complexes with different stoichiometries

Metal ion	Stoichiometry	pH	$\log\beta/\log K_{\text{app}}$	Refs.
Fe <sup>3+</sup>	1:1	8	5.50	Marković et al. (2011)
	1:2	4	9.56	
Al <sup>3+</sup>	1:1	Not stated	-5.79	Furia et al. (2014)
	1:2	Not stated	24.1	Erdogan et al. (2005)
Fe <sup>2+</sup>	1:1	7.2	6.65	Guo et al. (2007)
	1:2	7.2	10.7	
Pb <sup>2+</sup>	1:1	Not stated	4.87	Cornard et al. (2005)
	1:2	Not stated	7.71	

## 2.2 *Metal Complexation Sites in the Selected Flavone – Luteolin – and Structural Remarks*

Luteolin is a flavonoid which can bind metal ions in all three aforementioned coordination modes. For instance, in acidic solutions (pH = 3.5), two complexes with luteolin to Al(III) ratio of 1:1 and, respectively, 1:2 are formed. In the 1:1 complex, Al(III) is bound in the 4–5 chelating site. The 1:2 complex was isolated in the solid state under basic conditions, and a structure with a triple deprotonation of the ligand was proposed. Interestingly, while in the solid-state complex the Al–O bonds are considered to be ionic, the complex in solution is formed by coordinative bonding to metal ions (Rygula et al. 2013).

Luteolin binds Cu(II) ions via the 4–5 coordination site to form a complex with a 1:2 metal ion/flavonoid ratio in a solid state; however, the dihydroxybenzene moiety was protected first with boracic acid. Complex formation took place in methanol, although the final pH of the solutions is not mentioned in this study (Niu et al. 2009). The Cu(II)/luteolin 1:1 complex was formed involving the 3',4'-dihydroxy B-ring system; it should be stated, however, that the synthesis in this case took place at pH = 7.4 (Shi et al. 2011).

NMR spectroscopy and various levels of ab initio calculations confirmed the CO-4 carbonyl oxygen and the deprotonated C-5 OH group of luteolin as the Zn(II) chelation site, in a 1:1 molar ratio (Primikyri et al. 2015). The 4–5 chelation site has also been reported for a manganese (II)-luteolin 1:2 complex. Remarkably, the anti-oxidant, antibacterial, and hypoglycemic activities were enhanced after the complexation of manganese (II) cations with luteolin, as compared to the free ligand. Also, the luteolin-manganese (II) complex reversibly inhibited xanthine oxidase (XO) in a competitive manner, with a higher XO inhibitory activity than that of luteolin (Dong et al. 2017).

Regarding complexation of luteolin with trivalent metal ions, 1:1 rare earth-luteolin complexes with anti-inflammatory activities have been reported (Li et al. 2011), and the chelation between luteolin and Cr(III) ion was studied using theoretical methods. Cr(III) forms chelates at the 4–5 site to form luteolin-Cr(III) complex, resulting in the formation of a stable six-membered ring. The antioxidative activity of luteolin-Cr(III) complex is predicted to be higher than that of luteolin (Gao et al. 2013).

Luteolin has also been reported to bind the vanadyl ion, in a “catechol-like” coordination mode, in which case both luteolin and VO(lut) displayed similar anti-oxidant properties (Naso et al. 2016a). In contrast, the V<sup>IV</sup>O luteolin complex 1:2 in which an “acetylacetonone-like” coordination mode is involved exhibits superior anti-oxidant activity compared to that of the free ligand (Roy et al. 2015).

### ***2.3 Metal Complexation Sites in the Selected Flavanol – Quercetin – and Structural Remarks***

Due to the favorable spatial arrangement of the donor groups, quercetin can also bind metal ions in all three coordination modes, namely, in the 3–4, 4–5, and 3'-4' sites; additionally, depending on the metal ion and the conditions in which the reaction occurs, the 7-OH group can also be involved in metal coordination. Table 4.3 summarizes the data obtained from either solid-state or solution structural studies regarding the coordination modes and reaction stoichiometry of different quercetin metal complexes.

In alkaline solution,  $\text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$ , and  $\text{Al}^{3+}$  ions have the strongest affinity for the 3'-4' site of quercetin (Kasprzak et al. 2015); the formation of a 1:2  $\text{Fe}^{3+}$ -quercetin complex in acidic solution mainly involves the 3–4 or 4–5 sites (Marković et al. 2011).


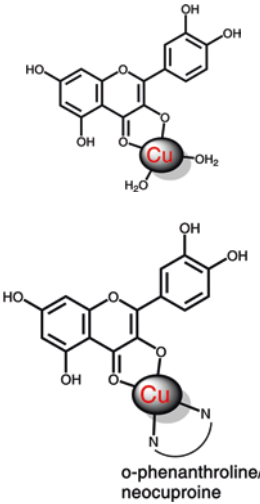
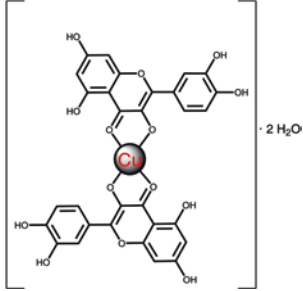
The Zn(II) chelation with quercetin in a 1:1 stoichiometry investigated by NMR spectroscopy and ab initio calculations revealed that the CO-4 carbonyl oxygen and the deprotonated C-5 OH group of quercetin are involved in coordination. DFT calculations of the 1:1 complex in the gas phase demonstrated that the C-3 O- and CO-4 sites are favored for quercetin (Primikyri et al. 2015).

These observations regarding the chemical structures of quercetin complexes are especially relevant for the antioxidant properties of the metal compounds, which are generally improved in comparison with the free flavonoid.

### ***2.4 Metal Complexation Sites in Selected Flavanone – Naringenin – and Structural Remarks***

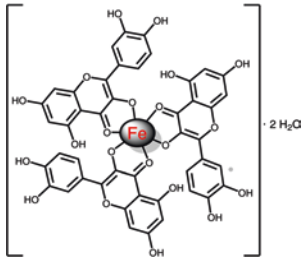
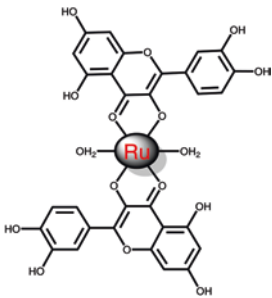
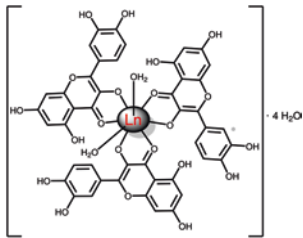

Naringenin possesses only one coordination site, the 4–5 site. Table 4.4 contains data on metal complexes formed by naringenin with various metal ions. The complexation at this site of Cu(II) and Zn(II) with naringenin in a 1:2 molar ratio and the addition of an ancillary ligand (o-phenanthroline and 2,2'-bipyridine) resulted in an exceptional inhibitory activity against cholinesterase enzymes (AChE and huBChE) higher than that of the parent flavanone and even than that of the reference standard galanthamine (Sarria et al. 2016). Similar complexes of Cu(II), Ni(II), and Zn(II) possess antioxidant activity, with the effect of the Cu(II) complex being the most remarkable and the average scavenger ability of the complexes against  $\text{OH}\cdot$  being higher than that of the ligand (Wang et al. 2006a).

**Table 4.3** Data regarding the coordinating groups, stoichiometry, chemical formula, and chemical structure of several quercetin metal complexes

Metal ion	Stoichiometry (metal ion: flavonoid)	Chemical formula	Structure	Refs.
<b>Studies regarding metal complex species in solid-state</b>				
<b>Quercetin coordinating groups: 4-oxo, 3-hydroxo</b>				
Me(II) Me: Fe, Mn, Co, Ni, Cu, Zn, Pb	1:2	C <sub>30</sub> H <sub>22</sub> O <sub>16</sub> Fe C <sub>30</sub> H <sub>22</sub> O <sub>16</sub> Mn C <sub>30</sub> H <sub>24</sub> O <sub>17</sub> Co C <sub>30</sub> H <sub>24</sub> O <sub>17</sub> Ni C <sub>30</sub> H <sub>22</sub> O <sub>16</sub> Cu C <sub>30</sub> H <sub>24</sub> O <sub>17</sub> Zn C <sub>30</sub> H <sub>22</sub> O <sub>16</sub> Pb		Raza et al. (2016) and Zhou et al. (2001a)
Cu(II)	1:1	Cu(Q)(H <sub>2</sub> O) <sub>2</sub> C <sub>15</sub> H <sub>13</sub> O <sub>9</sub> Cu		Vimalraj et al. (2018)
	1:1:1	Cu(Q)(o-phen) o-phen = o-phenanthroline C <sub>27</sub> H <sub>17</sub> N <sub>2</sub> O <sub>7</sub> Cu		
	1:1:1	Cu(Q)(neo) neo = neocuproine C <sub>29</sub> H <sub>21</sub> N <sub>2</sub> O <sub>7</sub> Cu		
Cu(II)	1:2	C <sub>30</sub> H <sub>22</sub> O <sub>16</sub> Cu		Jabeen et al. (2017)

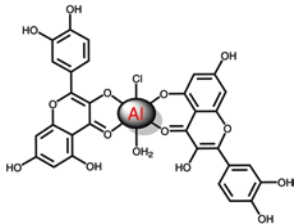
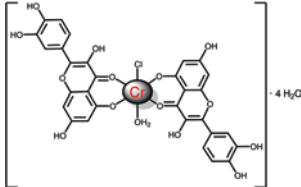
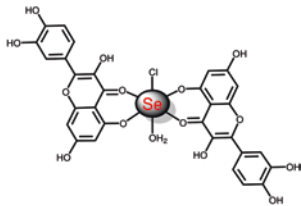
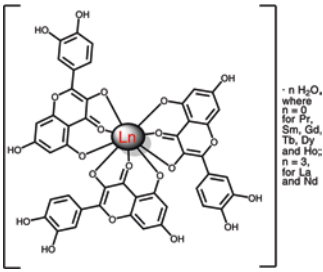
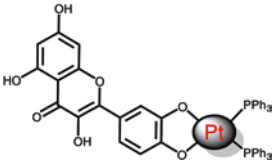
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**Table 4.3** (continued)

Metal ion	Stoichiometry (metal ion: flavonoid)	Chemical formula	Structure	Refs.
Fe(III)	1:3	$C_{45}H_{31}O_{23}Fe$		Jabeen et al. (2017)
Ru(III)	1:2	$C_{30}H_{22}O_{16}Ru$		Roy et al. (2018)
Ln(III) Ln: La, Nd, Eu, Gd, Tb, Dy, Tm, Y	1:3	$C_{45}H_{39}O_{27}Ln$		Zhou et al. (2001b)
Sn(IV)	1:1	$[(CH_3)_2Sn(Q)(val)]$ $C_{22}H_{24}NO_9Sn$ $[(C_6H_5)_2Sn(Q)(val)]$ $C_{32}H_{28}NO_9Sn$ val= valine		Parveen et al. (2016)
VO(IV/V)	1:1	Not given	Not given	Shukla et al. (2004)

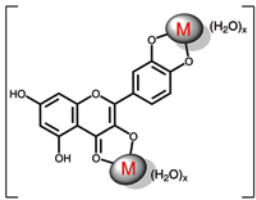
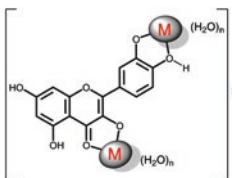
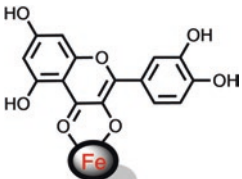
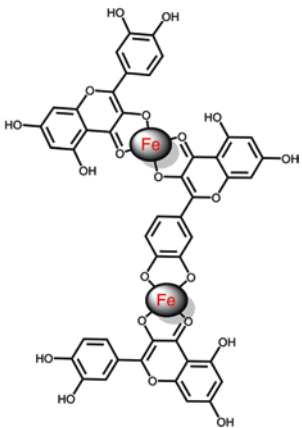
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**Table 4.3** (continued)

Metal ion	Stoichiometry (metal ion: flavonoid)	Chemical formula	Structure	Refs.
<b>Quercetin coordinating groups: 4-oxo, 5-hydroxo</b>				
Al(III)	1:2	$C_{30}H_{19}ClO_{15}Al$		Ahmedova et al. (2012)
Cr(III)	1:2	$C_{30}H_{27}ClO_{19}Cr$		Chen et al. (2009)
Se(IV)	1:2	$C_{30}H_{19}ClO_{15}Se$		Zhang and Chen (2012)
<b>Quercetin coordinating groups: 4-oxo, 3-hydroxo, and 5-hydroxo</b>				
Ln(III) Ln: La, Pr, Nd, Sm, Gd, Tb, Dy, Ho	1:3	$C_{45}H_{35}O_{25}La$ $C_{45}H_{29}O_{22}Pr$ $C_{45}H_{35}O_{25}Nd$ $C_{45}H_{29}O_{22}Sm$ $C_{45}H_{29}O_{22}Gd$ $C_{45}H_{29}O_{22}Tb$ $C_{45}H_{29}O_{22}Dy$ $C_{45}H_{29}O_{22}Ho$		Ansari and NMR (2008)
<b>Quercetin coordinating groups: 3', 4'-hydroxo</b>				
Pt(II)	1:1	$C_{27}H_{18}O_7 P_2Pt$		Michela et al. (2016)

(continued)

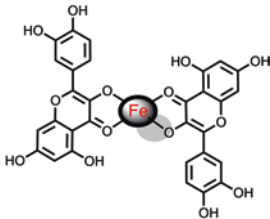
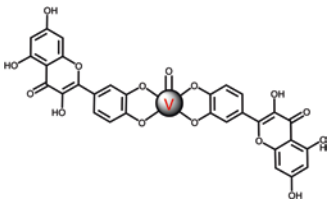
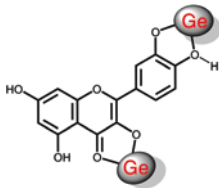
**Table 4.3** (continued)

Metal ion	Stoichiometry (metal ion: flavonoid)	Chemical formula	Structure	Refs.
<b>Quercetin coordinating groups: 4-oxo, 3, 3', 4'-hydroxo</b>				
Al(III) Zn(II)	<b>2:1</b>	$C_{15}H_{23}O_{15}Cl_4Al_2$ $C_{15}H_{15}O_{11}Cl_2Zn_2$	 <p>for Al(III) <math>x = n = 4, z = 0</math>; for Zn(II) <math>x = n = 2, z = 0</math>; for Co(II) <math>x = 2, n = 1, z = 4</math></p>	De Souza and De Giovanni (2005)
Co(II)		$C_{15}H_{23}O_{15}ClCo_2$		Bukhari et al. (2008)
Cu(II)	<b>2:1</b>	$C_{15}H_{20}O_{17}SCu_2$	 <p>for Cu(II) <math>n = 2</math>; for Mg(II) - <math>n</math> is not given</p>	Bukhari et al. (2009)
Mg(II)		Not given		Ghosh et al. (2015)
<b>Studies regarding metal complex species formed in solution</b>				
<b>Quercetin coordinating groups: 4-oxo, 3-hydroxo</b>				
Fe(II)	1:1	$C_{15}H_9O_7Fe$		Kim et al. (2013)
	2:3	$C_{45}H_{25}O_{21}Fe_2$		

(continued)



**Table 4.3** (continued)

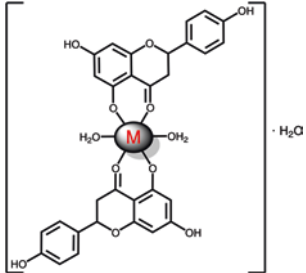
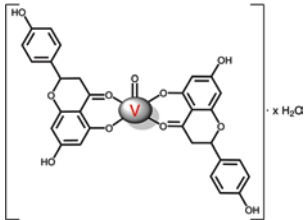
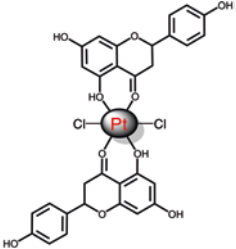
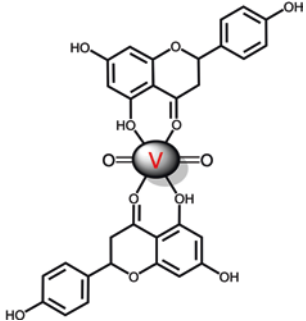
Metal ion	Stoichiometry (metal ion: flavonoid)	Chemical formula	Structure	Refs.
Fe(III)	1:2	C <sub>30</sub> H <sub>19</sub> O <sub>14</sub> Fe		Dimitrić Marković et al. (2011)
<b>Quercetin coordinating groups: 3', 4'-hydroxo</b>				
V <sup>IV</sup> O	1:2	C <sub>30</sub> H <sub>16</sub> O <sub>14</sub> VO		Sanna et al. (2014)
<b>Quercetin coordinating groups: 4-oxo, 3, 3', 4' - hydroxo</b>				
Ge(IV)	2:1	Not given		Li et al. (2012)

## 2.5 Metal Complexation Sites in Selected Flavan-3-Ol – Epigallocatechin Gallate (EGCG) – and Structural Remarks

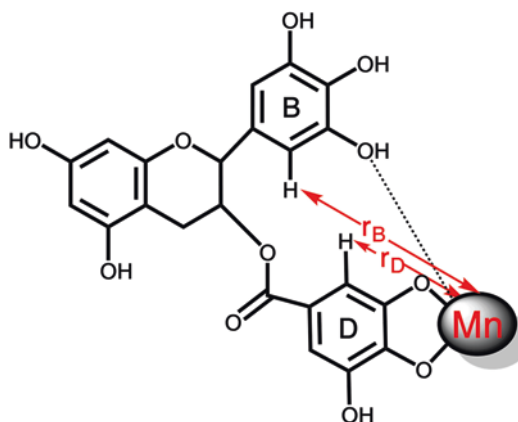
The two rings, B and D, in EGCG structure have been shown to possess the exact same local structure and to be able to participate in metal complexation (Fig. 4.2). The D-ring OH groups represent the preferred coordination sphere around a metal ion, the B-ring OH groups having a secondary effect on complexation. The D-ring of EGCG is capable of forming a diolate chelate ring with Mn(II) (Navarro et al. 2005) and Al(III) molar ratio 1:2 in the complexes (Inoue et al. 2002).

Another study regarding the interaction between Al(III) and EGCG in solution revealed the influence of the pH value over the stoichiometry of the resulted complex. Thus, at pH = 5.0 and 6.2, the ratio of Al<sup>3+</sup> to EGCG was proven to be 1:1. However, at pH = 6.2, when the ratio Al(III):EGCG was increased over 2, the com-

**Table 4.4** Data regarding the coordinating groups, stoichiometry, chemical formula, and chemical structure of several quercetin metal complexes

Metal ion	Stoichiometry (metal ion: flavonoid)	Chemical formula	Structure	Refs.
<b>Studies regarding metal complex species in solid-state</b>				
<b>Naringenin coordinating groups: 4-oxo, 3-hydroxo</b>				
Cu(II), Zn(II), Ni(II)	1:2	$C_{30}H_{28}O_{15}M$		Wang et al. (2006a) and Tan et al. (2009)
V <sup>IV</sup> O	1:2	$C_{30}H_{22}O_{12}VO$		Uivarosi et al. (2016) and Islas et al. (2015)
<b>Studies regarding metal complex species formed in solution</b>				
Pt(II)	1:2	Not given		Fazary et al. (2016)
V <sup>V</sup>	1:2	Not given		Fazary et al. (2016)

**Fig. 4.2** A proposed coordination scheme in Mn-EGCG 1:2 complex ( $r_D$  and  $r_B$ : interatomic distances between  $Mn^{2+}$  ion and the closest H atom of the D- and B-rings. (Navarro et al. 2005)



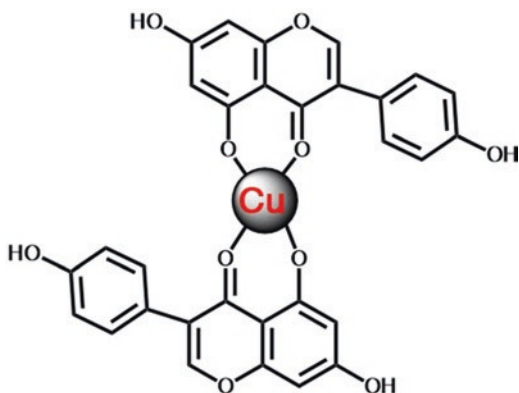
plex of Al-EGCG started polymerization, and the ratio in the polymer was 2:1. Moreover, the ability of EGCG to coordinate Al(III) is relevant in regard to the low absorption and the high levels of excretion of Al(III) observed after tea consumption, due to the presence of the green tea polyphenols (Tang et al. 2004).

Interestingly, it was found that the concentration of Ca(II) ions plays an important role on the mechanism of aggregation of  $\beta$ -lactoglobulin/EGCG complexes formed during digestion, in particular on the z-potential and on the particle size. Electrostatic interactions are thought to participate in the formation of the EGCG-calcium network, with an increasing particle size, which might improve the bio-availability of EGCG,  $\beta$ -lactoglobulin, and calcium (Carnovale et al. 2016).

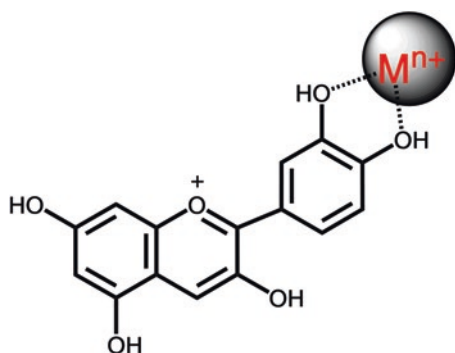
## 2.6 Metal Complexation Sites in the Selected Isoflavone – Genistein – and Structural Remarks

Due to its chemical structure, genistein can only bind metal ions at the 4-keto and the 5-OH site. Although the literature cites numerous studies in regard to its potential biological applications, only scarce data exists on genistein metal complexes or its interactions with metal ions. Spoerlein et al. reported on the synthesis and characterization of a copper (II)-genistein complex 1:2 (Fig. 4.3) with more potent cytotoxic activity than that of genistein alone (Spoerlein et al. 2013). The 1:2 metal/ligand stoichiometry has also been reported by Dowling et al. for the Cu(II) and Fe(III) chelates of genistein (Dowling et al. 2010).

**Fig. 4.3** Chemical structure of the Cu(II) complex of genistein. (Spoerlein et al. 2013)



**Fig. 4.4** Cyanidin metal complex structure. (Khaodee et al. 2014)



## 2.7 Metal Complexation Sites in the Selected Anthocyanidin – Cyanidin – and Structural Remarks

Certain aspects regarding complex formation of cyanidin with metal ions (Cu(II), Pb(II), Fe(III), Cr(III), Al(III), Cd(II), Ni(II), Zn(II), Co(II), and Mn(II)) have been studied in different buffer solutions with a pH value ranging from 3 to 7. Cyanidin formed complexes with Cu(II), Pb(II), Fe(III), and Al(III) at wide pH ranges, in the pH range of 5–7 for Cu(II), pH 5–6 for Pb(II), pH 3–4 for Fe(III), and pH 3–6 for Al(III). Spectrophotometric analysis revealed that the interaction between cyanidin and the metal ions involved the ortho-dihydroxyl group in the B-ring (Fig. 4.4) (Khaodee et al. 2014).

### **3 General Effect of Metal Complexation on the Biological Properties of Flavonoids with Special Reference to the Anticancer Activity**

Cancer is the second cause of death worldwide, responsible at this moment for one in three premature deaths from nontransmissible diseases. Cancer is, therefore, without any doubt, one of the most fearsome public health issues of our time. The efficacy of current chemotherapeutics is impeded by intrinsic and acquired resistance and dose-limiting side effects (see the case of cisplatin, for instance), which renders the search for new anticancer drug candidates an ongoing task. Several classes of natural compounds, including flavonoids, have been taken into consideration as novel anticancer agents due to their promiscuous affinity toward a plethora of biological targets. The molecular targets of flavonoids and their metal complexes will be briefly discussed in the following subchapters.

#### ***3.1 General Remarks Regarding the Biological Targets Relevant to the Anticancer Activity of Flavonoids and Their Metal Complexes***

Their cytotoxic activity involves the inhibition of several molecular targets and pathways: cyclin-dependent kinases (Casagrande and Darbon 2001), DNA topoisomerases I and II (Lopez-Lazaro et al. 2010), androgen receptor signaling (Khan et al. 2008), actin polymerization (Bohl et al. 2007; Sinha et al. 2015), activation of the tumor-suppressor protein p53, and inhibition of nuclear factor-kappa B (NF- $\kappa$ B) pathways (Erdogan et al. 2016). Figure 4.5 includes a schematic representation of several molecular targets and downstream signaling pathways of flavonoids. Consequently, flavonoids influence a series of cancer-related processes, such as cellular proliferation, differentiation, apoptosis, metastasis, angiogenesis, and reversal of multidrug resistance (Chahar et al. 2011). Interestingly, the modulation of these processes is, in most cases, the result of a fine balance between the antioxidant and prooxidant properties of flavonoids. The antioxidant properties are mainly exerted through direct free radical scavenging, metal chelation, primarily Fe(II), Fe(III), and Cu(II) (Pietta 2000). Noteworthy, flavonoid metal complexes have shown more potent free radical scavenging properties than the free corresponding flavonoids (Malešev and Kuntić 2007). Also, their antitumor activity has been reported to be superior to that of the parent flavonoids against several types of cancer cells. A very important and promising feature is that a number of them have proved selectivity toward cancerous over non-cancerous cell lines.

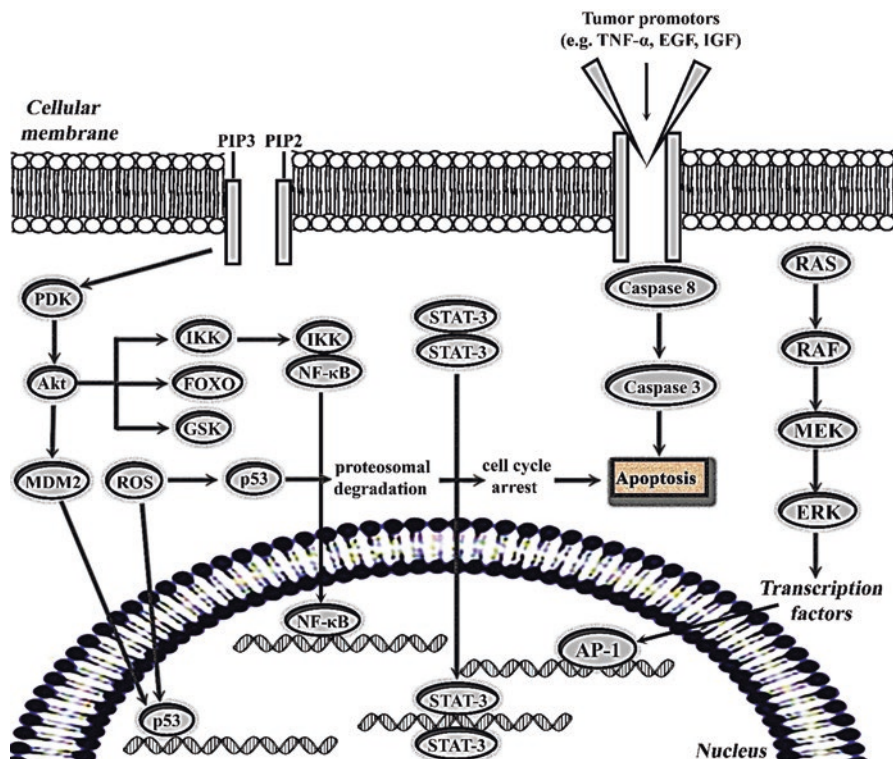


Fig. 4.5 Schematic diagram of several molecular targets and downstream signaling pathways of flavonoids. (Millimouno et al. 2014)

## 3.2 Flavones: Luteolin

### 3.2.1 General Remarks Regarding the Biological Targets Relevant to the Anticancer Activity of Luteolin

Luteolin has been reported to show potent anticancer activity on a wide variety of cancer cells, including multidrug-resistant cells. Briefly, luteolin mediates cell cycle arrest (Lee et al. 2012; Lim et al. 2012), triggers both intrinsic (Ham et al. 2014; Jiang et al. 2018) and extrinsic apoptosis pathways (Park et al. 2014), prevents tumor invasion and metastasis (Lee et al. 2017), and exerts strong antiangiogenesis activity (Ravishankar et al. 2015). A detailed work reviewing the molecular targets of luteolin as an anticancer agent can be found at ref. (Tuorkey 2016).

### 3.2.2 Metal Complexes of Luteolin with Anticancer Activity

Luteolin metal complexes have been investigated as DNA biosensors (Niu et al. 2009) and in regard to their anti-inflammatory (Li et al. 2011) and antioxidant activities (Kostyuk et al. 2004; Roy et al. 2015). The anticancer properties have only been marginally studied for the oxidovanadium (IV) complex of luteolin,  $[\text{VO}(\text{lut})(\text{H}_2\text{O})_2]\text{Na}\cdot 3\text{H}_2\text{O}$ . Noteworthy, in this case, the cis hydroxyl groups, not the 4-CO and 5-OH moiety, of luteolin are involved in metal binding, resulting in a “catechol-like” coordination mode. For this reason, both luteolin and VO(lut) displayed similar antioxidant properties (Naso et al. 2016a). In contrast, the  $\text{V}^{\text{IV}}$  luteolin complex 1:2 in which an “acetylaceton-like” coordination mode is involved exhibits superior antioxidant activity compared to that of the free, corresponding flavone (Roy et al. 2015). The results for the VO(lut) complex on MDAMB231 breast ( $\text{IC}_{50} = 17 \mu\text{M}$ ) and A549 lung ( $\text{IC}_{50} = 60 \mu\text{M}$ ) cancer cell lines were notwithstanding, apart from the fact that metal complexation has improved the activity of the free ligand in MDAMB231 cells (luteolin  $\text{IC}_{50} = 88.3 \mu\text{M}$ ). In mechanistic terms, the cytotoxic activity of both luteolin and VO(lut) was found to be due to oxidative stress processes, which caused cancer cells to undergo mitotic arrest. In addition, VO(lut) generates cytoplasmic and nuclear membrane damages (Naso et al. 2016a). Also, VO(lut) exerts stronger cytotoxic activity than luteolin on CT-26 colon cancer cell line ( $\text{IC}_{50} 0.9 \mu\text{M}$ ) and no toxic effects at concentrations up to  $10 \mu\text{M}$  on normal colon epithelial cells. In addition, VO(lut) prevents liver metastasis in a murine model of highly aggressive, orthotopic colon cancer (CT-26 cancer cell lines) (Naso et al. 2016b).

## 3.3 Flavonols: Quercetin

### 3.3.1 General Remarks Regarding the Biological Targets Relevant to the Anticancer Activity of Quercetin

The chemopreventive and anticancer activities of quercetin are well-documented in various cancer cell lines and animal models. Several reviews regarding these activities can be found at ref. (Murakami et al. 2008; Darband et al. 2018; Sharma et al. 2018; Kashyap et al. 2018). Also, an extensive review regarding the modifications of the quercetin scaffold leading to molecules with cytotoxic activity can be found in ref. (Massi et al. 2017). We will only refer here to the most recent work in respect to revealing novel mechanistic aspects underlying the cytotoxic activity of quercetin and to developing new metal complexes as anticancer agents.

Quercetin inhibits vascular endothelial growth factor receptors (VEGFR), targeting the AKT/mTOR/P70S6K pathway (Pratheeshkumar et al. 2012), and inhibits the expression of ErbB2 and ErbB3, influencing the ErbB/HER signaling pathway (Kim et al. 2005). Also, quercetin has been shown to inhibit the Wnt signaling pathway (Mojsin et al. 2014). A recent study has identified quercetin to be one of the

most active natural flavonoids in regard to the inhibition of the transmembrane member 16A (TMEM16A)-encoded  $\text{Ca}^{2+}$ -activated  $\text{Cl}^-$  channels (overexpression of TMEM16A is thought to be connected to cancer progression) (Xuan et al. 2017).

In the human leukemia HL-60 cell line, quercetin was found to induce cell cycle arrest in the G0/G1 phase; to inhibit the expression of cyclin-dependent kinases (CDK) 2 and 4; to induce CDK inhibitors, p16 and p21; to activate poly-ADP ribose polymerase (PARP)-1 cleavage (is involved in proapoptotic signaling); and to trigger caspase activation. Moreover, besides promoting apoptosis, quercetin also induced in HL-60 cells a cytoprotective autophagy process, proposed by the authors as a novel approach to enhance the anticancer activity of quercetin for future studies (Junn-Liang et al. 2017).

A comprehensive study was recently conducted on nine breast, colon, and colorectal cancer cell lines to assess the cytotoxic effects of quercetin. Apoptosis was found to be responsible for the cell growth inhibition, an effect that was evident in the colon carcinoma CT-26, pheochromocytoma PC-12, prostate adenocarcinoma LNCaP, and human prostate PC-3 cell lines. Moreover, quercetin treatment resulted in a significant decrease of the tumor volume in mice bearing MCF-7 and CT-26 tumors (Shedid et al. 2017).

Furthermore, quercetin was assessed as a potential candidate for oral squamous cell carcinoma (OSCC) chemoprevention. The mechanism underlying the antitumor activity in a 7,12-dimethylbenz(a)anthracene (DMBA)-induced hamster buccal pouch carcinogenesis model was attributed to the suppression of the NF- $\kappa$ B signaling pathway (Zhang et al. 2017).

Additionally, recent studies focused on assessing the synergistic behavior of quercetin with other drugs, suggesting a potential role in combination therapy. For instance, quercetin enhanced the efficacy of irinotecan and its metabolite, SN-38, in a human gastric cancer cell line. These results were confirmed in a corresponding xenograft mouse model (Lei et al. 2018). Furthermore, sequential treatment with quercetin and vitamin C enhances the potency of a combined treatment with doxorubicin (DOX) and paclitaxel (PAC), in breast cancer cells. This combination treatment resulted in a significant decrease of the  $\text{IC}_{50}$  value in comparison to the corresponding values resulted from the DOX + PAC and PAC treatments, in all breast cancer cells. Moreover, this treatment was more effective in the induction of the early stages of apoptosis than DOX + PAC (Ramezani et al. 2017). Additionally, *in vivo* experiments on mouse xenografts show that quercetin downregulates the splicing factors involved in enzalutamide resistance in prostate cancer cells and antagonizes androgen receptor (AR) signaling (Tummala et al. 2017).

### 3.3.2 Metal Complexes of Quercetin with Anticancer Activity

*In vitro* studies showed that a ruthenium quercetin complex induced apoptosis and DNA fragmentation in HT-29 cells, increased p53 expression, and decreased VEGF and mTOR expression. Results from the *in vivo* studies revealed that the ruthenium quercetin complex suppressed key transcription factors and hyperplastic lesions and



improved CAT, SOD, and glutathione levels. The complex was also shown to decrease cell proliferation and increase apoptotic events in tumor cells correlated to the downregulation of B-cell lymphoma 2 (Bcl2) protein and the upregulation of p53 and Bcl-2-associated X (Bax) protein expression (Roy et al. (2018)).

It has been proven that the combined treatment of quercetin and myricetin is more effective in causing DNA damage in K562 (human chronic myelogenous leukemia) cells than the stand-alone treatment with any of the two flavonoids. More importantly, the presence of copper ions increases cellular damage, suggesting the possible formation of a flavonoid metal complex (Das et al. 2017).

The ability of flavonoids and other small molecules to bind to double-stranded DNA ranks among the most significant mechanisms that underlie their antitumor activity. Quercetin binds DNA as a result of electrostatic interactions. Its bulkier complexes, on the other hand, display diverse mechanisms of binding toward DNA, including major or minor groove binding and/or intercalation (Uivarosi and Munteanu 2017). An increase of the DNA binding affinities has been observed for quercetin complexes with Fe(II) (Raza et al. (2016)), Cu(II) (Ni et al. 2007), Mn(II) (Jun et al. n.d.), Zn(II) (Jun et al. 2007), Tb(III) and Eu(III) (Li et al. 2009), valine quercetin diorganotin(IV) (Parveen et al. 2016), and Cu<sup>II</sup>-Sn<sub>2</sub><sup>IV</sup>-quercetin and Zn<sup>II</sup>-Sn<sub>2</sub><sup>IV</sup>-quercetin complexes (Tabassum et al. 2013). The GC-rich DNA binding propensity of a Ni(II)-quercetin complex has been correlated with its cytotoxic activity against human hepatocarcinoma HepG2 and SMMC-7721 and human lung (carcinoma) A549 cell lines. Following the treatment with the Ni(II)-quercetin complex, decreased levels of survivin and Bcl2 expression, and significantly increased levels of p53 were found in HepG2 cells, resulting in cell apoptosis (Tan et al. 2010). A quercetin/lanthanum complex showed considerable cytotoxicity on human cervical carcinoma cells. The complex also triggered dose-dependent pro-oxidative effects and DNA breakage (Durgo et al. 2011).

### 3.4 Flavanones: Naringenin

#### 3.4.1 General Remarks Regarding the Biological Targets Relevant to the Anticancer Activity of Naringenin

Naringenin is a citrus flavanone, with well-documented antioxidant, antimutagenic, anticancer, antiproliferative, anti-inflammatory, antiatherogenic activities and insulin-like properties (Farhan et al. 2015 and ref. therein, Patel et al. 2014). The next paragraphs will focus on the antitumor activity of naringenin and its metal complexes, with a special attention given to the most recent articles or book chapters published.

Naringenin has been found to impair cell proliferation and to induce apoptotic events in tamoxifen-resistant MCF-7 breast cancer cells through inhibition of PI3K and MAPK pathways, as well as a decrease of ERK and AKT expression. Furthermore, naringenin induced the translocation of the estrogen receptor (ER)- $\alpha$

to a perinuclear region, which indicates that naringenin acts as an antagonist to the ER (Ramos et al. 2017). The involvement of naringenin in the PI3K and MAPK pathways has also been reported in several other studies on bladder cancer cells (Liao et al. 2014), prostate cancer cells (Lim et al. 2017), and human placental choriocarcinoma (Park et al. 2017). The modulation of NF- $\kappa$ B expression has also been reported in benzo(a)pyrene-induced pulmonary carcinogenesis (Bodduluru et al. 2016). Noteworthy, naringenin prevents osteosarcoma progression and recurrence via improvement of the redox status of the cells (Zhang et al. 2018) and inhibits the migration of lung cancer cells via inhibition of MMP-2 and MMP-9, which play a key role in cancer cell invasion and metastasis (Chang et al. 2017).

The potential role of naringenin in combination therapy has been proven in co-treatment with ABT-737, a Bcl-2 inhibitor, on gastric cancer cells (ZHANG et al. 2016), with curcumin (in which case it induces cell cycle arrest and apoptosis through the interference with various pathways) on THP-1 cells (Shi et al. 2015), and by the enhancement of the cytotoxic effects of a histone deacetylase inhibitor, by transglutaminase activation (Ling et al. 2012).

### 3.4.2 Metal Complexes of Naringenin with Anticancer Activity

A naringenin complex with oxovanadyl (IV),  $[\text{VO}(\text{nar})_2] \cdot 2\text{H}_2\text{O}$  (VOnar), displayed more potent antiproliferative effects than naringenin, against lung and breast cancer cell lines. Moreover, the activity of the complex was accompanied by ROS generation, cell membrane damage, DNA degradation, cell cycle arrest, activation of caspase 3/7, and mitochondrial membrane potential decrease (Islas et al. 2015). Furthermore, a naringenin Schiff base La(III) complex has been proven to bind potently with calf thymus DNA, presumably via intercalation, which may be responsible for the cytotoxic effects of the complex on HL-60 and A-549 cells (Wang et al. 2006b).

## 3.5 Flavan-3-Ols: EGCG

### 3.5.1 General Remarks Regarding the Biological Targets Relevant to the Anticancer Activity of EGCG

The research on the biological effects of EGCG, particularly the anticancer activity, has attracted much interest mainly because of the well-documented beneficial properties of the green tea consumption. The most important bioactive components in green tea are catechins, with EGCG being the most abundant in nature. EGCG exhibits versatile activities on cancer cells, being able to induce apoptosis; inhibit growth/proliferation, adhesion, invasion, migration, and metastasis; reduce motility; induce antiangiogenic effects; and enhance antitumor immunity (Gan et al. 2018). Recent studies address the mechanistic aspects of its anticancer activity. For

instance, it was shown to inhibit heat shock protein 90 (HSP 90) (Yin et al. 2009), a molecular chaperone, with a key role in the prevention of protein misfolding and aggregation and in enhancing protein stability. HSP90 coordinates cancer-specific networks; it modulates the activity of various transcription factors relevant to prostate cancer, such as AR, ErbB2, Akt, VEGFR, and MMP-2 and MMP-9. Through improper interactions with some client proteins, HSP 90 is thought to promote a malignant state of the cancer cells (Moses et al. 2015).

Moreover, EGCG has been found to modulate the AKT/STAT3 pathway and to cause potent inhibition of the multidrug resistance 1 (MDR1) protein expression in cisplatin-resistant oral cancer CAR cells. As a result, EGCG triggered programmed death in these cancer cells, through apoptosis and autophagy.

Moreover, EGCG exerts synergistic effects with well-established anticancer agents (Yuan et al. 2017). Downregulation of STAT3-NF $\kappa$ B signaling pathway appears to be involved in the inhibition of cancer stem cell phenotype by curcumin in combination with EGCG (Chung and Vadgama 2015). In MCF-7 cells, EGCG enhances 5-fluorouracil's antitumor activity by modulating the expression of Bcl-xL (Sun et al. 2016). Additionally, combined EGCG and cisplatin treatment showed synergistic cytotoxic effects in five biliary tract cancer cell lines and antagonistic effects in other two (Mayr et al. 2015).

### 3.5.2 Metal Complexes of EGCG with Anticancer Activity

The combination of Zn<sup>2+</sup> with EGCG on androgen-insensitive prostate cancer cells (PC-3) resulted in growth inhibition of the cells in a time- and dose-dependent manner. These inhibitory effects were considerably reduced in the presence of EGCG. Therefore, it has been hypothesized that Zn<sup>2+</sup> complexation of EGCG might be responsible for the observed bioactivities on PC-3 cells (Chen et al. 2007). This hypothesis, however, has been rejected in a later study (Sun et al. 2008). Another study showed that Cd(II)-induced growth inhibition of PC-3 cells in a concentration- and time-dependent manner and EGCG improved the effect of Cd(II) on the previously mentioned cell line, although no proof of a EGCG-Cd(II) complex was observed in the system (Yu et al. 2007).

Moreover, EGCG is thought to induce the mobilization of copper ions bound to chromatin in human peripheral lymphocytes, causing oxidative DNA damage. Structure-activity relationship studies revealed that novel anticancer molecules based on the catechin backbone should possess as many hydroxyl groups as possible, which may facilitate cellular DNA binding (Farhan et al. 2015).

Furthermore, a Cu(II) complex of EGCG was found to inhibit the enzymatic activity of ribonuclease A (RNase A) in a noncompetitive manner, with inhibition constants in the micromolar range. More importantly, the copper complex is a more potent RNase A inhibitor than the parent flavonoid. Taking into account the fact that RNase A and angiogenin are homologous, the authors of this study hypothesize that these complexes could be used as antiangiogenic agents through copper chelation (Ghosh et al. 2006).

### 3.6 Isoflavones: Genistein

#### 3.6.1 General Remarks Regarding the Biological Targets Relevant to the Anticancer Activity of Genistein

Genistein (4',5,7-trihydroxyisoflavone) is well-known as a chemotherapeutic and chemopreventive agent. Genistein has been proven to be effective against various types of cancer cells in the breast, colon, prostate, liver, lung, ovarian, bladder, neuroblastoma, brain, and gastric and leukemia (Russo et al. 2016 and ref. therein). Other several studies have demonstrated the positive effects of genistein on pancreatic (Bi et al. 2018), thyroid (Ozturk et al. 2018), tongue (Ardito et al. 2017), and melanoma (Cui et al. 2017) cancers. Generally speaking, the cytotoxic effects of genistein emerge as a result of ROS-mediated mitochondrial apoptosis, inhibition of telomerase activity, inhibition of DNA topoisomerase II, cell cycle arrest, and inhibition of metastasis and angiogenesis. Genistein could also inhibit migration of the Mia-PaCa2 (pancreas carcinoma) cells, accompanied by the downregulation of metalloproteinases (MPP-2 and MPP-9) (Bi et al. 2018).

Briefly, genistein interacts at a molecular level with caspases, Bcl-2, Bax, nuclear transcription factor  $\kappa$ B (NF- $\kappa$ B) (Zhou et al. 2017), extracellular signal-regulated kinase 1/2 (ERK 1/2) (Huang et al. 2014), mitogen-activated protein kinase (MAPK) (Cui et al. 2017), Wnt/ $\beta$ -catenin (Zhang et al. 2013), phosphoinositide 3 kinase/Akt (PI3K/Akt) (Nakamura et al. 2009), FOXO3a transcription factor (Song et al. 2018), and STAT3 (Lian et al. 2004) signaling pathways. More recently identified molecular targets are kinesin-like proteins (Yan et al. 2012), long noncoding RNA HOTAIR (Imai-Sumida et al. 2017), and microRNAs (Xia et al. 2012; Chiyomaru et al. 2013; Hirata et al. 2013; Xia et al. 2014; Ma et al. 2013; Yuzhen et al. 2017).

MicroRNAs (miRNAs) are short, noncoding endogenous RNAs, involved in cancer progression. Among polyphenols, genistein, EGCG, curcumin, resveratrol, quercetin, and hesperidin decrease the expression of several types of miRNAs (Bodduluru et al. 2016 and ref. therein). These results might establish polyphenols targeting miRNAs as novel and promising agents in anticancer chemotherapy and chemoprevention.

Moreover, a recent study showed that isoflavones, especially genistein, induce apoptosis of colon cancer cells by reducing the formation of lipid droplets (LDs) (Liang et al. 2018). LDs are key cellular organelles which serve as storage of lipid surplus. Cancer cells are lipid-rich under normoxia and hypoxia and can store a large amount of LDs. Moreover, LDs mediate various stress response mechanisms of cancer cells, which is why inhibition of LD formation is correlated with decreased cancer cell proliferation (Koizume and Miyagi 2016). Isoflavones have been proven to inhibit oleic acid-induced LD accumulation by regulating LD-related factors, associated with the regulation of the expression of LD-associated genes (Liang et al. 2018).

Furthermore, genistein shows synergistic behavior with other drugs, suggesting a potential role in combination therapy. Several examples include anticancer agents

such as Adriamycin (Monti and Sinha 1994), 5-fluorouracil (Hwang et al. 2005), tamoxifen (Mai et al. 2007), indole-3-carbinol (Nakamura et al. 2009), anesthetics (propofol) (Yuzhen et al. 2017), or anti-inflammatory agents such as dexamethasone (Park et al. 2001).

### 3.6.2 Metal Complexes of Genistein with Anticancer Activity

Up to this moment, only few studies have reported on the anticancer-related activity of genistein metal complexes. Dowling et al. showed that the antioxidant ability of genistein is affected by metal complexation in a disparate manner: while copper binding results in an increase of the antioxidant effect in comparison to the free ligand, iron chelation leads to a prooxidant effect (Dowling et al. 2010).

The Cu (II) homoleptic complex of genistein, for instance, greatly enhances the cytotoxicity of isoflavone when tested on the fast proliferating metastatic 518A2 melanoma cells. Moreover, coordination of genistein to Cu (II) also led to the diminished expression and secretion of MMP-2 and MMP-9, remodeling of the actin cytoskeleton, an increase in cadherin-catenin complex formation (factors that favor cell-cell adhesion), and cell cycle arrest in the G2/M transition phase. The antimigratory and antimetastatic activities of the complex are much more pronounced when compared to those observed for the free ligand (Spoerlein et al. 2013). Also, Schiff base derivatives of 3-formylchromone and genistein and their copper (II) complexes display key interactions with amino acids in the pleckstrin homology (PH) and the kinase domain of the PKB (Akt) protein. In vitro evaluation of the copper complexes against hormone-independent and metastatic breast, prostate, and pancreatic cancer cells revealed that these complexes displayed PKB (Akt protein) inhibitory activities. Moreover, in a pancreatic tumor model using COLO 357 cells, the complexes caused NF- $\kappa$ B inactivation (Barve et al. 2006).

## 3.7 Anthocyanidins: Cyanidin

### 3.7.1 General Remarks Regarding the Biological Targets Relevant to the Anticancer Activity of Cyanidin

Anthocyanins, a class of water-soluble flavonoids, possess antioxidant, anti-inflammatory, cardioprotective, antidiabetes, and antitumor activities (Bo-Wen et al. 2016). Numerous studies have reported diverse biological activities, including anticancer, of anthocyanin-rich plant extracts. However, these studies will not be addressed in the present work, which will focus on the most recent investigations regarding the cytotoxic activity of cyanidin, the most abundant natural anthocyanin (Colditz et al. 1985), either in its aglycone or  $\beta$ -glucoside form.

Cyanidin-3-*o*- $\beta$ -glucoside isolated from mulberry exerts anticancer effects on MDA-MB-453 human breast cancer cells via caspase-3 cleavage and DNA

fragmentation through Bcl-2 and Bax pathway and in a MDA-MB-453 cell-inoculated animal model (Cho et al. 2017). Cyanidin-3-o- $\beta$ -glucoside also induced cytotoxic and apoptogenic effects in U87 (human glioblastoma multiforme) cell line. Apoptosis was associated with increased Bax and p53 expression and decreased Bcl2 expression leading to cell cycle arrest in G1/S and G2/M phases (Hosseini et al. 2017).

Cyanidin displayed significant inhibitory effects on the proliferation, migration, and invasion of renal carcinoma cell (RCC) lines. It was found to modulate the expression of various transcription factors involved in tumor suppression or cellular growth and differentiation, such as early growth response protein 1 (ERG1) and selenoprotein W (SEPW1), respectively. Also, cyanidin treatment led to increased activity of the apoptotic mediator caspase 3 and inhibition of E-cadherin activation. Furthermore, cyanidin regulates important proteins associated with autophagy, such as p62 and ATG4 cysteine protease. Moreover, these effects occurred in a selective manner in RCC tumor tissue, but not in the adjacent normal tissue samples. In vivo studies revealed that cyanidin significantly hindered the growth of xenografts in nude mice (Liu et al. 2018). Another in vivo study on a mouse model showed that anthocyanins prevent the formation and growth of colorectal cancer in azoxymethane/dextran sodium sulfate-treated Balb/c mice (Lippert et al. 2017).

Furthermore, cyanidin ameliorates cisplatin (Qian et al. 2018)- and doxorubicin (Petroni et al. 2017)-induced cardiotoxicity, a side effect which limits the clinical use of these drugs and increases the risk of cardiovascular disease. Cyanidin reverts cisplatin-induced cardiotoxicity by impeding ROS-mediated apoptosis; other processes and pathways, such as mitochondrial and extracellular regulated kinase signaling pathways, may also be involved (Qian et al. 2018).

### 3.7.2 Metal Complexes of Cyanidin with Anticancer Activity

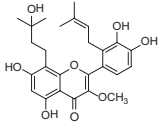
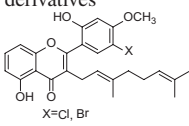
To the extent of our knowledge, the literature does not cite any anthocyanin metal complex with anticancer activity.

## 4 Patents Involving Flavonoids with Anticancer Therapeutic Applications Filed During 2015–2018

Natural products are well-known therapeutic agent due to their wide diversity of chemical and biological functionality. The process of evaluating natural products in the development of new drugs is considered to be attractive and reliable sources. Several recent reviews of patent highlight have revealed the significance of natural products toward drug discovery process (Yadav et al. 2018; Kashyap et al. 2016; Sharma et al. 2018; Chakrawarti et al. 2016; Di Martino et al. 2017a, b). Flavonoids represent the “polyphenol” natural products family and are secondary metabolites

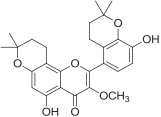
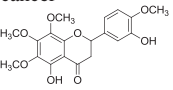
(phytochemicals) of plants. They are responsible for key functions in plant nourishment; by virtue of this, they are abundantly dispersed and found in various fruits, vegetables, stems, and flowers of plants, cereals, herbs, spices, beverages (red wine, beer, tea), beans, cocoa, and other numerous botanical foods and beverages. This diverse class of compounds has various human-benefited health significance because of their numerous antioxidant, antitumor, anticancer, anti-inflammatory, antiviral, immune supporting, antiallergic, and hormonal regulating activities. A large number of patents were filed between 2015 and 2018 for flavonoids; their synthetic analogs and pharmaceutical formulations have described their anticancer therapeutic applications. The patents presented in this study have been collected from WIPO, USPTO, SIPO, and EPO databases through multiple electronic databanks including Espacenet, Google patents, and Mendeley. A brief description of patents and findings is presented in Table 4.5.

**Table 4.5** Patents filed during 2015–2018 related to anticancer therapeutic applications of flavonoids

Patent no.	Title	Significance	Refs.
CN105130940B	Preparation method and application of prenyl flavonoids having an anti-breast cancer activity	Synthesized the prenyl flavonoids and evaluated their activity against human breast cancer MCF-7 cell line  HepG2 cells cytotoxic activity $IC_{50} (\mu M) = 22.6 \pm 1.7$	YanJun et al. (2015a)
CN105001191A	Derivative with 5,2'-dihydroxy-4'-methoxy-3-geranyl flavonoid skeleton and preparation method and application thereof	Synthesized 5, 2'-dihydroxy-4'-methoxy-3-geranyl flavonoid derivatives and studied its inhibitory effect on the cervical cancer cell growth (Hela cells). The chloro and bromo derivatives have slightly better activity, i.e., $IC_{50}$ values are less than 25 $\mu M$ among all derivatives  $X = Cl, Br$ $IC_{50} (\mu M) < 25$	Sheng et al. (2015)
CN104288223A	Method for preparing total flavonoids of Chinese mosla herb and application of total flavonoids of Chinese mosla herb	The extraction of flavonoids from Chinese mosla herb and observed in vitro antitumor effect toward tumor cell growth human lung cancer (GLC4) cell line and colon cancer (C0L0) cell line inhibition	Dongfeng and Chengdong (2015)

(continued)

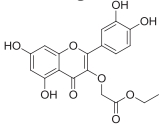
**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
KR101505175B1	A novel composition for suppressing metastasis of cancer	The Pharmaceutical composition consists of kaempferol as an active ingredient with carriers, excipients, or diluents and reported to suppress metastasis of cancer of TGF-beta1 in lung cancer cell line (A549) and controlled phosphorylation of Thr179 associated with Smad3 connection domain	Chul et al. (2015)
CN105131008A	Preparation method and application of prenylated flavonoid compound with anti-hepatoma activity	Synthesized the prenylated flavonoid compound and investigated their antitumor activity against human hepatoma cells (HepG2). These derivatives have showed promising cytotoxic activity on HepG2 and may be used as therapeutic agent for the treatment of liver cancer  $IC_{50} (\mu M) = 33.9 \pm 2.7$	YanJun et al. (2015b)
CN104922143A	EGCG (epigallocatechin gallate) and chitosan oligosaccharide composition as well as preparation method and application thereof	Preparation of pharmaceutical composition of EGCG (epigallocatechin gallate) and chitosan oligosaccharide. The suggested composition possessed potent antitumor synergistic effect against H22 hepatoma cell lines	Gengyuan et al. (2015)
US20160145230A1	Agent containing flavonoid derivatives for treating cancer and inflammation	Preparation of flavonoid-based pharmaceutical composition and their evaluation against various cancer cell lines including brain, breast, Kaposi sarcoma, leukemia, lung, melanoma, ovarian, pancreatic, colon, and prostate cancer  Most potent various cancer cell lines	Lowe et al. (2016)
CN105343158A	Fructus sophorae total flavonoid extract with broad-spectrum antitumor activity and preparation method and application of fructus sophorae total flavonoid extract	The total flavonoid in <i>fructus sophorae</i> extract comprises quercetin, genistein, kaempferol, and isorhamnetin. This extract has promising anticancer activity against human esophageal cancer cell (Qiao ca-109) and hepatoma cells (SMMC-7721). Mechanistic insight has revealed lower expressions of proteins including cyclin B1 and Bcl-2/ Bax ratio	Zhiling et al. (2016)

(continued)



**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN106176711A	Drug containing flavonoid compound composition and application thereof	The pharmaceutical composition of Formononetin, calycosin, and their glycosides has anticancer potential against breast cancer cells (MCF-7) and cervical cancer cells (HeLa). It has improved anticancer function and good synergistic effect	Yukun et al. (2016)
CN105669796A	Flavonoid compound TA34a and preparation method and application thereof	The extraction of 6,8,4'-trihydroxyflavone glycoside from <i>semen thlaspi</i> . It showed potent inhibition of the proliferation of tumor (HGC-27) cells. It also possessed antioxidant activity, antitumor activity, and immune enhancement activity in addition to PC-12 cell protection effect	Xiaoyan et al. (2016)
CN106008481A	Flavonoid compound targeting tumor cells and preparation method of flavonoid compound	The synthesis of compound from isoflavone and IR-783. This derivative has showed significant inhibitory effect on human breast cancer tumor (MCF-7) cell	Zhongqiu et al. (2016)
CN105503804A	Synthesis of quercetin-3- <i>O</i> -acetate and application of quercetin-3- <i>O</i> -acetate to tumor resistance	Synthesized quercetin-3- <i>O</i> -acetate and studied its inhibitory effect against four cell lines EC9706, EC109, B16-F10, and SGC7901 growth. This derivative has showed significant inhibition of EC9706 and EC109 cells than the parent drug quercetin (IC <sub>50</sub> = 31.884 μmol / L) and 5-FU (IC <sub>50</sub> = 41.738 μmol / L). In addition, inhibition of B16-F10 and SGC7901 tumor cells were also stronger than the parent drug quercetin 	Tingke et al. (2016)
CN105963246A	Genistein salt oral solution and its preparation method and use	Preparation of genistein salt-based oral solution from lysine, genistein, aspartame, and sodium bicarbonate. The solution drug has found to possess resistance reversion effects on a non-small cell lung cancer A549/DPP cells. It inhibits A549 lung cancer cell growth and showed promising antitumor effects	Chengxiong et al. (2016)
KR101678791B1	Usage of genistein as an anticancer drug in p53-mutated solid tumors or paclitaxel-resistant cancer	Preparation of composition containing genistein that have been effective in treating p53-mutated solid tumors or paclitaxel-resistant solid tumors. Genistein as PLK1 inhibitors promotes the cell death of taxol-resistant prostate cancer and lung cancer	Shin et al. (2016)

(continued)

**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN106214673A	Application of epigallocatechin gallate in preparing drug for preventing or treating bladder tumor	Prepared a formulation which contains epigallocatechin gallate as an active ingredient. The formulation was found to be effective in preventing or treating bladder tumor cancer cells. It showed the inhibitory action against the proliferation of bladder cancer cells SW780, 5637, and SV-HUC-1	Kewang et al. (2016)
KR20170014595A	Composition for preventing, improving, or treating hepatocellular carcinoma comprising flavonoid compounds isolated from fruit peels of <i>Citrus spp.</i>	The flavonoids compound derived from <i>citrus dermis</i> and studied in the prevention, amelioration, or treatment for liver cancer. The extract has showed increased expression of BaK protein and downregulation of Bcl-xL and the flavonoid compound derived from <i>citrus dermis</i> and studied in the prevention, amelioration, or treatment for liver cancer. The extract has showed increased expression of BaK protein and downregulation of Bcl-xL, inhibiting Akt P38, MAPK, and ERK phosphorylation in the liver cancer cells	Geon-seop et al. (2017)
CN106943438A	<i>Phellinus igniarius</i> anticancer active flavones compound PBF-1, preparation method, and application thereof	The extraction of flavones from <i>Phellinus igniarius</i> tested against human cervical carcinoma (Hela helmet) and human cancer cells (SGC-7901). There is no adverse effect on normal cells, such as human embryonic kidney cell HEK293 and mouse macrophage RAW264.7	Liangen and Mingming (2017)
CN107115372A	Antitumor pharmaceutical composition containing total flavonoids of <i>Apocynum venetum</i> leaves	The pharmaceutical composition contains total flavonoids of <i>Apocynum venetum</i> leaves and an anthracycline drug (pirarubicin or epirubicin) with better antitumor effect against MD-MBA-231 human breast cancer cells	Liqun et al. (2017)
CN107137619A	Anti-breast cancer healthcare product containing rice bran flavonoids	Preparation of anti-breast cancer healthcare product that consist of rice bran flavonoids = 50–70%; <i>Morinda officinalis</i> extract = 5–10%; radix aristolochiae extract = 5–10%; <i>Polyporus umbellatus</i> extract = 5–10%; <i>Epimedium</i> extract = 5–10%; <i>Bupleurum</i> extract = 5–10% (by weight). It has significant inhibitory action on human breast cancer (MCF-7) cell line proliferation	Xiangyu et al. (2017)

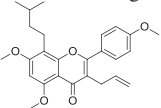
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**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN104945281B	Flavonoids acetic acid derivatives, pharmaceutical compositions thereof, and methods for their preparation and use	The prepared composition was found to induce a strong antitumor effect by blocking TNF- $\alpha$ signaling. Also it has showed antiangiogenic effect on chorioallantoic membrane and may be utilized as promising therapeutic formulation in the near future	Aihua et al. (2017)
US9808439B2	Use of tangeretin in cancer treatment	This pharmaceutical composition comprises a citrus methoxyflavone (tangeretin) and a chemotherapeutic drug (paclitaxel). It has been tested against human ovarian cancer (A2780) cells and its PTX-resistant (A2780/T) cell line and human non-small cell lung cancer (NSCLC) A549 and its PTX-resistant (A549/T) cell line. Results revealed that utilization of tangeretin with chemotherapeutic agents enhanced the drug efficacy in animal studies and clinical trials	Ma et al. (2017)
RU2619207C1	Biologically active food supplement with cancer-preventive action	Preparation of biologically active food supplement contains extracts from green tea leaves, turmeric roots, black cumin seeds, Japan ampelopsis root grass, <i>Sigesbeckia orientalis</i> grass, and Baikal skullcap roots, leaves, and natural honey. It has cancer-preventive action as well as cancer-protective effect	Gafurov et al. (2017)
CN107308270A	Anticancer drug composition	Preparation of anticancer drug composition that consists of raw material medicines in parts by weight: 10 to 50 parts of a propolis flavonoid extract, 5 to 40 parts of a fructus sophorae flavonoid extract, 8 to 50 parts of a fructus viticis flavonoid extract, and 0.03 to 0.12 part of 5-fluorouracil. This composition is active against human esophageal cancer cells (Eca-109), hepatoma cells (SMMC-7721), and breast cancer cells (MDA-MB-231) (MCF-7), while it showed the inhibition against (MCF-7) and (SMMC-7721) cancer cells	Jiejun and Chuan (2017)
US20170087125A1	Flavonoid compositions for the treatment of cancer	The suggested composition contains luteolin, quercetin, and kaempferol that are useful in inhibiting prostate cancer and head and neck cancer cell growth	Wu (2017)

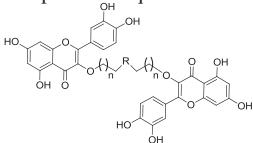
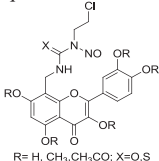
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**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN107459502A	Flavonoid compound and application thereof to preparation of antitumor drugs	Preparation of novel flavonoid derivatives as Hsp90 inhibitor and studied their application for the treatment of breast cancer, pancreatic cancer, colon cancer, and lung cancer 	Yonghua et al. (2017)
CN107441148A	<i>Euphorbia esula</i> total flavonoid extract as well as preparation method and antitumor activity application thereof	The extraction of <i>Euphorbia esula</i> flavonoids (quercetin, kaempferol, and their glycoside) and their antitumor actions against H22 tumor cell. The results have shown significant inhibitory effect toward solid tumors of H22 hepatoma as possessed by cyclophosphamide	Lishu et al. (2017)
CN107115372A	Antitumor pharmaceutical composition containing total flavonoids of <i>Apocynum venetum</i> leaves	The preparation of pharmaceutical composition of <i>Apocynum venetum</i> leaves which comprises flavonoids and an anthracycline drug (pirarubicin or epirubicin). It has been tested against human breast cancer MD-MBA-231 cells. In addition, this composition effectively alleviates or avoids the cardiac toxicity and other side effects in cancer patients	Liqun et al. (2017)
CN107397740A	Synergistic antitumor polyphenol composition and application	The preparation of polyphenol composition of two or more of tricin, quercetin, luteolin, and p-coumaric acid followed by evaluation of their in vitro proliferatory activity against human breast cancer MCF-7. EC <sub>50</sub> = 28.45 ± 0.91 μM (luteolin), EC <sub>50</sub> = 14.91 ± 0.34 μM (tricin and luteolin) EC <sub>50</sub> = 161.30 ± 1.48 μM (quercetin), EC <sub>50</sub> = 46 ± 1 · 46 μM alone (quercetin and tricin)	Zhengang et al. (2017)

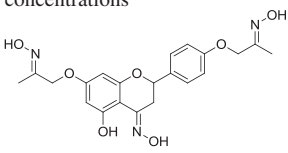
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**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN107056739A	Bola-type quercetin derivatives and preparation method and application thereof	<p>Synthesized the bola-type quercetin derivatives as amphiphilic molecule. It is formed by connecting two polar head groups via hydrophobic chain. These have a better stability and compatibility toward plasma membrane. The anticancer proliferation activity studied against prostate cancer (PC-3) cell, liver cancer (HepG2) cells, cervical cancer (Hela) cells, ovarian cancer cell (SKOV3, BxPC-3), and pancreatic cancer cells (Panc-I). At the same concentration of dosages, they have showed remarkable inhibitory effect on tumor cell growth as comparison of quercetin</p> 	Yi et al. (2017)
CN106674180 A	Quercetin derivative and preparation method and application thereof	<p>Synthesized the quercetin derivative and showed significant inhibition activity against human esophageal squamous cell carcinoma (EC109), (human glioma (U251) cell line, human hepatoma (Hep-2) cells, human gastric cancer (MGC-803) cells, and human prostate cancer (PC-3) cells growth than that of quercetin</p>  <p>R= H, CH<sub>3</sub>, CH<sub>2</sub>CO; X=O,S</p>	Baohua et al. (2017)
CN105601603B	Extraction of <i>Armillaria</i> genistein monomer compound and its application	Extraction of genistein from yellow- <i>Armillaria</i> fruiting body and evaluation of its inhibitory effect on A549 lung cancer and liver cancer HepG2 cell growth	Yaozhou et al. (2017)

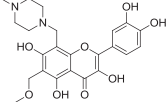
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**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN107412221A	<i>Paeonia suffruticosa</i> seed extract and application thereof as antitumor drug	The extraction of <i>Paeonia suffruticosa</i> seed which comprises suffruticosol A, suffruticosol B, trans-epsilon-viniferin, ampelopsin E, trans-resveratrol, trans-resveratrol-4'-O-beta-D-glucopyranoside, paeoniflorin, luteolin, luteolin-4'-O-beta-D-glucopyranoside, apigenin, kaempferol, oleanolic acid, betulinic acid, hederagenin, and caffeic acid. This extract can be useful for preventing and/or curing tumor diseases because it possessed promising antitumor activity against COLO205, HT-29, HepG2, AGS, and HL-60 tumor cells	Naisheng et al. (2017)
CN107007715A	<i>Eleocharis tuberosa</i> peel anticancer extract as well as preparation method and application thereof	The extraction of <i>Eleocharis tuberosa</i> peel which comprises luteolin, quercetin, and diosmetin. The water chestnut skin extract with fluorouracil has showed positive growth inhibitory effect against HCT-116, COLO205, A549 NSCLC, and HeLa cell lines	Jinfeng and Zhenhua (2017)
CN105037314 B	Multi-hydroxyimino naringenin derivatives, preparation method, and application	Synthesized the multi-hydroxyimino naringenin derivatives and studied their action against gastric cancer cells. Study confirmed the effective inhibition action against the cancer cell growth at low concentrations   <chem>CC(=O)NCCOC1=CC=C(C=C1)OC2=C(O)C(=O)N(O)C2=CC3=C(O)C(=O)N(O)C3=O</chem> IC50 (μMol/L) = 15.3	Zhiping et al. (2017)
CN106692049A	HUT-EGCG (11-hydroxyundecane-1-thiol-epigallocatechin gallate) nanoparticle solution system and preparation method thereof	Preparation of nanoparticle solution system of epigallocatechin gallate, 11-hydroxyundecane-1-thiol, and beta-lactoglobulin and evaluated their antitumor effect against human melanoma cancer cells A375, mouse hepatoma cells HepG2 cells, and human esophageal cancer TE-I. The antitumor activity of the EGCG was found to be improved significantly in in vivo as well in vitro study models.	Qizhen et al. (2017a)

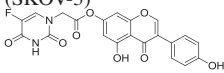
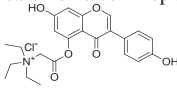
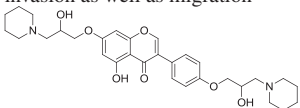
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**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN106729724A	3MH (3-mercapto-1-hexanol)-EGCG (epigallocatechin gallate) nanoparticle solution system and preparation method thereof	Preparation of nanoparticle solution system of epigallocatechin gallate, 3-mercapto-1-hexanol, and beta-lactoglobulin and studied against human melanoma cancer cells A375, mouse hepatoma cells HepG2 cells, and human esophageal cancer TE-I. The antitumor activity of the EGCG in vivo or in vitro is greatly improved	Qizhen et al. (2017b)
JP2017178813A	Cancer cell proliferation inhibiting composition	Prepared the composition comprises carnosic acid and epigallocatechin gallate and evaluated its proliferation inhibitory effect against colon adenocarcinoma DLD-1 cancer cells	Masahiro and Hironori (2017)
CN108017608A	Flavonoid derivatives and a preparation method and uses	<p>Flavonoid derivative possessed therapeutic applications against pancreatic cancer, stomach cancer, leukemia, esophageal cancer, cervical cancer, osteosarcoma, lung cancer, prostate cancer, colon cancer, breast cancer, liver cancer, glioma, and ovarian cancer</p>  <p>Most promising inhibitory effect on the proliferative activity of tumor cells including AsPC-1, BxPC-3, MV-4-11, and AGS. The cytotoxicity against normal cell lines is lesser as compared to cancer cells</p>	Zuohuan et al. (2018)
CN107722087A	Application of an antineoplastic <i>Gynostemma</i> flavonoids and their preparation	The plant extract of <i>Gynostemma</i> contains a variety of flavonoids and glycosides, mainly quercetin, rutin, kaempferol, ombuin, ombuoside, and isorhmnetin. The plant extract possessed significant antitumor effect toward human lung cancer (A-549) cell and human breast cancer (MCF) cell	Jianguo et al. (2018)
CN104352562B	Preparation method of total flavonoids of <i>Schizonepeta tenuifolia</i> Briq. and application of total flavonoids of <i>Schizonepeta tenuifolia</i> Briq. to resistance to tumors	The extraction of total flavonoids from <i>Schizonepeta tenuifolia</i> Briq. and significantly inhibited the tumor cells (Caco-2) growth	Xiansheng et al. (2018)

(continued)

**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN107595834A	Application of drug combination of quercetin and curcumin in preparation of product for treating prostatic cancer	Preparation of drug combination of quercetin and curcumin. This combination has showed synergistic effect with lower drug toxicity and better cancer inhibition effect against prostatic cancer prostate PC-3 cells or LNCaP cells	Nianzeng et al. (2018)
CN107892686A	A kind of genistein derivatives and preparation method and application thereof	Synthesized the genistein derivative bearing 5-fluorouracil. This derivative has showed promising growth inhibitory action toward hepatoma carcinoma (HepG2) and ovarian cancer cell line (SKOV-3) 	Chengxiong et al. (2018a)
CN107573316A	Acylated derivative of genistein and preparation process of acylated derivative	Synthesized the acylated genistein derivative with improved in vivo bioavailability as well as promising anticancer activity against hepatoma carcinoma cell HepG2 	Chengxiong et al. (2018b)
CN105732560B	Genistein derivatives, preparation method, and application in pharmacy	Synthesis of genistein derivative with promising antitumor effect. In addition, it has showed potent therapeutic effect for inflammation-related tumors AOM/DSS and the ability to inhibit tumor cell invasion as well as migration 	Rong et al. (2018)
CN107670052A	Luteolin-glycyrrhizic acid conjugated bovine serum albumin drug-loaded nanoparticles and preparation and application thereof	Preparation of luteolin-glycyrrhizic acid conjugated bovine serum albumin drug-loaded nanoparticles and studied the inhibition of proliferation of human hepatoma cell HepG2. The luteolin-loaded nanoparticle has significantly increased water solubility, absorption, and antitumor efficacy	Yongli et al. (2018)

(continued)



**Table 4.5** (continued)

Patent no.	Title	Significance	Refs.
CN108057034A	Combination of naringenin and asiatic acid for cancer	This patent claims a synergistic effect due to the combination of asiatic acid and naringenin. The combination of both significantly enhanced antitumor effect of NK cells. NK cell maturation and cytotoxicity action against cancer are due to the balance between Smad3 and Smad7	Huiyao (2018)
CN107625732A	Functional drug-loaded system used for treating lung tumor and preparation method and application thereof	Preparation of drug-loaded liposome and microsphere using hispidulin and/or epigallocatechin gallate as drug molecules. The nano-mediated formulation has showed potent antiproliferative effect on lung adenocarcinoma A549 cells	Xue et al. (2018)

## 5 Conclusions

In this chapter, we have summarized the knowledge gathered in regard to the metal complexation sites in flavonoids, highlighting the recent progress registered for the anticancer and chemopreventive activities of six selected natural flavonoids (luteolin, quercetin, naringenin, EGCG, genistein, cyanidin) and their metal complexes as well as patents related to flavonoids as anticancer agent. The patent literature (2015–present) relating the flavonoids and their analogs, derivatives, and pharmaceutical formulations as potential cancer-preventing agents and responsible to affect multiple essential survival proteins and pathways associated with human cancer cell growth. Various *in vitro* and *in vivo* studies report on the mechanisms of action of flavonoids, which modulate multiple signaling pathways and interact with diverse molecular targets. All of these data combined persuasively argue for the potential role of flavonoids in cancer prevention and treatment.

A large number of flavonoid derivatives have been developed in order to increase the therapeutic value of these natural compounds. Among them, the authors of this chapter have selected metal complexes, since the metal center can inflect the spectrum of activity or play a critical role in key interactions of the parent flavonoids with biological targets. However, only scarce data exists in the literature regarding the mechanisms of action of these metal complexes. Undoubtedly, additional studies are yet required to elucidate the mechanistic basis that underlies their anticancer activity in order to validate the potential use of flavonoid metal complexes as novel anticancer agents.

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# Chapter 5

## Flavonoids as Emerging Anticancer Agents: Current Trends and Recent Advances in Phytotherapy



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### 1 Introduction

Incidence of severe lethal diseases is continuously increasing due to unhealthy diet and stressful lifestyle (Kashyap et al. 2018). There are various effective allopathic treatment options available, but all are having long-term side effects (Kashyap et al. 2016a, c, e). Therefore, there is a need for alternative therapeutic strategies that

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could not only improve the therapeutic potential but also reduce possible side effects. Phytochemicals are emerging consistently in the field of medical health with promising therapeutic implications (Kashyap et al. 2016d, 2018).

Flavonoids, a class of bioactive compounds, in particular, have been tested against several human diseases including cancer, cardiovascular, diabetes, neurological disorders, etc. (Kashyap et al. 2016b, 2017). In various human cancers, flavonoids have been known as effective molecular targets for their key role in apoptosis, cell cycle, angiogenesis, metastasis, inflammation, and oxidative stress. Studies have suggested the role of flavonoids in cell cycle arrest by regulating the expression of cyclin-dependent kinases (CDKs) (Kashyap et al. 2016b). Angiogenesis and metastasis, the hallmarks of cancer, were also inhibited with flavonoids treatment via controlling protein kinase B/mammalian target of rapamycin/P70-S6 Kinase 1 (AKT/mTOR/P70S6K), plasminogen activator (uPA), and matrix metalloproteinases (MMPs) pathways. Further cancer-related inflammatory mediators such as interleukin 6 (IL-6), IL-8, interferon gamma (IFN- $\gamma$ ), inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) can be regulated by flavonoids (Wang et al. 2013a, b; Kumi-Diaka et al. 2000; Lou et al. 2012; Liu et al. 2013a, b; Balamurugan and Karthikeyan 2012a, b). In addition, flavonoids showed inhibitory effects against topoisomerase II and hTERT enzymes responsible for cancer progression and survival (Jagadeesh et al. 2006). The miRNA contributed as oncogenes and tumor suppressor roles in cancer are also known to be modulated by flavonoids (Ma et al. 2013). Therefore, in order to explore the potential of flavonoids in clinical settings, it is essential to understand their interactions with different cellular targets in tumor cells. This chapter will summarize the molecular mechanism of variety of actions of flavonoids in various cancer-associated molecular signaling pathways.

## 2 Apoptosis Induction

Apoptosis, a programmed cell death, is represented by the following cellular events including plasma membrane blebbing, loss of cell affixment, cytoplasmic contraction, DNA fragmentation, and activation of caspases through extrinsic/intrinsic pathways (Kashyap et al. 2016e). Naturally, apoptosis is required for various biological processes such as embryogenesis for morphological constructions and homeostasis. It is also required to destroy the cells that have damaged DNA so as to prevent defective cell division. Cancer initiation is known to hijack cellular homeostasis and apoptotic features (Kashyap et al. 2016a). Several chemotherapeutic drugs are found to activate apoptotic death of cancer cells (Kashyap et al. 2017). Experimental evidences have discovered the apoptosis-activating property of various flavonoids through modulations of multiple signaling (intrinsic and extrinsic) pathways (Kashyap et al. 2016d, 2017, 2018; H. S. Tuli et al. 2015c).



## 2.1 *Induction of Extrinsic Pathway of Apoptosis*

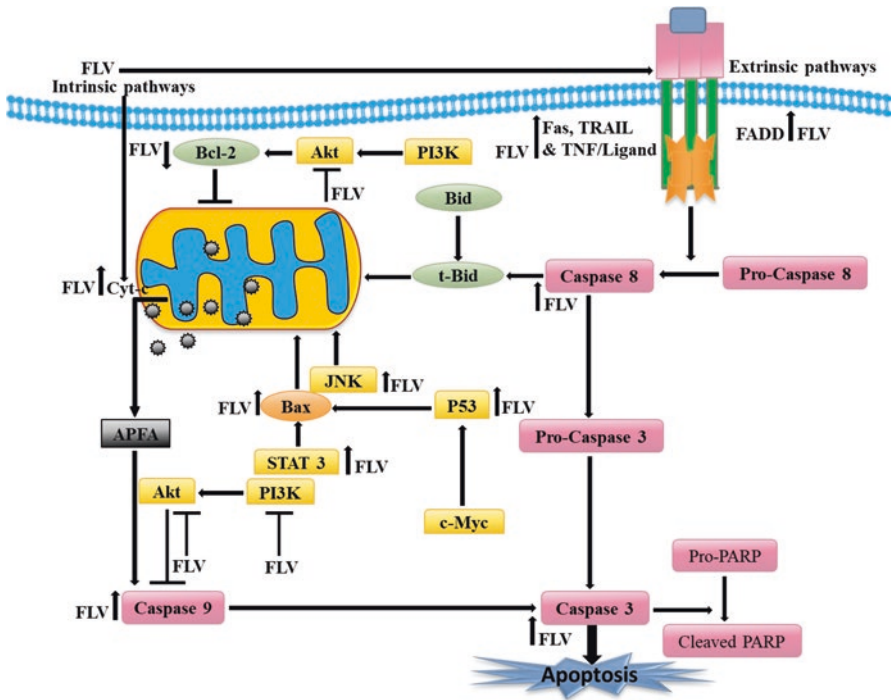
The extrinsic apoptotic pathways are known to receive death-inducing stimuli from outside the cell to trigger cell death (H. S. Tuli et al. 2015a). To receive these death-inducing signals, plasma membranes have specific receptors for each stimulus (H. S. Tuli et al. 2015c). The external stimuli for apoptosis are usually in the form of cytokines. Fas ligand (FasL) and TNF- $\alpha$  are the most studied cytokines produced in response to adverse conditions and bind to the Fas receptor and TNF-receptor, respectively, to activate apoptosis via regulating caspases-8, caspase-10, pro-apoptotic proteins (Ba, Bid, Bak, and Bad), and anti-apoptotic proteins (Bcl-2 and Bcl-Xl) (Kashyap et al. 2016b, c).

The role of flavonoids in the regulation of extrinsic pathway components in vitro and in vivo cancer models has been demonstrated by many studies. Luteolin (LUT), a member of flavone family, was found to markedly induce the expression of death receptor 5 (DR5) along with Bcl-2-interacting domain cleavage, resulting in the activation of caspase-8, caspase-10, caspase-9, and caspase-3 (Horinaka et al. 2005). Similarly, genistein (GEN) could also enhance the expression of DR5 and cause mitochondrial dysfunction in human gastric adenocarcinoma AGS cells (Jin et al. 2007). Additionally, LUT, in a dose-dependent manner, upregulated the levels of Smad 4 proteins, a downstream regulator of the transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) which further activated the Fas/Fas-L signaling pathway in Hep3B cells (Nam et al. 2007). Furthermore, quercetin (Quer) and apigenin induced caspase-dependent extrinsic apoptosis upregulating the levels of cleaved caspase-8, caspase-3, and poly(ADP-ribose) polymerase (PARP) in HER-2-overexpressing breast cancer cells (Seo et al. 2015, 2016). Kaempferol stimulated p53&ATM-mediated death receptor signals [Fas/CD95, DR4, and DR5] in HUVECs (Lee et al. 2016). Poncirin is another flavonoid which is shown to induce extrinsic apoptotic pathway through Fas ligand upregulation in AGS human gastric cancer cells (Saralamma et al. 2015).

## 2.2 *Induction of Intrinsic Pathway of Apoptosis*

The intrinsic pathway of apoptosis involves the swelling of the mitochondrial membrane, which increases the membrane permeability. The apoptotic proteins further create membrane pores in mitochondria resulting in the leakage of cytochrome c (cyt c) into the cytoplasm. In the cytoplasm, cyt c binds with the apoptotic protease-activating factor-1 and ATP which in turn binds with procaspase-1, forming the apoptosome complex (Fig. 5.1). Apoptosome is further known to cleave and activate caspase-9 which subsequently activates the effector caspase-3.

Evidence has suggested that flavonoids can initiate apoptosis via regulating mitochondrial pathway (Russo et al. 1999; Wang et al. 1999). Exposure of narin-



**Fig. 5.1** Diagrammatic representation of flavonoid-induced modulation of different proteins of intrinsic and extrinsic pathways and activation of the apoptotic cascade

genin (Nar) to rat C6 glioma model was reported to modulate Bcl-2/Bax ratio followed by release of cyt c, caspase-9 and caspase-3 upregulation, and enhanced expression of Cx43 (Sabarinathan et al. 2010). Quer-mediated induction of apoptosis through the mitochondrial pathway has been reported in a variety of human cell lines, including breast cancer MCF-7 cells, nasopharyngeal carcinoma CNE2 and HK1 cells, leukemia HL-60 cells, thymus-derived HPB-ALL, and oral squamous carcinoma SCC-9 cells (Chou et al. 2010; Haghiac and Walle 2005; Lautraite et al. 2002; Mozghan Farzami Sepehr 2011; Niu et al. 2011; Russo et al. 2014). In vitro studies with human breast cancer MDA-MB-231, HaCaT keratinocytes, epidermoid carcinoma KB, and KBv200 cells showed decreased mitochondrial membrane and increased expression of pro-apoptotic protein Bax and cyt c after exposure to Quer (Chien et al. 2009; Hu et al. 2015; Shen et al. 2012; Zhang et al. 2013). LUT exerted the inhibitory effect against gastric cancer proliferation through the intrinsic apoptotic pathway via cytoplasmic release of cyt c and subsequently led to increase in the levels of caspase-3 and caspase-9 (Lu et al. 2017). In another study using human leukemia THP-1 cells, it was noticed that Nar induces apoptosis by increasing hyperpolarization of the mitochondrial membrane potential (Arul and Subramanian 2013; Park et al. 2008). Furthermore, it has now been confirmed that flavonoids induce cell death in a variety of carcinoma cells via inducing ROS generation,

mitochondrial depolarization, nuclear condensation, DNA fragmentation, and caspase-3 activation (Ahmad et al. 2014).

### 2.3 Induction of Common Components of Both Apoptotic Pathways

The other molecular proteins that are downstream to the intrinsic pathways, such as pro-apoptotic (Bax, Bid, Bak, or Bad), anti-apoptotic (Bcl-X<sub>L</sub> and Bcl-2), and caspases, are considered as major stimuli to cause apoptotic cell death (Reed 2000). These proteins form homodimers required for alterations in mitochondrial membrane permeability for the release of caspase activators cyt c (Rastogi et al. 2009). Caspase enzymes are found to be crucial for transduction of endoplasmic reticulum (ER)-mediated apoptotic death signals generation (Reed 2000). Effector caspases degrade the tumor cell intracellular proteins to carry out the cell death program (Cohen 1999).

Flavonoids alter the ratio of pro-apoptotic/anti-apoptotic proteins and activate the caspases to initiate the apoptosis process in tumor cells (Fig. 5.1). For instance, LUT increased the expression of pro-apoptotic proteins (Bid, Bak, Bax, Bad) and activated caspase-3, with a concomitant increase in the levels of cleaved PARP in different human cancer cells such as GBM 8401, U87 cells, gastric cancer, lung A549, HCC cells (HepG2), and SCC-4 cells (Li et al. 2016; Lu et al. 2017; Meng et al. 2016; Tsai et al. 2013; W. Wang et al. 2017; Yang et al. 2008). Similarly, LUT and GEN are known to activate caspase-3, increase the levels of Bax protein, and decrease Bcl-X<sub>L</sub> levels in five human hepatoma cell lines, namely, HepG2, SK-Hep-1, PLC/PRF/5, Hep3B, and HA22T/VGH, as well as human breast adenocarcinoma MCF-7 cells (Chang et al. 2005; Park et al. 2014; Yeh et al. 2007). The apoptosis activation of GEN was assessed in the cervical cancer cell lines HeLa, CaSki, and C33A, through activation of caspase-3, caspase-8, and caspase-9 (Kim et al. 2009). Moreover, LUT, in Neuro-2a mouse neuroblastoma cells, induced activation of caspase-12, caspase-9, and caspase-3 and showed its anticancer effects (Choi et al. 2011). In another investigation, Nar was analyzed to have apoptotic effects on rat C6 glioma model and SGC-7901 cells *via* altering Bcl-2/Bax ratio, downregulation of survivin proteins, upregulation of caspase-3 and caspase-9, and enhanced expression of Cx43 (Bao et al. 2016). Quer and GEN can also decrease the ratio of Bcl-x<sub>L</sub> to Bcl-x<sub>S</sub> and increase the translocation of Bax to the mitochondrial membrane reported in human prostate cancer cells DU145 and LNCaP (Granado-Serrano et al. 2006; Kumi-Diaka et al. 2000). Similarly, GEN caused the downregulation of Bcl-2, upregulation of Bax and p21<sup>WAF1</sup> expressions, p53 expression, DNA ladder formation, caspase-3 activation, and PARP cleavage in MDA-MB-231, MCF-7, HT-29, and NSCLC cancer cell lines (J. Chen et al. 2015a; Li et al. 1999; Lian et al. 1999; Yu et al. 2004). GEN inhibits proliferation and differentiation of neuroblastoma (N2A, JC, SKNSH, MSN, and Lan5) cells by inducing

apoptosis and modulating protein tyrosine kinase (PTK) activity and N-myc proto-oncogene expression (Brown et al. 1998).

## 2.4 Induction of Other Apoptotic Pathways

In addition to the abovementioned pathways, flavonoids could regulate several other apoptosis-related cancer survival signaling pathways too. The nuclear factor- $\kappa$ B (NF- $\kappa$ B) is a transcription factor for a large group of genes that are involved in several different pathways (Kaltschmidt et al. 2000). For example, NF- $\kappa$ B activates its own inhibitor (I $\kappa$ B) as well as groups of pro-apoptotic and anti-apoptotic genes (Fan et al. 2008). Several studies have revealed that Quer can modulate cellular signaling proteins involved in apoptosis, like NF- $\kappa$ B, Cox-2, suppressing Bcl-xL and Bcl-2 anti-apoptotic proteins and up-regulating Bax and pro-apoptotic proteins (Banerjee et al. 2002; D. Chen et al. 2005a; Cheong et al. 2004; Mutoh et al. 2000). Nar-induced apoptosis may be correlated to the activation of NF- $\kappa$ B and degradation of I $\kappa$ B $\alpha$  (Kanno et al. 2006). Another study demonstrated that GEN could induce apoptosis in human colon cancer LoVo and HT-29 cells through inhibiting the NF- $\kappa$ B pathway, as well as downregulation of Bcl-2 and upregulation of Bax (Qin et al. 2016). Furthermore, the inhibition of the MEK5/Erk5/NF- $\kappa$ B pathway may be an important mechanism behind GEN-mediated suppression of MDA-MB-231 cell growth via apoptosis induction (Li et al. 2008a). In another study using lung cancer cells, it was found that LUT effectively suppressed NF- $\kappa$ B and potentiated the c-Jun N-terminal kinase (JNK) to increase apoptosis via TNF $\alpha$  (Ju et al. 2007; Yan et al. 2012). In addition, LUT inhibited TNF $\alpha$ -induced activation of NF- $\kappa$ B which activates JNK and results in the elevation of pro-apoptotic proteins and suppression of anti-apoptotic gene expression in NSCLC (Cai et al. 2011; Shi et al. 2004). Many investigations have suggested that activation of phosphoinositide-3-kinase (PI3K) Akt pathway could mediate the protective effect in cancer cell progression (Franke et al. 2003). It has been observed that LUT and Nar acted as potential chemotherapeutic agents against gastric cancer by exerting a dual inhibition on the mitogen-activated protein kinase (MAPK) and PI3K signaling pathways and induced apoptosis (Bao et al. 2016; Lu et al. 2017). A significant elevation in the expression of the endocannabinoid receptor (CB1-R) has been observed in human colon cancer PTEN-null cell lines after Quer treatment, which further promotes the inhibition of survival signals such as PI3K/Akt/mTOR (Gulati et al. 2006; Refolo et al. 2015). Moreover, a sustained inhibition of survival signals like PI3K/Akt and extracellular regulated kinases (Erks) and cross-communication between PI3K and Erk were also described in Quer-treated liver carcinoma HepG2 cells (Granado-Serrano et al. 2006). The induction of apoptosis via inactivation of PI3K/Akt pathway was also studied using Nar and GEN treatment in anaplastic large-cell lymphoma (ALCL) and THP-1 cell lines (Park et al. 2005, 2008). Results of other studies have revealed that LUT exerted an antiproliferative effect in a dose- and time-dependent manner in A549 lung adenocarcinoma and HepG2 cells via elevation of phosphorylated

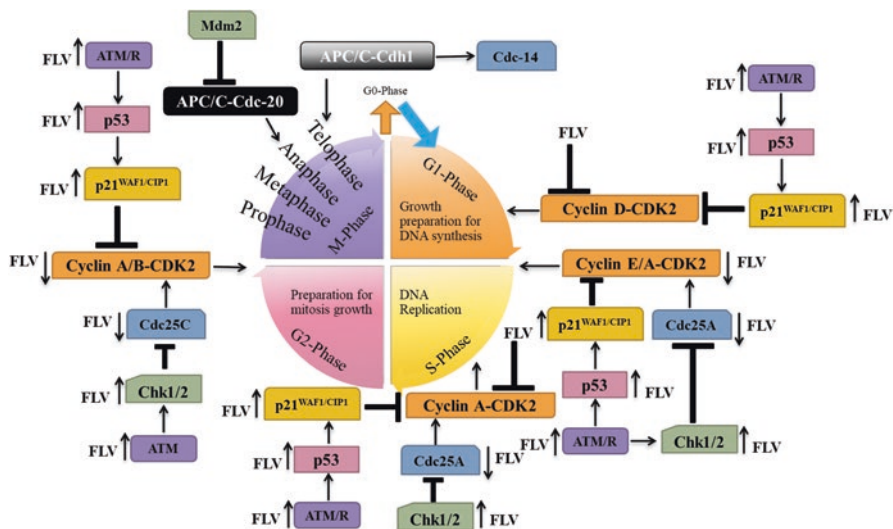
MEK and its downstream kinases (Meng et al. 2016; W. Wang et al. 2017; Wu et al. 2008). Data have also suggested that LUT induces a caspase-dependent and caspase-independent apoptosis *via* nuclear translocation of apoptosis-inducing factor (AIF) and activation of Erk and p38 in breast cancer cells (Kim et al. 2012). Moreover, p53-dependent mitochondrial apoptosis was also studied in Quer and cyanidin (Cy-g)-treated human cervical cancer HeLa cells, lung cancer A-549 cell line, and Jurkat T cells, respectively (Chan et al. 2013; Fimognari et al. 2004; Vidya Priyadarsini et al. 2010). It was additionally suggested that Quer activates and increases the expression levels of JNK and p53-dependent Bax in *in vitro* studies using bronchial epithelial BEAS-2B cells (Lee and Yoo 2013). In addition, evidence suggests that apoptotic effects of Quer may be due to the inhibition of heat shock protein (Hsp) (Aalinkeel et al. 2008). Similarly, LUT promoted the degradation of Tyr<sup>705</sup>- and Ser<sup>727</sup>-phosphorylated signal transducer and activator of transcription 3 (STAT 3) through interacting with Hsp90 and induced apoptosis of cancer cells (Fu et al. 2012). In a study using A549, Avinaba Mukherjee et al. described that Quer causes mitochondrial depolarization via downregulation of IL-6/STAT 3 signaling pathway (Mukherjee and Khuda-Bukhsh 2015). Moreover Quer and Nar inhibit p38/MAPK signaling pathway, which in turn inhibits transient receptor potential cation channel subfamily M member 7 (TRPM7) channels and activates pro-apoptotic protein expression, caspase-3 activation, and PARP cleavage in AGS cells (Kim et al. 2014; Totta et al. 2004). Similarly, a combination of GEN and TRAIL in human hepatocellular carcinoma Hep3B cells triggered the inhibition of p38- $\beta$ /MAPK activation leading to apoptosis initiation (Jin et al. 2009; Shafiee et al. 2016). Finally, flavonoids significantly modulated the expression levels of various transcription factors, such as Bax, Bcl-2, MAPK, Akt/mTOR, c-Jun and c-Myc, and early growth response-1 (Egr-1) in a variety of human cancer cells to modulate the apoptosis activation (Nam et al. 2007). Hence it is quite evident that flavonoids can modulate different proteins of intrinsic and extrinsic pathways and activate the apoptotic process in a cancer cell (Fig. 5.1).

### 3 Cell Cycle Arrest Potential of Flavonoids

Cell cycle is defined as the sequential changes of a cell from one phase to another phase ( $G_1 \rightarrow S \rightarrow G_2 \rightarrow M$ ) during cell division (Mukherjee et al. 2010). In normal cells, the cell cycle is controlled by a complex series of signaling pathways by which a cell grows in size, replicates its DNA, and finally divides (C. Gérard et al. 2015). This whole process is well regulated and under the control of cyclins and cyclin-dependent kinases (CDKs) (Gérard et al. 2015). These cell cycle regulatory proteins ensure that all errors have been corrected and if not, the cells will commit suicide (apoptosis) (Pietenpol and Stewart 2002; Wang et al. 2007). In cancer, as a result of gene mutations and epigenetic modifications, these regulatory processes are found to malfunction that result in uncontrolled cell proliferation (Alberts et al. 2014).

Flavonoids were noted to have antitumor effects via cell cycle arrest. For example, Quer and GEN were reported to upregulate p21<sup>WAF1/CIP1</sup> along with Cdc2-cyclin B1 downregulation in MCF-7 and human esophageal squamous cell carcinoma KYSE-510 cells (Choi et al. 2001; Davis et al. 1998; Zhang et al. 2009). Similarly, LUT inhibited cancer cell growth through perturbation of cell cycle progression at the sub-G1 and G1 phases of MCF-7 cells (Park et al. 2014). Furthermore, results revealed that LUT reduced the viability of SCC-4 cells and induced apoptosis by decreasing the expression of CDKs, cyclins, and phosphor retinoblastoma (p-Rb) anti-apoptotic protein (Yang et al. 2008). LUT, at a concentration of 100 $\mu$ M (IC<sub>50</sub>), decreased the expressions of non-P- $\beta$ -catenin, phosphorylated glycogen synthase kinase-3 $\beta$  (GSK-3  $\beta$ ) and cyclin D1 expression while promoting substantial cell cycle arrest at the G2/M phase of HCT-15 cells (Ashokkumar and Sudhandiran 2011). LUT also induced cell cycle arrest at G<sub>0</sub>/G<sub>1</sub> phase of five human hepatoma cell lines, namely, HepG2, SK-Hep-1, PLC/PRF/5, Hep3B, and HA22T/VGH (Chang et al. 2005). LUT and GEN exhibited an inhibitory effect on the proliferation of LoVo and MDA-MB-231 cells by causing cell cycle arrest at G2/M phase transition with an inactivation of cyclin B1 (Chen et al. 2018; Li et al. 2008b). Quer-mediated induction of G1 cell cycle arrest as a consequence of cyclin D1/CDK4 and E/CDK2 downregulation and p21 upregulation has been successfully demonstrated in vascular smooth muscle cells (Moon et al. 2003). GEN and Quer-mediated upregulation of p21, p27, p53, and Chk2, downregulation of CDK1 and cyclin B1, and phosphorylation of pRb followed by arrest of the cell cycle at G1 and G2/M phase have been found in a variety of cancer cell lines (Han et al. 2013; Jeong et al. 2009; Mu et al. 2007). LUT arrested human CCA, KGU-M156 cells, GBM 8401, and U87 cell cycle progression at the S and G2/M phase in a dose-dependent manner as assessed by downregulation of cyclin A and Cdc25A (Aneknan et al. 2014; Tsai et al. 2013). Treatment of different cancer cells (PC-3, AGS, MCF-7, and MDA-MB-231) with LUT and GEN for 24 h caused stimulation of c-Fos gene expression and significant inhibition of the cell cycle pathway (CCP) genes (CCNA2, CCNE2, CDC25A, CDKN1B, and PLK-1) and p21<sup>Cip1</sup> that results in G2/M arrest (Choi et al. 1998; Frey et al. 2001; Raffoul et al. 2006; Wu et al. 2008) (Choi et al. 2000). Results demonstrated that GEN activated ATM-Chk2-Cdc25 and ATR-Chk1-Cdc25 DNA damage checkpoint pathways and could arrest ovarian cancer (HO-8910) cells in the G2/M phase (Ujiki et al. 2006). Another study showed that GEN caused cell cycle arrest in the G2/M phase accompanied by activation of ATM/p53, p21<sup>WAF1/CIP1</sup>, and GADD45 $\alpha$  as well as downregulation of cdc2 and cdc25A in colon cancer HCT-116/SW 480 and HepG2 cells (Chang et al. 2004; Yuan 2013). In nasopharyngeal carcinoma (NPC), GEN elevated p21<sup>Cip1</sup> and ATR (ataxia telangiectasia and Rad3 related) and induced the expression of p15<sup>Ink4b</sup> that resulted in Caco-2 cell cycle arrest (Han et al. 2010). In addition, GEN induced the expression of Ras and Raf-1 proteins and upregulated both c-Jun and c-Fos suggesting that the Ras/MAPK/AP-1 signal pathway may be involved in GEN-induced G2/M cell cycle arrest in MDA-MB-231 breast cancer cells (Li et al. 2008b). Treatment of Hs578T with peonidin 3-glucoside or cyanidin 3-glucoside, a derivative of cyanidin, has shown a strong inhibitory effect on Lewis lung carcinoma cell





**Fig. 5.2** Cell cycle arrest by flavonoids. Flavonoids affect multiple signaling molecules and key players involved in cell cycle G1, S, G2, and M phases

growth via G2/M arrest due to downregulation of protein levels of CDK-1, CDK-2, cyclin B1, and cyclin D1 (P. N. Chen et al., 2005). Furthermore, dose- and time-dependent treatment with Quer led to arrest of MCF-7 cells in the S phase as a result of downregulation of CDK2 and cyclin A and B and upregulation of p53 and p57 (Chou et al. 2010; Duo et al. 2012). Nar was shown to inhibit the proliferation of HepG2 cells partly in the G0/G1 and G2/M phases of the cell cycle and resulted in a rapid accumulation of p53 (Arul and Subramanian 2013). Moreover, LUT-treated Hep3B cells have shown significant upregulation of the expression level of CDK inhibitor, p27<sup>KIP1</sup>, via the TGF- $\beta$ 1 signaling pathway (Li et al. 2016). The induction of G2/M cell cycle arrest in GEN-treated SGC-7901 and BGC-823 cells involved phosphorylation of Akt and upregulated PTEN expression (Y.-L. Liu et al. 2013a). In conclusion, flavonoids have the ability to cause cell cycle arrest and could be utilized as promising approach toward chemoprevention (Fig. 5.2).

## 4 Inhibition of Angiogenesis by Flavonoids

Angiogenesis, characterized by the formation of new vessels from a pre-existing microvascular network, is a crucial step in proliferation and metastasis (Guo et al. 2010; Olsson et al. 2006; Tuli et al. 2015a). Blood circulation is required by tumor cells for food and exchange of waste and gases (Nishida et al. 2006; Semenza 2003). The crucial process of angiogenesis requires angiogenic proteins including vascular endothelial cell growth factor (VEGF), fibroblast growth factor (bFGF), epidermal growth factor (EGF), and MMPs (Battagay 1995; Klagsbrun and Moses 1999; Kong

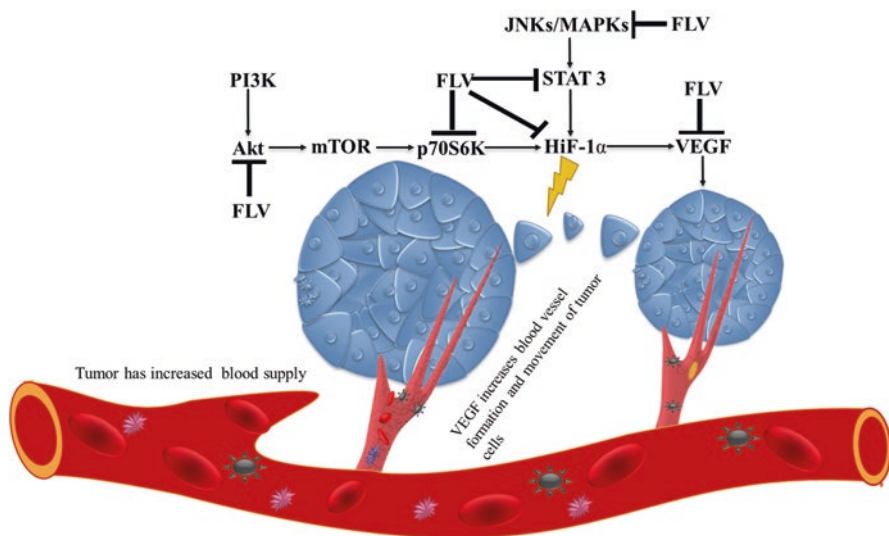
et al. 2005). Thus, the inhibition of angiogenesis has become a promising strategy for cancer treatment (Fig. 5.4).

There are several reports on the anti-angiogenic effects of flavonoids (Argyriou et al. 2009; Hayashi et al. 2000; Igura et al. 2001). The flavonoids like Quer were found to inhibit several steps of angiogenesis including proliferation, migration, and tube formation of human microvascular dermal endothelial cells (ECs) in a dose-dependent manner (D. Zhao et al. 2014a) (Igura et al. 2001). Previous literature suggested that LUT blocked VEGF production as well as KDR activity, thereby inhibiting tumor cell migration in triple-negative breast cancer (TNBC) (Cook et al. 2016). Further, LUT at a concentration of 10 mg/kg/d significantly reduced CD31 and CD34 markers and reduced the microvessel density (MVD). Similarly, the inhibitory effects of LUT on the activation of MMP-2 and MMP-9 and VEGF/VEGF receptor 2 and their downstream protein kinases Akt, Erk, mTOR, and P70S6K were also observed in human prostate tumor (Pratheeshkumar et al. 2012). In another study, LUT decreased the formation of capillary-like structure by inhibiting VEGF mRNA expression and transcriptional activity of nuclear transcription factor NF- $\kappa$ B (Cai et al. 2012). Furthermore, the anti-angiogenic effects of Quer were also revealed by chicken chorioallantoic membrane (CAM) mediated through regulation of similar type of cellular signaling pathways (Mojzis et al. 2008). In HUVECs, Quer inhibited the expression of VEGF R2 and tube formation in a dose-dependent manner (D. Zhao et al. 2014a). In addition, flavonoids were found to be involved in suppressing the Erk signaling pathway in both *in vivo* and *in vitro* studies resulting in the inhibition of angiogenesis (Article et al. 2014; F. Li et al. 2015b). Treatment of ECs with GEN induced VEGF-loaded endothelial apoptosis by inhibiting the expressions and activities of MMP-2, MMP-9, JNK, and p38 (Yu et al. 2012). Thrombospondin-1 (TSP-1), an endogenous anti-angiogenic factor, was found to upregulate after treatment with flavonoids resulting in the antagonizing of prostate cancer PC-3 cell growth (Yang et al. 2016). Thus, we could say that flavonoids stop angiogenesis in tumors and inhibit proliferation as well (Fig. 5.3).

## 5 Inhibition of Metastasis by Flavonoids

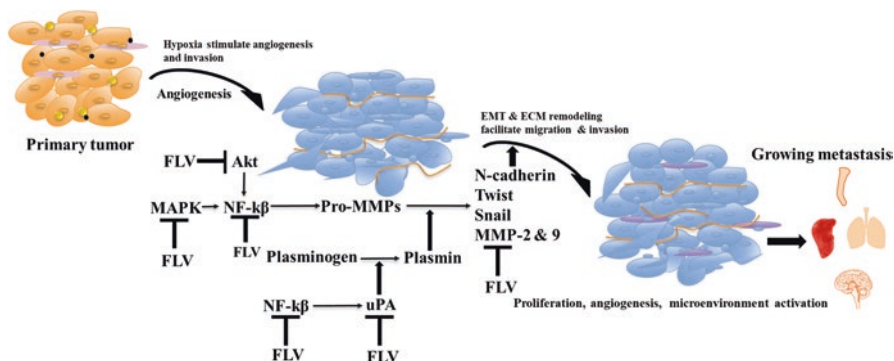
Most of the cancer-related mortality has been associated with complex metastasis process which is accomplished by activation of various regulatory proteins (Leber and Efferth 2009; Steeg 2016). There are multiple targets in the metastasis process, which are known to be inhibited by flavonoids making them promising molecules for anticancer therapy (Brooks et al. 2010; Steeg 2016). For instance, GEN significantly induced the expression of KAI1, both at the mRNA and protein levels, and decreased the invasiveness of TRAMP-C2 cells (El Touny and Banerjee 2007). It also significantly regulated the FAK/paxillin/vimentin and epithelial to mesenchymal transition (EMT)-related transcription factor Snail and MAPK signaling pathways in MHCC-97H and B16F10 cells (Cui et al. 2017; Gu et al. 2009). The exposure of HeLa cells to GEN resulted in effective inhibition of cancer migration by modulating the expression of MMP-9 and metalloproteinase inhibitor 1 (TIMP-1)





**Fig. 5.3** Anti-angiogenic effect of flavonoids in cancer cells through affecting target genes involved in angiogenesis process, e.g., AKT, p70S6K, and VEGF

(Hussain et al. 2012). Further, GEN also inhibited TGF- $\beta$ -mediated phosphorylation of MAPKAPK2 and Hsp27, MMP-2 activation, and thus cell invasion of PCA cells (Xu 2006). In addition, flavonoids also exhibited a dose-dependent inhibition of VEGF, platelet-derived growth factor (PDGF), urokinase, uPA, and MMP-2 and MMP-9 and upregulated angiogenesis inhibitors plasminogen activator inhibitor-1, endostatin, angiostatin, and thrombospondin-1 and  $\beta$ -catenin (Nakamura et al. 2012; Su et al. 2005). The results from another study demonstrated that LUT exerted an anticancer effect against NCI-H460 cells through Sirt1-mediated apoptosis and the inhibition of cell migration (Ma et al. 2015). Similarly Nar downregulated epithelial to mesenchymal transition of EMT markers such as vimentin, N-cadherin, MMP-2, and MMP-9 expression through inhibiting TGF- $\beta$ 1/Smad3 signal pathway in the pancreatic and SGC-7901 cancer cells (Bao et al. 2016; Lou et al. 2012). In melanoma, Quer was found to inhibit STAT 3 signaling and further downregulated its targeted genes such as Mcl-1, MMP-2, MMP-9, and VEGF involved in cell growth, migration, and invasion (Cao et al. 2014). Further, LUT acts as an anti-metastatic agent by suppressing MMP-9 and MMP-2 release and upregulating TIMP-2 expression. It also inhibited Raf and PI3K activities and subsequently attenuated phosphorylation of MEK and Akt in Balb/C mice and colorectal cancer cells (Kim et al. 2013; Pandurangan et al. 2014). Free intracellular  $\text{Ca}^{2+}$  is a central signal amplifier triggering lymph endothelial cell (LEC) retraction which enhances intravasation. MMP1 induces  $\text{Ca}^{2+}$  release and causes the phosphorylation (activation) of FAK at Tyr397 in LECs. LUT inhibited MMP1-induced  $\text{Ca}^{2+}$  release and prevented MMP1-induced FAK activation revealing that it reduces the metastasis of breast cancer cells to lymphoid system (Hong et al. 2018). Quer significantly suppressed TPA-induced activation of the PKCd/Erk/AP-1-signaling in breast cancer



**Fig. 5.4** Anti-metastatic effect of flavonoids through inhibition of several target proteins involved in cellular metastasis, including Akt, NF- $\kappa$ B, and MAPK proteins

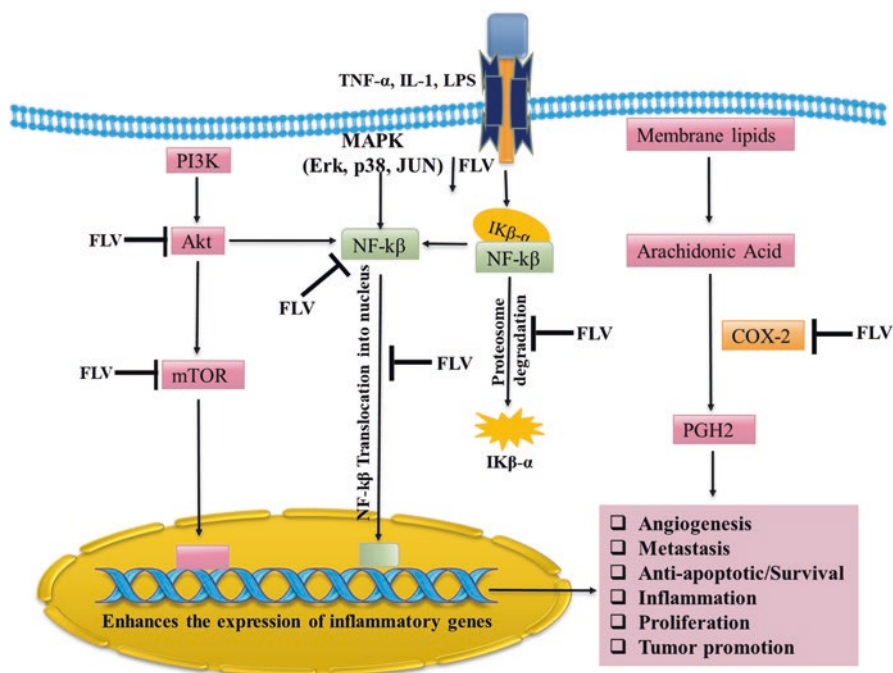
(Lin et al. 2008). Researchers further investigated the downregulation of PKC and RhoA by flavonoids in human cancer cells by modulating multiple targets such as MAPK, PI3K/AKT, NF- $\kappa$ B, and uPA (Lai et al. 2013). One study demonstrated that LUT prevents the migration of glioblastoma cells (U-87 MG cells) by affecting PI3K/AKT activation, modulates expression of Cdc42, and facilitates their degradation via proteasome pathway (Cheng et al. 2013). The effects of Nar and GEN on TSGH-8301 bladder cancer cells resulted in reduced cell viability and MMP expression. In this study the anti-metastatic potential of flavonoids was accomplished by multiple signaling pathway regulation such as Akt, Erk1/2, and JNK and blockage of nuclear translocation of NF- $\kappa$ B and AP1 in different cancer cells (PC-3 and DU145 cells, HepG2, Huh-7, and HA22T) (Liao et al. 2014; Wang et al. 2014; Yen et al. 2015). In MDA-MB-231 cells, a significant increase in connexin 43 (Cx43) levels was identified, which ameliorated gap junctional intercellular communication (GJIC) and hence suppressed the growth and metastasis in human breast cancer (Conklin et al. 2007). Flavonoids also downregulated the HGF/c-Met signaling pathway, which proved its anti-metastatic action for the inhibition of cancer (Cao et al. 2015). The potential chemopreventive role of Quer in colon cancer including the molecular mechanisms related to metastasis has been well reviewed by Darband et al. (Darband et al. 2018). Thus, flavonoids inhibit a number of metastatic targets (Fig. 5.4) and may be used as potential candidates for cancer therapy.

## 6 Anti-inflammatory Effects of Flavonoids for Cancer Prevention

Inflammation has been recognized a tumor-promoting process during cancer development (Nishida et al. 2006; Coussens and Werb 2002). There have been a number of reports from epidemiological studies that chronic inflammation is associated with the risk of cancers (Hold and El-Omar 2008; Mantovani et al. 2008). Tumor

microenvironments (TME) contain many different inflammation mediators (cytokines and chemokines) that modulate cancer-associated signaling. Therefore, this complex network could be targeted for inflammation associated malignant disease.

Nar significantly decreased the number of metastatic tumor cells in the lung and extended the life span of tumor-resected mice via increased proportion of IFN- $\gamma$  and IL-2 expressing T cells. In vitro studies further demonstrated that relief of immunosuppression caused by regulatory T cells might be the fundamental mechanism behind metastasis inhibition by Nar (Qin et al. 2011). In colorectal cancer cells, it potently suppressed anchorage-independent growth by inhibiting COX-1 activity and acted as a potential preventive agent (Li et al. 2014). The results showed that IL-6-induced JAK/STAT 3 activation in KKV-M156 cells was suppressed by the treatment with LUT (Yen et al. 2015). Furthermore, pro-inflammatory cytokines such as IL-1 $\beta$ , IL-6, IL-8, and TNF- $\alpha$  level were significantly reduced by the treatment of PC-3 cells with LUT (Pratheeshkumar et al. 2012). Apigenin is shown to inhibit IL-1 $\alpha$ - and TNF- $\alpha$ -induced CCL2 release in human triple-negative breast cancer cells via IKK and ERK signaling (Bauer et al. 2017). Thus, flavonoids possess anti-inflammatory potential and may reduce the inflammation (Fig. 5.5) associated with cancer risk.



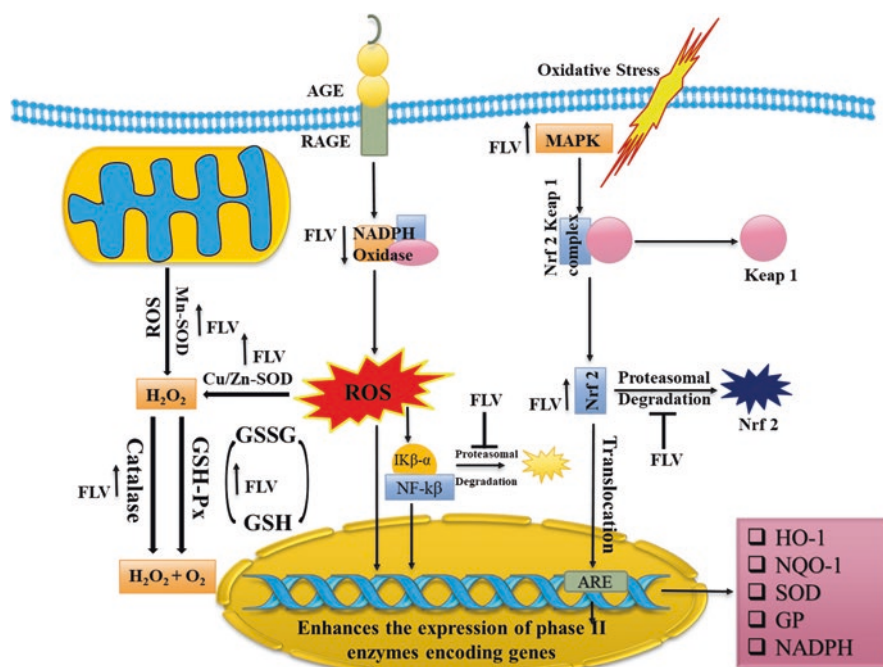
**Fig. 5.5** Diagrammatic representation of anti-inflammatory potential of flavonoids. The anti-inflammatory effect of flavonoids occurs through inhibition of NF- $\kappa$ B, AKT, mTOR, and many other important genes in the inflammatory process

## 7 Antioxidant Potential of Flavonoids for Cancer Prevention

Oxidative stress arising from exogenous and endogenous origins put body under abnormal physiological condition (Ozben 2007). It is closely associated with every aspect of carcinogenesis, like tumor-bearing state, as well as treatment and resistance (Klaunig et al. 2010). The increased oxidative stress results in an imbalanced cellular oxidation-reduction arrangement, which can lead to further alterations such as gene mutations and genetic instability and can affect intracellular signal transduction and transcription factors (Schieber and Chandel 2014). It has been observed that tumors have abnormal expression levels of various antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), manganese superoxide dismutase (MnSOD), and copper-zinc superoxide dismutase (CuZnSOD) (Martindale and Holbrook 2002) (Yanishlieva et al. 2001). Nuclear factor erythroid 2-related factor-2 (Nrf2), a well-known transcriptional factor that controls the expression of above mentioned antioxidant molecules, has been found to be suppressed during the carcinogenesis (Ma 2013). Furthermore, anticancer agents and radiation therapy for cancer treatment are also known to exert oxidative stress, which may result as one of the reasons for drug resistance and failure of cancer therapy (Rendic and Peter Guengerich 2012).

Quer stabilized Nrf2 protein by obviating its degradation and decreased post-translational levels of Keap1 protein, without affecting the dissociation of Keap1-Nrf-2 intricate (Tanigawa et al. 2007). Similarly, the time-dependent effect of Quer on nuclear translocation of Nrf-2 and its increased expression at the mRNA and protein levels in HepG2 cells and malignant mesothelioma (MM) MSTO-211H and H2452 cells were recently reported (Y.-J. Lee et al. 2015; Ramyaa et al. 2014). Evidence has shown that flavonoid-induced ARE-dependent transcriptional gene activation is mediated by the activation of sundry intracellular signaling cascades, including the MAPK. Among MAPK signaling pathways, p38 and Erk-mediated Quer-derived Nrf-2 translocation into nuclei has been shown to be responsible for subsequent induction of HO-1 expression and activity (Chow et al. 2005; Lee et al. 2008, 2011; Yao et al. 2007). In addition, treatment with Nar significantly modulates lipid peroxidation (LPO), pro-inflammatory cytokines (TNF- $\alpha$ , IL-6, and IL-1 $\beta$ ), and activities of antioxidant enzymes (SOD, CAT, GPx, GR, and GST) and nonenzymatic antioxidants (glutathione (GSH) and vitamin C (Vit C)). Moreover Nar treatment effectively negates B[a]P-induced expression of CYP1A1, PCNA, and NF- $\kappa$ B, further substantiating the chemopreventive potential against lung cancer in mice (Bodduluru et al. 2016). Similarly, LUT also decreased cell viability in human colon cancer by increasing the level of GSH and the expression of GSH synthetase in HT-29 cells (Kang et al. 2017). The decreased activities of the antioxidant enzymes such as SOD, CAT, GPx, and GR in mouse colon cancer and HCC in male Wistar albino rats were also found to be ameliorated after LUT treatment (Ashokkumar and Sudhandiran 2008; Balamurugan and Karthikeyan 2012a, b). In addition, GEN promoted the decreased levels of MnSOD, CuZnSOD, and TrxR

mRNA expression while increasing GPx expression levels in breast cancer (Prietsch et al. 2014). Furthermore, Fork-head box O (FOXO) transcription factors, Akt downstream effectors, are important regulators of cell growth. The various adverse processes activated upon FOXO suppression include increased generation of reactive oxygen species (ROS). Quer suppressed the cell growth in EGFR overexpressing squamous cell carcinomas of the head and neck cancer cells over inhibiting the EGFR/Akt activation with a concomitant induction of FOXO1 activation (Huang et al. 2013). Apigenin has revealed an anticancer effect on transgenic adenocarcinoma mouse prostate (TRAMP) mice via FOXO3a- and FOXO-responsive proteins BIM p27/Kip1 (Shukla et al. 2014). Furthermore, inhibition of FOXO3a and related proteins has been investigated in pancreatic cancer model in vivo, and (-)-epigallocatechin-3-gallate (EGCG) induced the apoptosis EGCG induced apoptosis by upregulating Bim and activating caspase-3. EGCG modulated markers of cell cycle (p27/KIP1), angiogenesis (CD31, VEGF, IL-6, IL-8, SEMA3F, and HIF1 $\alpha$ ), and metastasis (MMP2 and MMP7) (Shankar et al. 2013). The efficacy of antioxidants in the prevention of carcinogenesis is currently under investigation. A schematic representation of mechanistic insight on antioxidative activity of flavonoids is given in Fig. 5.6.



**Fig. 5.6** Flavonoids induce antioxidant effects through modulating multiple signaling pathways involved in oxidative stress

## 8 Synergistic Potential of Flavonoids

There are several examples where synergistic effects of flavonoids have been demonstrated as promising therapeutic strategy (Kashyap et al. 2017). The flavonoids in combination with other natural and synthetic drug molecules are found to enhance their antitumor effects. In addition, the synergism of flavonoids with radiation therapy has also been investigated and reported to have significant improvement in cancer cell sensitization toward radiotherapy (Brito et al. 2015).

### 8.1 Chemosensitizer Effect

Indeed, it was demonstrated that Quer can increase the cisplatin-induced apoptosis by 16.3% in human laryngeal carcinoma Hep-2 cells. Cumulated effect of Quer and EGCG was found to suppress the JAK/STAT cascade in human embryonal rhabdomyosarcoma CCA cells (Senggunprai et al. 2014). Synergistic effect of Quer was tenacious with two pharmaceutical molecules, sulforaphane and GTC, which induced miR-let7a-mediated inhibition of K-ras in pancreatic ductal adenocarcinoma PDA (Appari et al. 2014). In LNCaP and PC-3 human prostate cancer cell lines, Quer and 2-methoxyestradiol (2-ME) showed antiproliferative and proapoptotic activities via increased G2/M phase population of cells and decreased Bcl-2/Bax ratio (G. Wang et al. 2013a). Quer, in association with two platinum drugs, cisplatin and oxaliplatin, overcame drug resistance in cancer (Nessa et al. 2011). The co-administration of 2.5  $\mu\text{M}$  of EGCG, GEN, and Quer flavonoids suppressed proliferation synergistically in CWR22Rv1 cells by modulating the expression of androgen receptor, NQO1 (Hsieh and Wu 2009). Imperatorin and Quer, two potent apoptosis inducers, were found to block Hsp27 expression especially when they act synergistically (Bądziul et al. 2014). A synergistic effect of resveratrol and Quer revealed the modulation of number of metabolic pathways involved in adipose tissue triacylglycerol accumulation (Arias et al. 2016). In another study, Quer and Dox induced G2/M cell cycle arrest in HT29 cells (Atashpour et al. 2015). Cumulated utilization of cyclophosphamide plus Quer not only minimized the toxicological symptoms but was also found to improve the fatigue behavior in advanced bladder cancer patients (Lorenzo et al. 2016). Storniolo et al. proposed that Quer, affecting the Hsp70-IRE1 $\alpha$  axis, may represent an efficacious adjuvant in antileukemia-based therapy (Storniolo et al. 2015). In addition, studies using a variety of other drugs have revealed that Quer can either decrease or increase their bioavailability by modulating CYP3A4 and/or P-glycoprotein (P-gp) (L. R. Zhao et al. 2014b). The combination of Nar and LUT in a couple of studies were found to enhance the efficiency of paclitaxel to suppress the progression of prostate cancer cells and oral squamous cancer (SCC-4 cells), respectively, via exerted apoptotic effects (Lim et al. 2017; Yang et al. 2008). The combination of LUT and paclitaxel



activated caspase-8 and caspase-3 and increased the expression of Fas due to the blocking of STAT 3 in an orthotopic tumor model (Yang et al. 2014). Furthermore, Nar and Hesperetin (HP), as HER2-TK inhibitors, sensitized HER2-positive cancer cells to cell death (Chandrika et al. 2016). Combined treatment with 4-OH-TAM (tamoxifen) and LUT synergistically sensitized the TAM-R cells to 4-OH-TAM by targeting the expression level of CCNE2 and could be a novel strategy to overcome TAM resistance in breast cancer patients (Tu et al. 2013). Combinatorial drug studies further showed that LUT could synergize the antitumor effects of 5-fluorouracil (5-FU) against HepG2 and Bel7402 cells by enhancing Bax/Bcl-2 ratios and p53 expression and PARP cleavage (H. Xu et al. 2016). Results suggested that the combination of 5-FU and GEN also showed a chemotherapeutic effect in colon cancers (Hwang et al. 2005). GEN and hydroxycamptothecin (HCPT) synergistically inhibited bladder cancer cell growth and proliferation and induced G<sub>2</sub>/M phase cell cycle arrest and apoptosis (Y. Wang et al. 2013b). Combination of Nar-Tam inhibited both PI3K and MAPK pathways in MCF-7 cells (Hatkevich et al. 2014). The data further support the chemosensitizing activity of flavonoids.

## 8.2 Radiosensitizer Effects

Evidences have indicated that LUT acts as a radiosensitizer by enhancing apoptotic cell death through activation of a p38/ROS/caspase cascade in a xenograft model of tumor growth (Cho et al. 2015). In addition, GEN has also been shown to be a radiosensitizing agent in prostate cancer cells through the inhibition of NF- $\kappa$ B, which in turn altered the expression of cyclin B and/or p21<sup>WAF1/Cip1</sup> and caused G<sub>2</sub>/M cell cycle arrest (Hwang et al. 2005; Raffoul et al. 2006). The combined treatment with GEN and X-rays have upregulated the phosphorylation of ATM, Chk2, Cdc25c, and Cdc2, leading to permanent G<sub>2</sub>/M phase cell cycle arrest, and apoptosis via upregulation of Bax and p73 and downregulation of Bcl-2 (X. Liu et al. 2013b). Similarly, the combination of LUT and IR enhanced apoptotic cell death through the activation of a p38/ROS/caspase cascade (Cho et al. 2015), further establishing the role of flavonoids as radiosensitizers.

## 9 Role of Flavonoids in miRNA-Mediated Cancer Inhibition

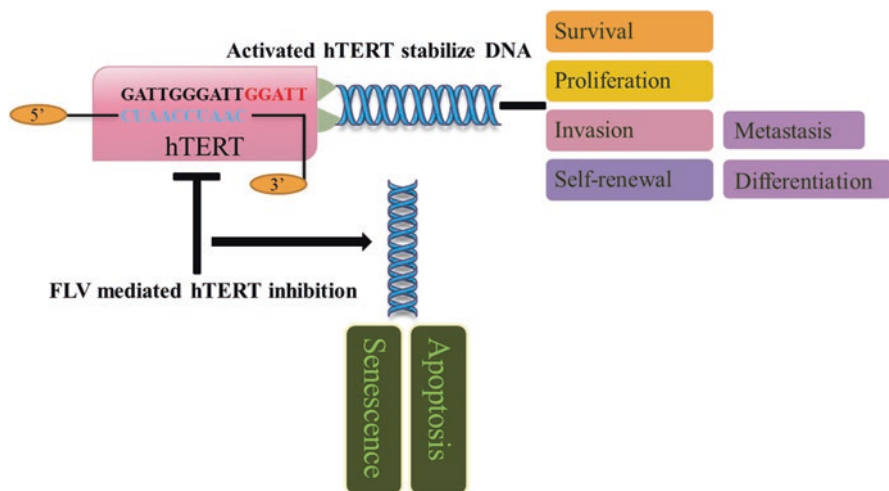
MicroRNA, also called noncoding RNA, regulates epigenetics of gene expression, which further controls several biological processes (D. Kashyap et al. 2018) (Esquela-Kerscher and Slack, 2006). By acting as oncogenic or tumor suppressors, these single-stranded molecules are known to modulate a variety of cancer-signaling pathways. For instance, LUT upregulates miR-34a expression, which in turn downregulates Bcl-2 expression, and thus induced apoptosis in gastric cancer cells (Wu

et al. 2015). In other studies using PCa, LUT was found to regulate the expression of the pro-apoptotic gene *DEDD2* through downregulation of miR-301 (Han et al. 2016). In addition, LUT inhibited tumorigenesis and induced apoptosis of NSCLC cells by upregulation of miR-34a-5p, which targets MDM4 in tumor cells (Jiang et al. 2018). Results clearly demonstrated that overexpression of miR-7-1-3p improved the antitumor potential of LUT and SIL to reduce autophagy and provoke apoptosis for controlling growth of human glioblastomas (Chakrabarti and Ray 2016). One study demonstrated that GEN exerts growth-inhibitory activities in human uveal melanoma cells by miR-27a and its target gene ZBTB10 (Sun et al. 2009). GEN led to the upregulation of miR-34a, inhibited cell growth, and induced apoptosis with concomitant downregulation of Notch-1 signaling pathway in prostate cancer cells (Xia et al. 2012). It has also been suggested that GEN exerts its antitumor activity partly through downregulation of miR-223 and hence upregulation of Fbw7 in PC cells (Ma et al. 2013). Similarly, GEN upregulates expression of other tumor suppressors, miR-574-3p and miR-1260, which directly bind to the 3' UTR of several target genes such as RAC1, EGFR, EP300, sFRP1, Dkk2, and Smad 4 that are involved in Jak-STAT and Wnt signaling pathways (Chiyomaru et al. 2013b; Hirata et al. 2013). Moreover, using MDA-MB-435 and Hs578t cells, GEN downregulated miR-155 which resulted in the upregulation of FOXO3, PTEN, casein kinase, and p27 and contributed as promising anticancer agent (De La Parra et al. 2016). Chiyomaru et al. identified that GEN inhibited PCa cell growth through tumor suppressor miR-34a and downregulation of oncogenic HOTAIR (Chiyomaru et al. 2013a). In PC3 cells, GEN mediated inhibition of miR-1296 and upregulation of *MCM2* mRNA, causing the S-phase cell cycle arrest (Majid et al. 2010). Further studies revealed that GEN downregulated oncogenic miR-27a expression which was accompanied by significant increase in the expression of Sprouty2 gene in ovarian cancer (Xu et al. 2013). Using HCC, Quer-mediated upregulation of miR-34a resulted in the activation of p53/miR-34a/SIRT1 signal feedback loop and apoptosis (Lou et al. 2015). Moreover, Quer enhanced cisplatin sensitivity by modulating the miR-217-KRAS axis in human osteosarcoma 143B cell line (Zhang et al. 2015). Consequently, flavonoids bear a strong ability to regulate miRNA and could be suggested as potential anticancer molecules.

## 10 Regulation of Topoisomerase II and Telomerase by Flavonoids

Topoisomerase II are ubiquitous enzymes that have crucial functions, including DNA replication, transcription, and chromosome segregation (Salti et al. 2000). They regulate DNA winding processes and resolve knots and tangles in the genetic material (Schmidt et al. 2008). Type II topoisomerases are known to fragment the genome during their catalytic cycle by generating DNA double-strand breaks





**Fig. 5.7** Diagrammatic illustration of flavonoid-mediated inhibition of telomerase activity in cancer cells

(Nagase et al. 2009). Therefore these are not only essential for the survival and proliferation of cells but also have significant genotoxic effects. Thus the genotoxic effect of type II topoisomerase has been exploited for the development of several classes of anticancer drugs that are widely being employed for the clinical treatment of human malignancies. On the other hand, telomerase is also considered to be an important therapeutic target for the treatment of cancer (Fig. 5.7). In the majority of cancer cells, the telomeric length of chromosomes is maintained by telomerase which further supports cancer initiation and survival.

Flavonoid molecules have been found to interfere with topoisomerase II and telomerase activity. For instance, GEN was found to induce cytotoxicity and inhibited cancer cell growth by increasing DNA/topo II complex formation (Schmidt et al. 2008). In colon cancer cells, GEN activated topo II-mediated DNA cleavage (Salti et al. 2000). Further results revealed that GEN activates HeLa cell apoptosis by modulating topo II $\alpha$  expression through the regulation of specificity protein 1 and specificity protein 3 (Zhou et al. 2009). Similarly GEN reduces telomerase activity in prostate cancer cells via repressing hTERT transcriptional activity through c-Myc/Akt and posttranslational modification of hTERT (Jagadeesh et al. 2006). GEN inhibits the growth of glioblastoma and medulloblastoma cells by arresting cells at the G2/M phase along with telomerase inhibition via suppressing the expression of TR and TERT mRNA (Prietsch et al. 2014). EGCG is shown to regulate the cross talk between JWA, a novel microtubule-binding protein, and topoisomerase II in NSCLC (Li et al. 2015a, b).

## 11 Conclusions and Future Perspectives

All the evidences that have been discussed in this chapter have pellucidly supported the utilization of flavonoids as therapeutic agents for cancer inhibition. Flavonoids are found to modulate a variety of extracellular as well intracellular signaling pathways associated with tumor progression and survival reflecting a wide variety of action interplay by flavonoids; however, the underlying therapeutic applicability still needs thorough scientific investigation. The use of nano-mediated techniques can further boost the bioactive potential of flavonoids by increasing bioavailability and targeted delivery (Ban et al. 2015). Similarly, synthesis of novel derivatives of flavonoids may also overcome drug resistance mechanisms in cancer therapy. Another promising aspect of future study could be the docking-based investigations of flavonoids with various recognized cellular targets (Harsa et al. 2015) (Rashid and Iftikhar 2014). In addition to these, metal complexation behavior of flavonoids may also be implemented to enhance their therapeutic activity. However, inclusive information about the above objectives and mechanisms of activity of flavonoids can also be regained by utilizing systems biology, transcriptomics, proteomics, and metabolomics tools. Therefore future research should preoccupy on the synergistic methods of flavonoids with other existing anticancer drugs.

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# Chapter 6

## Absorption, Metabolism, and Disposition of Flavonoids and Their Role in the Prevention of Distinctive Cancer Types



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and Hardeep Singh Tuli

### 1 Introduction

The term flavonoid originates from the Latin word “flavus,” meaning yellow. Thousands of flavonoids have been discovered from the plant stems, flowers, fruits, nuts, seeds, vegetables, herbs, spices, green tea, and red wine. They are also found in most of the vascular plants as phenylbenzo-pyrones (phenylchromones). Citrus fruits are the prominent source of flavonoids (Kefford and Chandler 1970; Brouillard and Cheminant 1988). Due to their potent antioxidant (viz., free-radical scavenging capacity) and anti-inflammatory properties, flavonoid-rich diets are promoted for maintaining good health and well-being and prevention of diabetes, obesity, cardiovascular diseases, and neurodegenerative disorders and as anticancer agents. Chemically, flavonoids consist of a basic three-ring nucleus (Fig. 6.1), and the molecular weight of these polyphenolic compounds ranges from 270 to 320 mol. They are categorized into a number of subclasses according to their substitutions, and such classes are known as flavones, flavanols, flavonols, flavanones, isoflavones, anthocyanidins, and chalcones (Figs. 6.1 and 6.2). The basic structure encompasses two benzene rings linked through a heterocyclic pyran or pyrone (with a double bond) ring in the center. The subclassification is principally based on the keto group on position 4 of the C (middle) ring or its absence, the double bond

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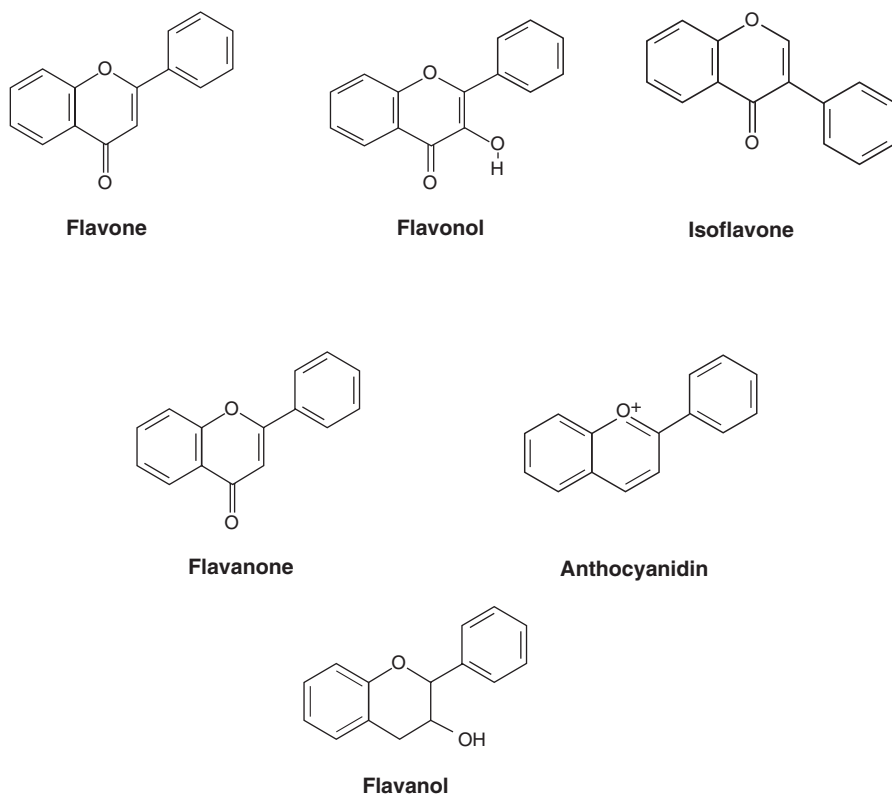
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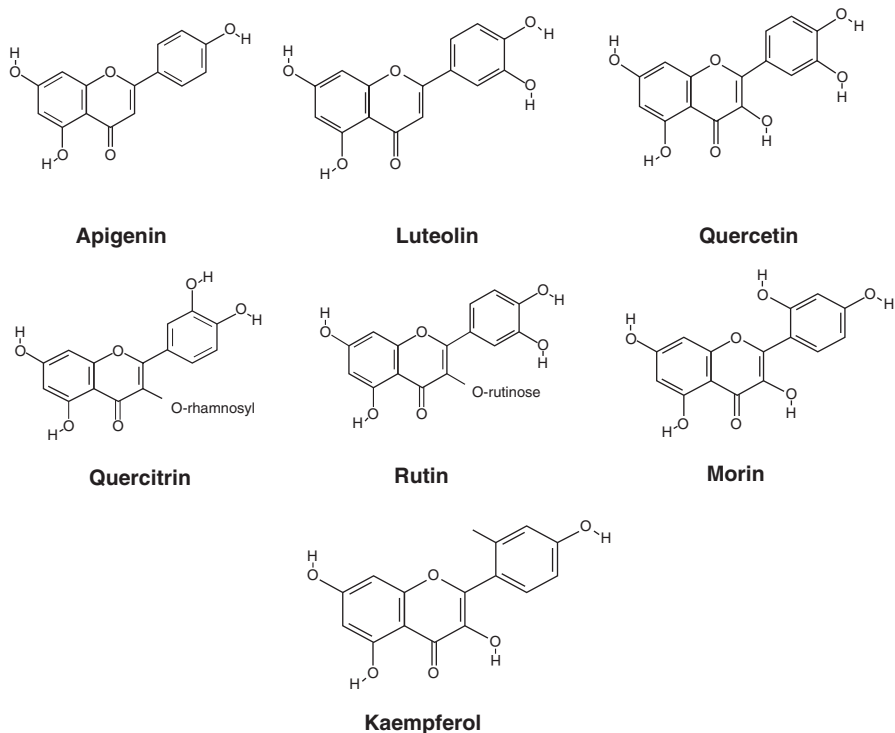




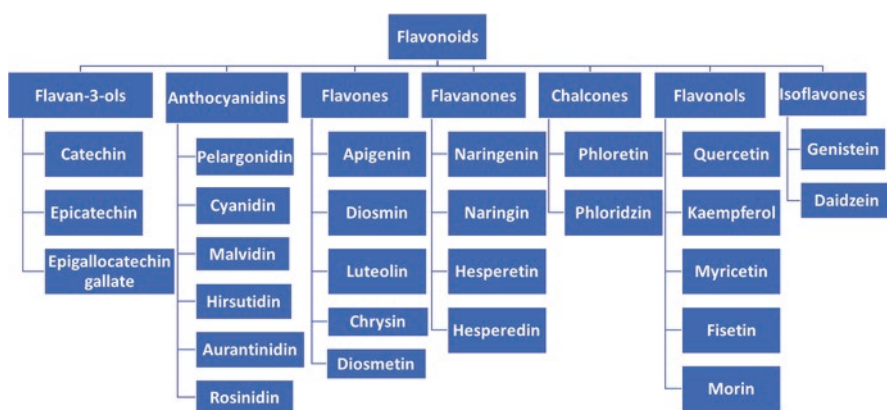
**Fig. 6.1** Chemical structures of different subclasses of flavonoids such as flavones, flavonols, isoflavones, flavanones, and anthocyanidins

between carbon atoms 2 and 3 of the C ring or its absence, and the occurrence of hydroxyl groups in the B ring or their substitutions. A phenyl group is typically substituted at the second position of the pyrone ring. In isoflavonoids, the substitution is at the third position (Hertog et al. 1992; Bilyk and Sapers 1985; Rice-Evans and Packer 1998). Some examples of subclasses of naturally occurring flavonoids are given in Fig. 6.3.

Since flavonoids seem to have existed for over billion years, they must have played significant bioactive roles in nature. There appears to be a long interactive association between the plant flavonoids and the existence and well-being of various animal species. The evolutionary researchers have hypothesized several biological effects of flavonoids and their subclasses (Swain 1975). For example, quercetin showed a strong inhibitory effect on gamete membrane fusion in sea urchins during egg fertilization (Eckberg and Perotti 1983) and its modulatory effect on mammalian sperm motility (Nass-Arden and Breitbart 1990). Prenatal exposure to isoflavone genistein influenced sexual differentiation in rats (Levy et al. 1995). These observations pose the possibility of parallel effects in humans. Indeed,



**Fig. 6.2** Chemical structures of some most common flavones (apigenin, luteolin) and flavonols (quercetin, kaempferol, morin, quercitrin, rutin)



**Fig. 6.3** Examples of subclasses of naturally occurring flavonoids. (Hertog et al. 1992; Bilyk and Sapers 1985; Rice-Evans and Packer 1998)

flavonoids have long been recognized to possess important biological activities in human cells. Several *in vivo* and *in vitro* studies have shown anti-inflammatory, hepatoprotective, antithrombotic, cardioprotective, neuroprotective, antiallergic, antioxidant, antiviral, antibacterial, and anticarcinogenic activities of flavonoids (Gabor 1986; Havsteen 1984; Farkas et al. 1986; Cody et al. 1986; Welton et al. 1988).

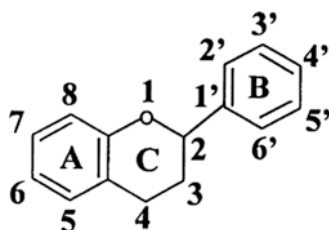
A well-balanced diet; intake of micronutrients, unsaturated fats, fruits, and vegetables; low intake of salt and sugar; and moderate physical activity are all essential for maintaining good health and prevention of noncommunicable ailments. Consumption of balanced and flavonoid-rich diet that is low in carbohydrates and saturated fats, lesser alcohol consumption, and non-smoking are critical factors to reduce the risk of cancer (Morales and Haza 2012; Chien et al. 2010; Sato et al. 1994). There is an overwhelming evidence that inclusion of fruits and vegetables and fiber-rich diet along with maintaining the physical activity can reduce the incidences of cancer by 30–40% (Bulzomi et al. 2012; Dai et al. 2010). Also, there are several studies which show less risk of cancer in the vegetarians as compared to the meat-eating population (Yin et al. 2001). Flavonoids have been demonstrated to be a predominant factor in the reduction of cancer risk (Table 6.1) (Chien et al. 2010). Diets rich in whole grains, fruits and vegetables, olive/flaxseed/perilla oils, fish and omega-3 fatty acids, and low-fat dairy products and moderate wine consumption (e.g., Mediterranean-type diet) are linked with lower incidence of cardiovascular diseases and cancer (Griffiths et al. 2016). Lifestyle modifications, namely, regular physical activity (about 30 min/day), restriction of caloric and sodium intake, healthy body mass index (BMI around 25–28 kg/m<sup>2</sup>), smoking cessation, and moderate alcohol consumption, are recommended for improving cardio-metabolic function and quality of life. Ingestion of functional foods, vitamins, minerals, and amino acids may assist to improve the overall health beyond basic nutritional functions. Emerging evidence suggests that dietary supplements containing flavonoids, carotenoids, and antioxidants can modulate gene and protein expression and thereby modify endogenous metabolic pathways and homeostasis and consequently reduce the risk of cardiovascular disorders, cancer, and chronic diseases multifactorial in their origin.

### ***1.1 Structure–Activity Relationship of Flavonoids***

Flavonoids are benzo- $\gamma$ -pyrone derivatives comprising of two phenolic rings and a pyran ring (Fig. 6.4) which are classified as per the substitutions. Arrangement of hydroxyl (OH), methoxy (O-CH<sub>3</sub>), and glycosidic side groups and conjugations among the rings A and B make the difference between the dietary proteins. During the metabolism in man, hydroxylation, methylation, sulfonation, or glucuronidation takes place. Dietary flavonoids exist principally as 3-O-glycosides and their polymers. Polymerization of flavanols to tannins during enzymatic oxidation is thought to adapt their antioxidant activity. Condensed tannins and proanthocyanidins

**Table 6.1** Role of flavonoids in various types of human cancers

Flavonoid group	Subgroup	Major sources	Anticancer properties
Flavanols	Flavan-3-ols:		
	Catechin	Grapes, apple, pear, cherries, strawberries, blueberries, raspberries and green tea	Breast cancer (Li-Ping Xiang 2016)
			Rectal and prostate cancer
	Gallocatechin	Green tea	Human stomach cancer (MK-1) cells (Kinjo J, et al. 2002)
	Catechin-3-gallate	Green tea	Prostate and breast cancers (Liao S, et al. 1995)
			Human esophageal cancer (Hallman K, et al. 2017)
	Epicatechin	Buckwheat, grapes	Cervical cancer (Mukherjee S, et al. 2017)
	Epigallocatechin	Green tea	Non-small cell lung cancer (Yu C, et al. 2017)
	Flavan-4-ols	Sorghum	Urinary bladder carcinoma (Truong HH, et al. 2017; Beydokthi SS, et al. 2017)
	Flavan-3,4-diols	Grape skin	Prostate cancer (Ananga A, et al. 2017)
Flavones	Epigenin, chrysin, luteolin	Parsley, celery, capsicum, pepper, broccoli	Lung cancer, leukemia, stomach, colon, thyroid, oral and laryngeal cancer, breast cancer
Flavonol	Kaempferol, myricetin, quercetin, rutin	Brussel sprouts, apples, onion, curly kale, leek, beans, cherries	
Flavanones	Eriodictyol, hesperitin, naringenin	Orange juice, grape fruit juice, lemon juice	
Flavanols	Taxifolin, catechin, epicatechin	Milk thistle, red onion, acai palm, Siberian larch tree	
Anthocyanidins	Cyanidin, delphinidin, malvidin, petunidin, peonidin, pelargonidin	Aubergine, black berries, black currant, blue berries	Colorectal cancer
Isoflavonoids	Isoflavones: daidzein, genistein, glycitein	Soy flour, soy beans, soy milk, miso, tempeh, beer	Breast cancer, prostate cancer, colon, kidney and thyroid cancer
	Isoflavane: equol	Metabolized from daidzein by intestinal bacteria	Breast cancer, prostate cancer, colon, kidney and thyroid cancer



**Fig. 6.4** Nucleated flavonoid structure. Conjugation of the aromatic rings, glycosidic and methoxy groups to the flavanoids, which makes them diverse. Polymerization of this nuclear structure yields tannins and other composite species, which are believed to have different pharmacological effects based on the substitution types

contain flavanol units, of which procyanidins are most significant in the human diet. The procyanidin dimers, trimers, and oligomers appear in red wine, apple, grape seeds, and cocoa. Also, the galloyl moieties present in the tannins and the monomeric catechins in green tea are moderately responsible for the chelation and radical scavenging activities of these compounds (Cook and Samman 1996).

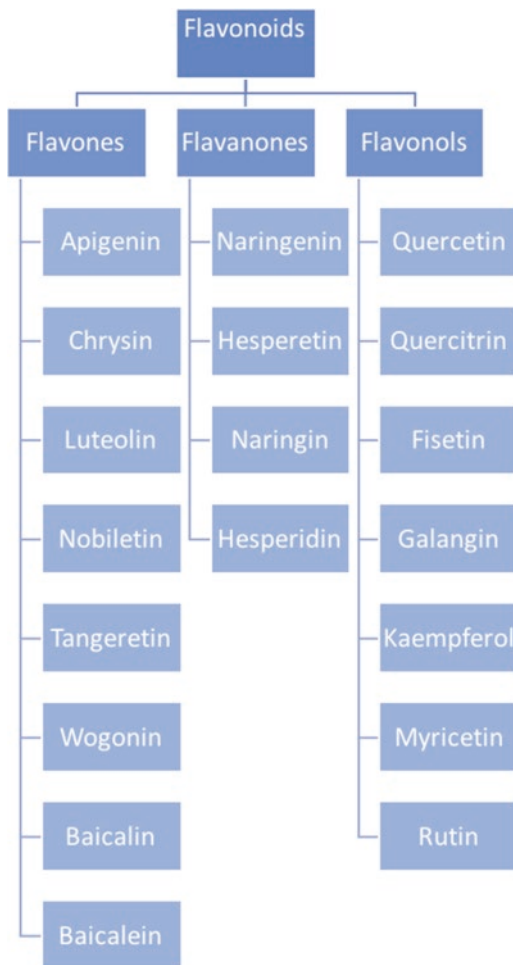
## 2 Modulating the Metabolism of Carcinogen

Activation of a procarcinogen to carcinogen is an important step in carcinogenesis and can be modulated by flavonoids. Flavonoids can exhibit their effects in two possible mechanisms. First is by interacting with phase I enzymes that are involved in metabolic activation of procarcinogens. The other mode of action is the detoxification and elimination of carcinogens through the induction of phase II enzymes such as UDP-glucuronyl transferase. CYP–flavonoid interactions are one of the multiple ways through which flavonoids can affect enzymatic activities, i.e., from regulation of gene expression to direct binding to the processed enzymes. Flavonoids can induce or eventually inhibit the biosynthesis of CYP1A1 via interactions with the aryl hydrocarbon receptor (AhR), a cytosolic protein that, once activated by a ligand, translocates to the nucleus and, in association with the AhR translocator, forms a transcription factor for CYP1A1 (Jitka Křížková et al. 2009) (Fig. 6.5)

## 3 Absorption, Distribution, Metabolism, and Excretion of Flavonoids

After the flavonoids are administered orally, it is important to understand their pharmacodynamics. Also, suitable extrapolation of existing structure–activity relationship (SAR) information can be useful for identifying the pharmacological activities of the flavonoids. Adding on to the SAR attributes of the flavonoids,

**Fig. 6.5** Major flavonoid aglycones and their glycosides



pharmacokinetics, biotransformation, and metabolism are significant determinants for the pharmacological action of the flavonoids. To correlate SAR of flavonoids with human nutrition and medicine, further research is needed to elucidate the rate of absorption, pharmacokinetics, characterization of metabolites, and effects of these metabolites on human physiology. Flavonoids are present in food items mostly as *O*-glycosidic compounds conjugated with glucose, glucorhamnose, arabinose, galactose, or rhamnose units (Hammerstone et al. 2000; Cook and Samman 1996). The  $\beta$ -linkages in these sugars resist their hydrolysis by pancreatic enzymes, but some intestinal microbiota is responsible to carry out  $\beta$ -hydrolysis. Also, some microbiota like *Peptococcus*, *Peptostreptococcus*, and *Clostridia* ferment these sugars in the colon (Cummings and Macfarlane 1991). Some  $\beta$ -endoglucosidases like lactase phlorizin hydrolase are believed to perform deglycosylation of flavonoids to allow the site for conjugation (Leese and Semenza 1973; Day et al. 2000; Daniels

et al. 1981; Gopalan et al. 1992). Quercetin-3-glucoside, luteolin-7-glucoside, and kaempferol-3-glucoside undergo absorption and hydrolysis in the small intestine by  $\beta$ -glucosidase action (Gopalan et al. 1992). Flavonoids are abundant, potent, diverse, and widely studied and thus provide insight into the absorption and metabolism studies of these polyphenols. The absorption kinetics vary considerably among flavonoids due to heterogeneity of sugars and functional groups in the flavan nucleus (Hollman et al. 1999, Hollman and Katan 1999). The variation in absorption of flavonoids can also be due to dosage, route of administration, vehicle of administration, colon microbiota, diet, food matrix, and sex differences (Erlund et al. 2001). Along with hydrolysis of flavonoid glycosides, cecal microflora contributes to degradation of monomeric flavonoids to monophenolic acids. 3,4-Dihydroxyphenylacetic acid and phloroglucinol are quercetin metabolites, which are produced by intestinal bacteria via cleavage of the C3–C4 bond of the heterocycle (Winter et al. 1991). In a study conducted by Baba S et al., rats on exposure to rutin produce traces of 3,4-dihydroxytoluene, phenylacetic acids, and 3-(*m*-hydroxyphenyl) propionic acid in the urine (Baba et al. 1983). Colonic microflora is essential for hydrolysis of rutinoides (quercetin-3-rutinoid) as compared to quercetin glycoside (quercetin-3-glucoside), which explains low bioavailability of rutin in human studies (Olthof et al. 2000). Absorption of quercetin in the small intestine was increased to 52% when a glucose unit was present in the structure as compared to 24% for the aglycone unit and 17% for rutin-based compounds. Quercetin glycoside reportedly interacts with epithelial glucose transporters (Gee et al. 1998), which suggests that there is a rapid uptake and bioavailability of glycosides after ingestion.

Biodegradation of larger flavone molecules to smaller low molecular weight compounds is required for crossing the intestinal epithelium. Dimers and trimers of procyanidin are capable of translocating through the epithelium of the small intestine (Déprez et al. 1999). As these molecules generally consist of (+) catechin and (–) epicatechin subunits, it is possible that catechins are the predominant by-products of degradation. The degradation process is conceded by the cecal bacterial colony (Groenewoud and Hundt 1986) and lower gastric pH (Spencer et al. 2000). The hydrolysis of proanthocyanidin oligomers into catechin dimers and free catechins expends 3.5 h in the gastric environment. After three catechin units, exposure to degradation increases correspondingly to the degree of polymerization. Although it is suggested that catechins are accountable for the pharmacological consequences of proanthocyanidins with high molecular weight, 3.5 h or more surpasses the average human gastric emptying rate of 30–90 min, and the influence of acid hydrolysis is undoubtedly less significant than successive metabolic events (Winter et al. 1991). In a study conducted by Doostdar et al. in the year 2000, it was found that the flavones acacetin and diosmetin could inhibit the ethoxyresorufin *O*-dealkylase (EROD) activity of CYP1B1 and CYP1A. Substitutions at the 3' and 4' positions mainly hydroxy and/or methoxy functional groups in the flavonoid structures were majorly involved in the selectivity of distinctive cytochrome P450 enzymes. It was also discovered that flavonoids like naringenin, eriodictyol, and homoeriodictyol were poor inhibitors of human CYP1A EROD activity. Selective inhibition of

human CYP1A1 and CYP1B1 was carried out by hesperetin and homoeriodictyol, where homoeriodictyol could selectively inhibit human CYP1B1. Hesperetin *O*-demethylation was carried out by both human CYP1A1 and CYP1B1 to formation of eriodictyol. It was observed that hesperetin could not be metabolized by human cytochromes CYP1A2 or CYP3A4. A study conducted *in vitro* on the human liver and intestinal microsomes suggested that luteolin was primarily glucuronidated at the seventh position in the liver cells and at the third and fourth positions in cells from the intestine. The conjugation of luteolin to the intestinal microsomes occurred nearly three times as much as liver microsomes. On testing the enzymes individually, it was found that some glucuronidated luteolin was much more efficient than the others. Human uridine diphosphate (UDP) glucuronosyltransferase family 1 member A (UGT1A) 1, UGT1A8, and UGT1A9 were the most efficient (Hostetler et al. 2017). A recent study has demonstrated that in myricetin, the C2 and C3 double bond, the aromatic ring B at position C-2, and the hydroxy groups in ring B may be responsible for the cytotoxic action of the compound (Semwal et al. 2016). It was believed that quercetin was excreted into feces by failing to get absorbed in the intestine, but recent studies have demonstrated the absorption of quercetin into the intestine and conversion to its metabolites (Murota et al. 2002). The involvement of the lymphatic system is seen in the transportation of the quercetin metabolites (Terao et al. 2008). A study has demonstrated that recurring ingestion of onions in female population has resulted in accumulation of metabolites of quercetin in blood and different tissues, i.e., total plasma concentration of 0.6  $\mu\text{M}$  post 1 week of treatment (Moon et al. 2000). Rutin expresses bioavailability in different *in vitro* systems. The poor bioavailability of rutin limits its biological effect. There are many drug delivery systems which are being developed to increase the bioavailability of rutin. Nanoparticulate systems, sulphonation and carboxylation of rutin, enzymatic oligomerization, and producing cyclodextrin complexes enhance the aqueous solubility of rutin. Phospholipid complexation, enzymatic and chemical acylation, and nanoparticulation are performed to enhance the lipid solubility of rutin (Gullón et al. 2017). In 1972 and 1998, Griffiths and Barrow (1972) and Hollman and Katan (1998), respectively, reviewed the fate of orally and parenterally administered flavonoids in mammals. Limited information is available regarding the metabolism of flavonoids in animals and in humans (Hackett 1986; Scheline 1991). Myricetin, kaempferol, quercetin, apigenin, and luteolin are the most consumable flavonoids obtained from plants. The average daily intake of these antioxidant flavonoids is approximately 23 mg/day, which is more than the intake of other acquainted antioxidants such as  $\beta$ -carotene whose average intake is 2–3 mg/day and vitamin E whose average intake is 7–10 mg/day and also encounters to be one-third of the average intake of vitamin C (70–100 mg/day) (Hertog et al. 1992; Hertog et al. 1993). The most significant contributor to the dietary consumption of flavonoids is quercetin. It is mainly obtained from apples and onions (Knekt et al. 2000; Gibellini et al. 2011). Preclinical and clinical studies have indicated that quercetin glucosides, cinnamate conjugates, and flavanols are readily absorbed in the small intestine (Olthof et al. 2003; Cermak et al. 2004), while quercetin galactosides, quercetin, rutin, and naringenin are not absorbed in the intestine. The mechanism of absorption



has not been completely explained, but the membrane transport process of flavonoids is a vital part of their bioavailability in plants and animals. The current research suggests the contribution of both ATP-dependent pumps and ATP-independent transporters (Passamonti et al. 2009).

## 4 Discussion and Conclusions

Flavonoids are reported to have oncolytic effects on different types of cancer cells (Table 6.1). They have low bioavailability and also undergo rapid metabolism in the liver. Whether or not the flavonoid metabolites also have anticancer targets needs to be ascertained. Additive and synergistic interactions have been reported between two or more dietary flavonoids. There is a need to understand their mechanisms of action as anticancer agents (Androutopoulos and Spandidos 2013). Polyphenolic flavonoids like quercetin, apigenin, myricetin, luteolin, and chrysin have been reported to exert anticancer properties. Additive effects were observed after the combined administration of flavonoids with synthetic anticancer drugs. Such combination would not only help in the dose reduction of anticancer drugs but also their toxic side effects. Further, chemotherapy-induced genotoxicity to the non-cancerous cells would be reduced through such combination, thus preventing the development of secondary cancerous manifestations (Strouch et al. 2009). Further research is needed to discover new effective anticancer flavonoids using sophisticated in vitro and in vivo molecular biology techniques of the structure–activity relationships of flavonoids. Structural alterations may enhance the bioavailability, prolong circulatory half-life, and increase oncolytic activity as well as improve the safety and efficacy profiles of more potent anticancer flavonoids.

**Conflict of Interest** The authors declare no conflict of interest.

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# Chapter 7

## Emerging Trends in Flavonoid Research and Associated Toxicity



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### 1 Introduction

Most of the widely available bioactive compounds present in vegetables and fruits are flavonoids (Pascual-Teresa et al. 2010). These biological molecules have a diversity of medicinal properties and biochemical consequences such as free radical scavenging effects, and anticancer, antiviral, and anti-inflammatory actions (Middleton et al. 2000). Flavonoids can prevent tumors, and can delay aging and prevent heart and blood vessel- related disorders; hence, they are assumed to be helpful for human well-being (Adhami and Mukhtar 2006; Seifried et al. 2007). Although much research on the health effects of flavonoids has been conducted in model organisms, the properties of these molecules in human individuals remain ambiguous (Halliwell 2007). The current focus of biomedical research has been on the constructive activities of flavonoids; however, their excessive consumption may lead to adverse effects on human health (Skibola and Smith 2000; Ross and Kasum 2002; Galati and O'Brien 2004). Generally, people are not able to recognize the ill effects of a natural supplement when it is taken in excess. As numerous individuals are not aware of the ill effects of natural molecules, this may be the reason that flavonoid intoxication is still under reported. There is a need for the investigation of toxicity and safety levels for flavonoids.

Flavonoids have been used in the food industry as nutritional supplements or functional foods (Chaudhary et al. 2018), and so there is an essential need for

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investigation in this area to determine the best uses and routes of administration of flavonoids and their enrichment to avoid adverse effects in humans.

Despite the positive effects of these molecules, there are a few reports that specify their genotoxicity or mutagenicity in mammalian and microbial test models (Popp and Schimmer 1991; Suzuki et al. 1991; Jurado et al. 1991). This strength was attributed due to initiation of oxidative anxiety in addition to reticence antioxidant coordination (Dickançaité et al. 1998). This behavior, i.e., initiation of oxidative anxiety may be responsible for generating free radicals, which may cause DNA damage and inhibition of enzymes associated with DNA, such as topoisomerase. DNA damage involves DNA thread rupturing and alteration, which leads to permanent preneoplastic abrasions (Breimer 1990). Also, these molecules have diverse pharmacological properties, and therefore high intakes may alter amino acid metabolism and act on other important chemical transformations by biocatalysts.

## 2 Mutagenicity and Genotoxicity of Flavonoids

The mutagenicity of the flavonoid quercetin was first documented by researchers (Bjeldanes and Chang 1977; MacGregor and Jurd 1978; Brown and Dietrich 1979) in regard to its capacity to cause frame-shift mutations and base-pair substitutions in the Ames test. Quercetin showed the exchange of genetic material between identical chromatids and genetic abnormality in Chinese hamster ovary (CHO) cells (Carver et al. 1983), and the generation of micronuclei in human lymphocytes (Rueff et al. 1986; Caria et al. 1995). Hodnick et al. (1986) documented the structure-activity of mitochondrial succinoxidase inhibition by flavonoids, which demonstrated that quercetin, quercetagenin, and myricetin triggered mitochondrial respiratory rupturing, which led to autoxidation and resulted in radical formation by hydrogen peroxide, superoxide, and hydroxyl ions at a biological pH.

Mostly, the oxidation state of molecules changed by many basic flavonoids was found to be lower than that of superoxide and alkyl peroxy radicals (Jørgensen et al. 1998), but the usefulness of basic flavonoids in creating DNA adducts, and in the oxidative degradation of lipids and alterations in living organisms, seems to be meaningful. The effect of flavonoids on mitochondrial enzymes during the generation of reactive oxygen species at slightly alkaline pH plays a role in the cytotoxic and antineoplastic ability related to the prevention and development of a neoplasm (Pritsos et al. 1982; Pritsos and Pardini 1984; Doroshov et al. 1980; Olson et al. 1981). A similar study, by Rahman et al. (1989), showed that quercetin caused DNA strand scission by the temporary reduction of copper from cupric oxide to cuprous oxide and oxygen species. The reactive oxygen species for DNA degradation was extensively studied in the nuclei of livers from rats treated with naringenin, morin (Sahu and Gray 1997), and myricetin (Sahu and Gray 1993). The effective oxygen scavengers, superoxide dismutase, mannitol, and catalase, had a lower effect on DNA damaged by naringenin and morin, while mannitol moderately hindered the peroxidation of nuclear covering. Also, naringenin, morin, and myricetin have the

possible capacity to damage components of the nuclear protective system, such as glutathione-S-transferase and glutathione (Sahu and Gray 1996).

### 3 Flavonoids as Topoisomerase Inhibitors

As many flavonoids, e.g., myricetin, genistein, equol, biochanin A, and quercetin, have an action on topoisomerase II blockage at low concentrations, they have cytotoxic potential that is similar to the blocking of epipodophyllotoxins, which are extensively employed as anticancer agents (Austin et al. 1992; Chang et al. 1995; Azuma et al. 1995). These topoisomerase II inhibitors cause the accumulation of cleavable complexes, i.e., enzyme-DNA covalent intermediates, which may cause lesions in double-stranded DNA at the required region of topoisomerase. For instance MLL gene involved in translocations of the chromosome in the victim having minor blood cancer successive powerful drug to cure the disease along with topoisomerase II inhibitors (adriamycin and etoposide) (Dassonneville and Bailly 1998). Consequently, a high intake of a flavonoid-rich diet by a pregnant mother could lead to infant leukemia.

The chemical processing of flavonoids in the body engages various pathways, which are the main factors in determining possible DNA topoisomerase II lessening action. Toxicological investigations must be carried out to determine whether neonatal genistein management could possibly alter the genetic material engaged with the MLL gene. Broad arrays of flavonoid-enriched foods and tablets are available in retail shops (Espin et al. 2007) and preclinical observations in mammals have shown that foods enriched with polyphenols have desirable outcomes as they are provided in large quantities (Thomasset et al. 2007). Additionally, a large number of people take nutritional flavonoids along with trace elements or conventional drugs (Cermak 2008). This affiliated ingestion of supra-nutritional flavonoids along with ordinary drugs has triggered scientific discussion about flavonoid drug consumption (Cermak 2008). Nearly 380,000,00 individuals in the United States (18.9% of the population) consume flavonoids, along with other polyphenols, in plant-derived supplements, but only one-third of them report their use of these supplements to their doctor (Kennedy et al. 2008). The lack of herbal formulations that have fewer or no adverse effects of various components present in crude form enhances the probability of diseases being caused by the intake of these nutritional compounds. The threat of these types of interactions may create toxic exposure to pharmaceutical drugs and medication malfunction. Pharmacotoxicity and treatment failure concerns can be overcome by initiating and discussing the route of drug administration, which may further guide quicker clearance.

The pharmacokinetic effects of phytochemical trace compounds (epigallocatechin; EGC), silibinin, rutin, and quercetin) in relation to possible detrimental effects due to their long-term use were investigated after single oral measurements in rats. For the trial, there were five groups of rats; controls were given olive oil and each of the other four groups was given one of the polyphenols, EGC, silibinin, rutin, or



quercetin. Evaluated the long-term use of EGC, silibinin, rutin, and quercetin on the ingestion and tissue transportation of copper, zinc, and iron after single oral measurements in the rats. On day 30, solutions of copper, iron, and zinc sulfate were given orally to the rats; after 3 h, blood samples, kidney sections, and liver and brain samples were acquired for the determination of the amounts of these elements. The findings revealed that, in contrast to the control, the polyphenols facilitated improvements in both the tissue and serum concentrations of these compounds. The outcomes were comparatively diverse due to the structural variations among the flavonoids. However, decreases in vital compounds (copper, zinc) and in the performance of the associated biological catalysts were observed with the excessive intake of flavonoids.

## 4 Flavonoids and the Gastrointestinal Tract

### 4.1 *Diarrhea*

*Glycyrrhiza glabra* root contains flavonoids that have been exploited for their effects on gastric function in traditional medicinal systems. A dose-related gastrointestinal effect was found to be due to isoliquiritigenin present in liquorice root (Chen et al. 2009). Flavopiridol, a cyclin-dependent kinase inhibitor, is a semisynthetic flavone analogous to rohitukine, which is found in the extracts of an Indian tree. Flavopiridol is one of the few flavonoids that has undergone a number of toxicological studies and it is used in the treatment of chronic lymphocytic leukemia. The dose-limiting toxicity observed with this flavone is associated with severe diarrhea. It has the ability to modify chloride secretory responses of the human colonic epithelial cell line T84. It also has a direct stimulatory effect on chloride secretion, which is likely due to an increase in cyclic adenosine monophosphate (Kahn et al. 2001).

### 4.2 *Colitis*

Though flavonoids have been reported to have anti-inflammatory effects, they have also been found to induce inflammation in the alimentary canal (Thiolet et al. 2003; Karrasch et al. 2007). Daflon, a modified hesperidin A micronized purified flavonoid, has been reported to cause lymphocytic colitis (Menecier et al. 1999). Similar studies by Rassiat et al. (2001) demonstrated that Cirkan, a dietary supplement, also caused lymphocytic colitis.



## 5 Flavonoids and Hepatic Side Effects

Most flavonoids are metabolized in the liver, and the liver plays a critical role in various physiological and pathophysiological processes in many diseases. Many properties of flavonoids have been highlighted as hepatoprotective, but the adverse effects of these agents on the liver need to be understood. Nowadays, the use of alternative and complementary medicines is becoming more widespread, but there is no guarantee of their safety, and the adverse effects of flavonoids on the liver are more prevalent in populations that consume these medicines.

### 5.1 *Hepatotoxicity and Tea*

The literature suggests that many important hepatic reactions occur when the leaves of *Camellia sinensis*, i.e., black, green, and oolong tea, are consumed (Mazzanti et al. 2009). Numerous clinical cases have demonstrated cholestasis, hepatic necrosis, and hepatitis with a high intake of flavonoids. The levels of various liver biomarkers, such as alanine aminotransferase (ALT), bilirubin transaminases, alkaline phosphatase (ALP), and bilirubin were found to be altered with high intakes of flavonoids. In several cases, the reactions were found to be reversed when the use of these products was minimized. As suspected products cause adverse reactions in the body, certain agencies have provided nutraceutical vigilance (García-Cortés et al. 2008; Menniti-Ippolito et al. 2008).

Toxicity in the liver and oxidative stress subsequent to the consumption of flavonoids was found to be associated with some of these compounds and their metabolites, viz., EGC, EGC-3-gallate, epicatechin-3-gallate, and epicatechin gallate (Galati et al. 2006). Various signs of green tea toxicity were reported in hepatocytes; findings showed potential mitochondrial membrane collapse, glutathione depletion, the formation of reactive oxygen species, and the formation of a conjugate of EGC, EGC-glutathione (Galati et al. 2006).

A dose-dependent effect in hepatocytes treated with an extract of green tea at concentrations >1000 µg/ml showed necrosis and leakage of lactate dehydrogenase. However, the concentrations of these flavonoids in the human liver after the consumption of green tea have not been elucidated (Takami et al. 2008). It has been found that concentrated extract of green tea, when consumed on an empty stomach, can cause significantly higher levels of adverse effects in comparison to effects in the fed state (Sarma et al. 2008).

## **5.2 *Dietary Estrogens and Liver Disease***

Phyto Soya, *Glycine max*(L.) which is generally rich in the flavonoid phytoestrogens genistein and daidzein, was found to increase ALT, aspartate transaminase, ALP, and gamma-glutamyl transpeptidase levels, and it induced cytolytic hepatitis (Borghesi-Scoazec et al. 2002).

## **5.3 *Isoflavones and Hepatocellular Carcinoma***

The levels of daidzein and genistein, and their risk for the development of hepatocellular carcinoma, were studied in Japanese men and women and showed a greater risk in Japanese women as compared with males (Kurahashi et al. 2009).

## **5.4 *Flavonoids and the Kidney***

Jaeger et al. (1979a, b) first reported acute renal failure with cyanidanol. Similar cases of such failure were also reported for flavonoid consumption resulting from *Taxus celebica* and cyanidanol use (Jaeger et al. 1980; Heim et al. 1982; Rotoli et al. 1985; Lin and Ho 1994). The consumption of small or large doses of flavonoids may lead to signs and symptoms such as fever, digestive system upset, jaundice, choloria, hemolysis, cholestatic hepatitis, and proteinuria.

# **6 Flavonoids and Blood Disorders**

## **6.1 *Hemolytic Anemia***

Various types of blood disorders, e.g., immune hemolytic anemia and thrombocytopenia, have been found to be associated with cyanidanol (Rotoli et al. 1985; Gandolfo et al. 1992). Cyanidanol binds to erythrocyte membranes and has been found to be responsible for the development of autoantibodies and other types of antibodies (Salama and Mueller-Eckhardt 1987).

## 6.2 Safe Flavonoid Intake

The increased consumption of vegetables, fruits, and soy products has decreased the possibility of heart diseases and various types of cancers – in the prostate, breast, lung, stomach, and colon (Tajima and Tominaga 1985; Severson et al. 1989; Koo 1988; Lee et al. 1991; Garcia-Closas et al. 1999). In Asian populations, the daily consumption of flavonols, i.e., 68 mg, and isoflavones, i.e., 20–240 mg, is generally good for health. However, in the human population overall, the effects of excessive use of these supplements have not yet been elucidated. The cytotoxicity and mutations triggered by flavonoids may not occur through dietary sources. The use of herbal mixtures and antioxidant formulas with flavonoids in gram doses, rather than in milligram doses, may lead to toxicity. Therefore, the indefinite use of flavonoids or their mixtures, which is important from the commercial point of view, can have various effects on human health.

## 7 Conclusion

Although flavonoids have a number of beneficial properties that have been exploited by the food and pharmaceutical industries, there is a need to explore safe levels of these agents in the diet for improving human health. The consumption of vegetables, beverages, and various fruits containing flavonoids is recommended, but it is still too early to make decisions on the recommended daily intake of these agents.

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# Chapter 8

## Role of Nanotechnology in Flavonoid-Mediated Anticancer Therapy



Saumya Srivastava and Anjana Pandey

### 1 Introduction

Herbs can be defined as any form of plant comprising stem, leaves, roots, or flowers having medicinal value. For exploiting the herbaceous properties of these plants, either they are used as raw or in the form of extract. Plant extracts can be prepared by softening of plant materials in different types of solvents including water, alcohol, or other solvents providing large source of phytochemicals like flavonoids, saponins, alkaloids, fatty acids, etc. (Bent 2008).

Flavonoids are polyphenolic compounds having a phenyl benzopyrone structure. These phytochemicals can be categorized into flavones, flavonols, flavanones, and chalcones. This categorization has been built up on the basis of their pattern of C ring substitution and central pyran ring opening (Middleton et al. 2000). The diverse structural patterns exhibited by these flavonoids help them to be predicted as potential anticancer compounds.

In new studies, to deal with the different types of cancer, viz., ovarian, breast, prostate, etc., flavonoids and their similar compounds have been intensely explored. Some flavonoids, viz., quercetin, flavopiridol, or genistein, have been used in clinical trials during late phase for various oncogenic signs (Ferry et al. 1996; Lin et al. 2006; Lazarevic et al. 2011). Several protein kinases which are involved in cancer pathology, for example, epidermal growth factor receptors (EGFRs), platelet-derived growth factor receptors (PDGFRs), and cyclin-dependent kinases (CDKs), have been found to be modulated by flavonoids (Singh and Agarwal 2006). These phytochemicals also play a significant role in enzyme inhibition, such as COX

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(cyclooxygenase) and LOX (lipoxygenase) that are associated with the cancer and inflammatory pathologies. Another chemoprevention strategy exhibited by flavonoids involves the phase I metabolizing enzyme inhibition, i.e., cytochrome P450, etc., and induction metabolizing enzymes of phase II such as GST, quinone reductase, etc., which are involved in the activation of pro-carcinogens (Tsyrov et al. 1994) and carcinogen metabolism, respectively (Bu-Abbas et al. 1998; Sun et al. 2010). Flavonoids show anticancer property by scavenging the reactive oxygen species (ROS) and growth stimulating oxidants that are mainly responsible for tumor formation showing the anticancer. These features highlight the flavonoids' potential to be developed as antiproliferative agents.

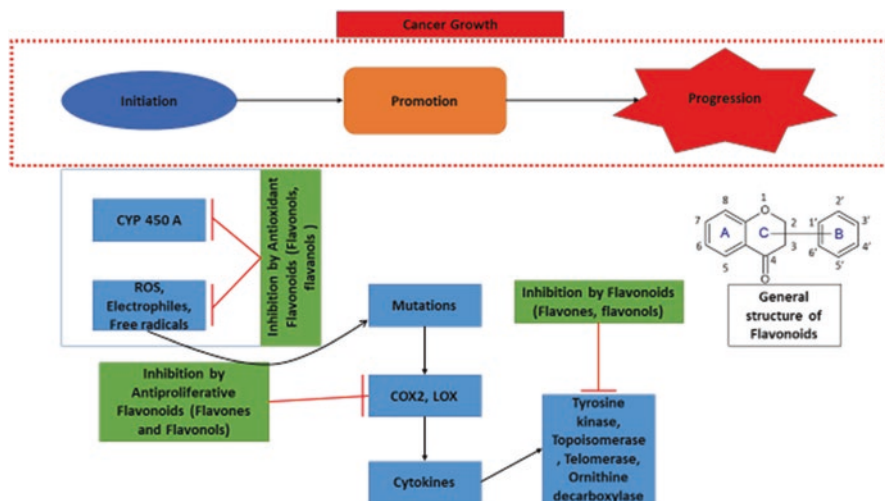
In past few years, the development of novel drug delivery systems for herbal drugs has withdrawn significant amount of attention. These drug carries target the specific part of body/organ for disease treatment. These drug delivery systems are capable of delivering the drugs in a regulated way, thus enhancing the drug bioavailability (Sharma 2014; Biju et al. 2006). Amalgamation of these novel drug delivery system (NDDS) technology and bioactive materials from plant sources results in reduction of drug degradation and toxicity (Sharma 2014). Numerous NDDS including liposomes, nanosomes, microspheres, and phytosomes have been studied for different herbal drugs. NDDS helps to increase stability, solubility, and enhanced pharmacological activity (Yadav et al. 2011).

## 2 Flavonoids in Anticancer Therapy

### 2.1 Protein Kinase Inhibition

Protein kinases (PKs) are the enzymes that catalyze the phosphorylation of different substrates resulting in regulation of several processes occurring in cells. They are the vital component of various cellular functions and under stringent regulation of homeostasis system. PKs can be deregulated under diseased situations resulting in uncontrolled cell division (Shchemelinin et al. 2006) which leads to causation of fatal diseases such as cancer, diabetes, etc. (Lapenna and Giordano 2009; Wagner and Nebreda 2009). Therefore, PKs inhibition during cancer treatment has become a potential therapeutic approach. Flavonoids have been found to inhibit several protein kinases including protein tyrosine kinases (PTKs), protein kinase C (PKC), cyclin-dependent kinases (CDKs), etc. In an in vitro experimental design conducted to estimate p40 protein tyrosine kinase inhibition, it was found that polyhydroxylated flavonols and flavones inhibit PTK activity with higher affinity than other classes of flavonoids due to their ring structure (see Fig. 8.1). Further this also suggests that the double bond formation among C2 and C3 and C2 of ring B has increased the inhibitory ability of flavonoids for PTK inhibition (Geahlen et al. 1989). For the inhibition of PKC, flavonols such as fisetin, myricetin, and quercetin have been found to display maximum inhibitory action. Existence of hydroxyl groups at C3, C4, and C7 and a coplanar structure of flavones are obligatory for the





**Fig. 8.1** Mode of action of different classes of flavonoids (*CYP450A* cytochrome P 450 A, *ROS* reactive oxygen species, *COX2* cyclooxygenase 2, *LOX* lipoxygenase)

inhibition of PKC (Gamet-Payraastre et al. 1999; Ferriola et al. 1989). Likewise, experiments have reported that the C2–C3 and C4-oxo double bond and existence of hydroxyl groups at C3 and C4 places of flavonoids help them inhibit the CDK activity. 3'-OH group at B ring of luteolin and quercetin is associated with the G1 block, whereas its absence in apigenin and kaempferol leads to a G2 block (Casagrande and Darbon 2001).

## 2.2 Topoisomerase Inhibition

Topoisomerases maintain the DNA topology at the time of replication, transcription, and recombination processes. The potential drug candidates exhibiting the property of inhibition or interference of these processes are called chemotherapeutic agents (Topcu et al. 2008). Due to their ability to form a planar, conjugated A-C ring system, flavones and flavonols are able to inhibit topoisomerase I.

## 2.3 Antiangiogenic Activity

Flavonoids show antiangiogenic activity by regulating the vascular endothelial growth factor (VEGF) and endothelial growth factor receptor (EGFR) expressions and by inhibition of ERK  $\frac{1}{2}$  and PI3-K/Akt signaling pathways (Mojzisa et al. 2008). Both quercetin and luteolin have been found to suppress the phosphorylation of VEGFR 2 and respective downstream processes (Pratheeshkumar et al. 2012a, b). Genistein is

found to show antiangiogenic activity in different studies by inhibiting the VEGF-induced cell activation (Yu et al. 2012) and proliferation processes (Piao et al. 2006).

## 2.4 Antioxidative Activity

The protective mechanism of flavonoids also includes the antioxidative effects. They prevent the tissue injury from free radicals by directly scavenging (Procházková et al. 2011) them and also inhibit oxidases such as cyclooxygenase, lipoxygenase, microsomal monooxygenase, etc. which produce the superoxide anions (Cos et al. 1998; Heim et al. 2002). Free iron and copper are mainly responsible for the production of reactive oxygen species. Flavonoids chelate these free ions resulting in inhibition of free radical development.

## 3 Flavonoid-Based Anticancer Formulations

Even though flavonoids have tremendous health benefits, the pharmacokinetics of these compounds need improvement after their administration for therapeutic purposes. They have less water solubility and lower bioavailability and can be effortlessly altered due to effects such as pH, temperature, etc. Flavonoids have complex gastrointestinal absorption mechanism due to susceptibility of being degraded by microorganisms or enzymes present in the gut. As a result, flavonoids get poor bioavailability (Bilia et al. 2014). These problems can be addressed by the use of nanocarriers that are found to be useful for enhancing the bioavailability and efficacy of flavonoids because nanocarriers have the potential to increase the solubilization potential and check the degradation in the gastrointestinal tract due to metabolic changes. These nano-sized drug carriers lie in the range 10–1000 nm and are commonly divided into polymer and lipid-based systems. It imparts significant impact on the absorption profile of the loaded particles due to its particle size and surface properties that help in the uptake in the gastrointestinal mucosa. The ideal size of nanocarriers for increasing the uptake across the gastrointestinal tract is 50–300 nm (Roger et al. 2010). Nanocarriers have the ability to increase the solubility and mucoadhesion and to interact with tight junction proteins and lymphatic absorption that play very important role in elevating the absorption by the enterocytes (Thanki et al. 2013).

## 4 Effects of Nanoformulations on Bioavailability of Drug

**Nanosuspensions** Nanosuspensions are a type of nanotechnology design having the drug which has poor water solubility without any suspended form of matrix material. The problem of poor solubility of some drugs such as itraconazole, simvastatin,

and carbamazepine in aqueous and nonaqueous environment is very high. To avoid this problem, use of nanosuspensions has become a promising approach. The compounds with higher melting points, dose, and large logP values are the most suitable combinations for using this approach (Dongsheng et al. 2011).

Cui et al. (2009) conducted a study for increasing in vivo bioavailability and demonstrated nanosuspensions that have shown 6.1 and 5.0 times rise in the maximum concentration (C<sub>max</sub>) and area under curve (AUC<sub>0 → 12</sub>) value, respectively, than commercial tablets in rats. Nanosuspensions of dried itraconazole (ITZ) have increased the bioavailability by 1.5- to 1.8-fold than commercial products.

**Nanoemulsions** Nanoemulsions are shear-induced ruptured nanoscale droplet dispersions. This formulation is oil-in-water (o/w) type, having the mean droplet size of 50–100 nm. It has greater commercial importance than lyotropic microemulsions due to exploiting less surfactant in its formulation and more kinetic stability (Mason et al. 2006).

Ramipril nanoemulsions made up of sefsol 218, carbitol, oil, surfactant, Tween 80, co-surfactant, and standard buffer solution increased the absorption 2.94 times as compared to conventional tablets in geriatric and pediatric patients (Shafiq et al. 2007).

Self-emulsifying drug delivery system (SEDDS) is a good methodology to elevate the drug bioavailability. Upon in vivo testing studies, it has been found that optimized formulation increased the AUC and C<sub>max</sub> of CoQ10 (Coenzyme Q10) in comparison with powdered formulation. Hence, it can be said that SEDDS are effective for bioavailability improvement of lipophilic drug (Balakrishnan et al. 2009).

After the use of self-nanoemulsifying drug delivery system (SNEDDS), it has become the recent approach nowadays consisting of 100 nm size range of globule. In animal studies, zedoary turmeric oil (ZTO) SNEDDS for oral delivery to rats has increased both AUC and C<sub>max</sub> values by 1.7- and 2.5-fold, respectively, of germacrone (GM) which is a biomarker of ZTO in comparison with unformulated ZTO (Rao and Shao 2008).

**Phytosomes** The pharmacokinetic studies of silybin-phosphatidylcholine complex were performed at a dosage of 80 mg (silybin equivalent) in healthy individuals. The peak values of free and conjugated drug concentrations were obtained at 2.4 and 3.8 h, respectively, whereas half-life of silybin in both free and conjugated state was observed to be 1.6 and 3.4 h, respectively (Savio et al. 1998). In case of soft gelatin dosage of silybin-phosphatidylcholine complex (80 mg), the C<sub>max</sub> and AUC were elevated by two- and threefold, respectively, as compared to the gelatin capsule.

Another study analyzed the pharmacokinetic effects of hesperetin on phospholipid complex in a noncompartmental model (Maiti et al. 2009). In the study the male albino Wistar rats were alienated into two groups, i.e., oral administration of free and complex drug at a dosage of 100 mg/kg of hesperetin equivalents. The

phospholipid complex was observed to have increased absorption of hesperetin along with a sustained release of the drug with increase in both  $C_{max}$  and  $T_{max}$  (Time at which  $C_{max}$  is observed) values. The increase in AUC of hesperetin was also found in complex as compared to the free form.

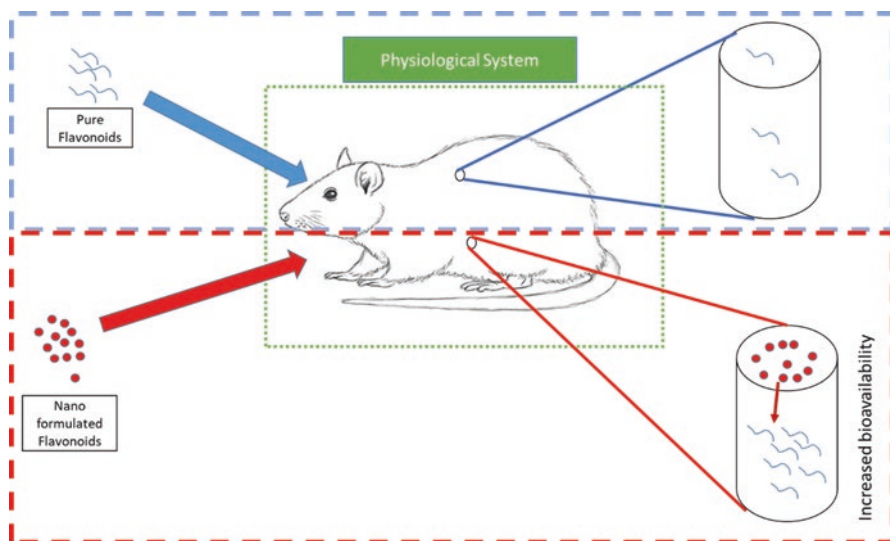
**Solid Lipid Nanoparticles (SLNs)** In another study the pharmacokinetics of quercetin encapsulated into SLNs was analyzed on rats after administration of quercetin (50 mg/kg body weight) orally in both the forms i.e. SLNs and suspension. The results exhibited that the relative bioavailability of quercetin in SLNs was increased by 571.4% in comparison to quercetin suspension along with enhancement in both  $T_{max}$  and mean residence time of quercetin in serum/plasma, thus suggesting that SLNs have the potential to act as effective drug delivery systems and to elevate the absorption of drugs that are poorly soluble (Li et al. 2009).

The pharmacokinetics and bioavailability of ellagic acid (EA), a poorly water-soluble phytochemical, were observed to be enhanced in phospholipid complex of the drug. EA at a dosage of 80 mg/kg and EA phosphatidylcholine (80 mg/kg body weight dose equivalents) complex were given orally to the test animals. The peak serum concentrations of the drug were observed to be 0.21 mg/mL at 0.5 h in case of EA and 0.54 mg/mL at 2 h in case of complex, thus suggesting the enhanced bioavailability of the drug in complex form along with increase in the  $C_{max}$  and  $T_{max}$  and half-life values (Mukherjee et al. 2015).

Similar studies have been performed by other researches on different flavonoids in different dosage forms including micelles (Dian et al. 2014), encapsulation in polymeric nanoparticles, metallic nanoparticles, nanospheres, etc., where in each case the drug bioavailability along with increase in  $C_{max}$  and AUC was observed as summarized in Fig. 8.2. Different flavonoid-based nanoformulations and their mode of action have been summarized in Table 8.1.

## 5 Conclusion and Future Prospects

The anticancer and antitumor activity of various flavonoids have been well studied and established by a number of researchers, and the mode of action of flavonoids in their anticancer activity is well studied. Also the nanoencapsulation/nano-based dosage forms are known to improve the drug kinetics in the physiological systems, thus leading to increased  $C_{max}$  and area under curve (AUC) values. All these values lead to enhanced bioavailability of the drug. Hence the efficacy and bioavailability of the flavonoids can be enhanced by nanoformulations. There is a need to study the effects of these nanoformulated flavonoid dosage forms in both animal models and in humans in vivo.



**Fig. 8.2** Effects of nanoformulations on drug bioavailability

**Table 8.1** Different flavonoids in nanoformulations and their mode of action

S. no.	Compound	Formulation type	Mode of action	References
1.	Cyanidin	Nanoencapsulation	Regulation of p53-mediated apoptosis in mice	Liu et al. (2018)
2.	Apigenin	Carbon nanopowder solid dispersion	Modulation of cell cycle, induction of apoptosis	Yan et al. (2017) and Ding et al. (2014)
3.	Fisetin	Nanoemulsion	Induction of apoptosis	Ragelle et al. (2012) and Lee et al. (2002)
4.	Kaempferol	Nanoparticles	Modulation of metabolic pathways	García-Mediavilla et al. (2007) and Luo et al. (2012)
5.	Luteolin	Nanoparticles	Regulation of p53 pathway, induction of apoptosis	Majumdar et al. (2014) and Lin et al. (2008)
6.	Naringenin	Nanoemulsion	Suppression of free radicals	Mir and Tiku (2015) and Khan et al. (2015)

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# Chapter 9

## Flavonoids as Potential Anticancer Agents in Clinics: Where Have We Reached So Far?



Balbir Singh, Hasandeep Singh, Davinder Singh, Amrit Pal Singh, Harpal Singh Buttar, and Saroj Arora

### 1 Introduction

Cancer is one of the leading causes of mortality, and every sixth death globally is due to cancer. About 8.9 million people died from different types of cancer in 2016. According to a recent report, the majority of deaths in cancer patients are predominantly due to cancer of the prostate, colorectum, breast, and lungs (Siegel et al. 2017). Even with the progress in the discovery of novel anticancer medications, cancer is still the leading cause of mortality worldwide (May 2014; Siegel et al. 2017). There is an increased incidence of cancer along with the unwanted side effects of chemotherapeutic agents. This has enforced the scientists to explore the future anticancer agents from natural sources. Notably, plant-derived anticancer agents have gained attention because of their low toxicity and better therapeutic efficacy (Pan et al. 2013). The plant-derived drugs have diverse mechanisms of action, but most of them cause apoptotic cell death by caspase or p53-dependent as well as p53-independent mechanisms. In addition, plant-derived drugs exhibit their anticancer activity through certain novel mechanisms such as autophagy, mitotic catastrophe, and senescence leading to cell death and necrosis-like programmed cell death (Gali-Muhtasib et al. 2015).

Plants synthesize a wide array of chemical compounds like flavonoids, alkaloids, glycosides, terpenoids, etc. Many of these compounds are produced by plant as

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secondary metabolites, which help the plants to respond against various environmental stimuli and stresses as well as genetically programmed developmental signals. It is estimated that more than 50% of modern pharmaceuticals have originated from the plant sources. In the recent times, there is an increasing contemplation in the scientific community about the importance of phytomedicines, phytochemistry, and pharmacological investigations of natural health products and diets for treating noncommunicable diseases, especially cancer, type 2 diabetes, obesity, cardiovascular, and neurodegenerative disorders. Partly, this is due to the realization that in folklore medicine, herbal remedies have been used effectively for treating different ailments. At present, there exists data from an overwhelming number of in vitro and in vivo studies showing beneficial effects of plant-based extracts and their bioactive ingredients. Many clinical studies with isolated ingredients from plants have revealed multiple health benefits in boosting immune function, anti-inflammation, antimicrobial, and antioxidant activities.

Flavonoids are polyphenolic substances, which are widely found in fruits, vegetables, and certain beverages. They are associated with various therapeutic activities and are present in various nutraceutical, pharmaceutical, medicinal, and cosmetic preparations. The basic structure of flavonoid contains flavan nucleus having 15 carbon atoms arranged in three rings ( $C_6-C_3-C_6$ ). The various classes of flavonoids exist as flavones (e.g., apigenin and kaempferol), flavanones (e.g., hesperetin and fisetin), catechins (e.g., catechin and epigallocatechin gallate), and anthocyanins (e.g., cyanidin and delphinidin). The basic nucleus of flavonoid and its various subtypes is given in Fig. 9.1. In addition, the various food sources of various types of flavonoids are summarized in Table 9.1.

## 2 Flavonoids as Pharmacological Agents

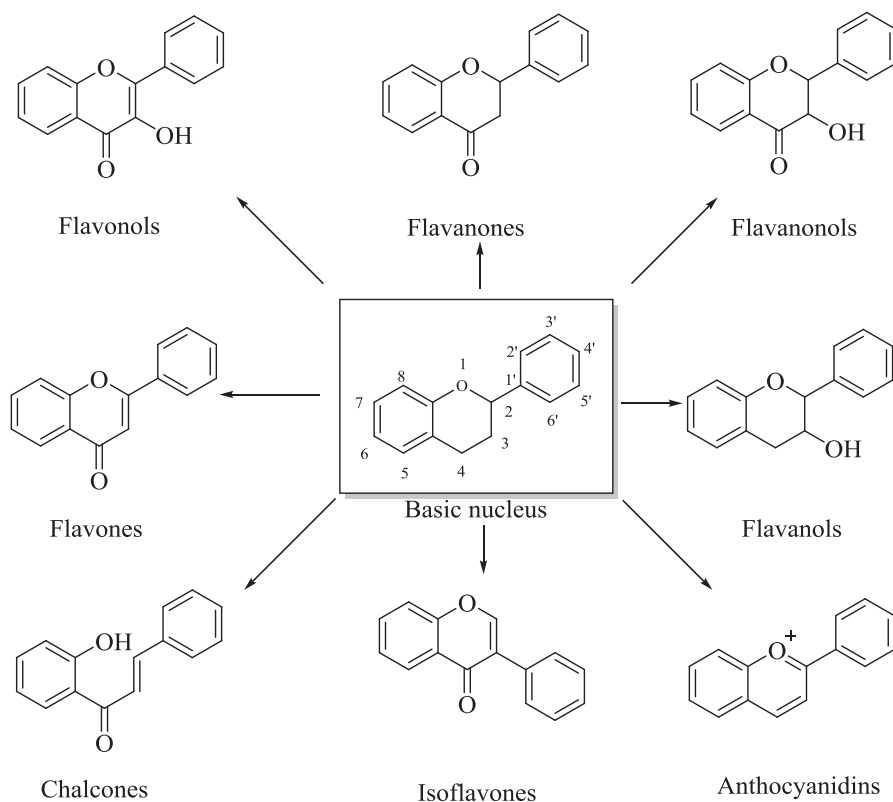
Several types of flavonoids, flavanols, flavones, and flavanonols isolated from plants, vegetables, and fruits have shown multifarious biological activities, such as antioxidant, anti-inflammatory, antidiabetic, cardioprotective, as well as anticancer activity. Various pharmacological activities of flavonoids are depicted in Fig. 9.2.

## 3 Flavonoids as Anticancer Agents

### 3.1 Flavanols

#### 3.1.1 Myricetin

Myricetin is a phenolic compound isolated from *Myrica nagi* Thunb. bark belonging to family Myricaceae (Lau-Cam and Chan 1973). It is found mostly in vegetables, berries, wines, and teas prepared from different plants mainly from families



**Fig. 9.1** Chemical structures of various types of flavonoids

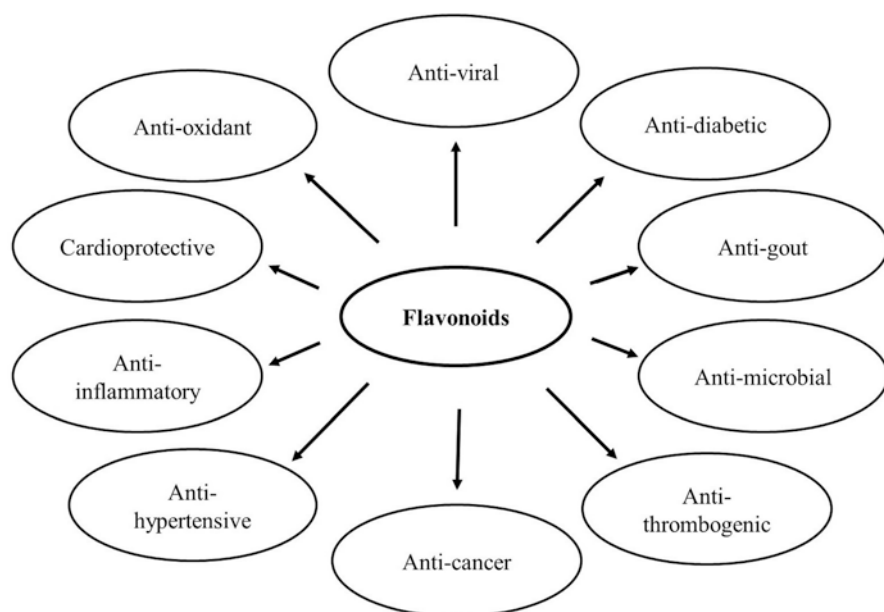
Primulaceae (Chua et al. 2011), Polygonaceae (El-Kader et al. 2013), Myricaceae (Jones et al. 2011), Pinaceae (Hergert 1956), and Anacardiaceae (Umadevi et al. 1988). It is 3,5,7-trihydroxy-2-(3,4,5-trihydroxyphenyl)-4-chromenone and occurs in free and bound form such as myricetin-3-O-(4''-acetyl)- $\alpha$ -L-arabinopyranoside, myricetin-3-O- $\beta$ -D-xylopyranoside, myricetin-3-O-(3''-O-galloyl)- $\alpha$ -L-rhamnoside, etc. (De Leo et al. 2006; Kong et al. 2014). Myricetin exhibits its anti-cancer activity against several types of cancers. Myricetin affects Akt signaling in EGF-induced cell transformation by competing with ATP and thereby inhibits the expression of Akt. Thus, it is demonstrated that myricetin is an inhibitor of Akt which is overexpressed in the cancer cells. Moreover, it inhibits the cancer cell growth by inhibiting their entry into mitotic phase by targeting the kinase activity of cyclin B/CDK1 complexes. Studies suggest its antimetabolic potential in treating liver cancer and its apoptotic cell death-promoting activity in various cell lines. In addition, myricetin targets the tumor metastasis and angiogenesis mechanisms by targeting several proteins including MMP-9, MMP-13, VEGF, and HIF-1 (Devi et al. 2015).

**Table 9.1** Food sources of different flavonoids

Groups	Compounds	Food sources
Flavanols	Isorhamnetin Kaempferol Myricetin Quercetin Quercetagenin	Apple, black grapes, blueberry, broccoli, cherry, curly kale, green and black tea, leek, tomato, yellow onion
Flavones	Apigenin Chrysin Diosmetin Heptamethoxyflavone Nobiletin Luteolin Quercetagenin Sinensetin Tangeretin Tricetin	Capsicum pepper, celery, parsley
Flavanones	Dihydrofisetin Dihydroquercetin Eriodictyol Hesperetin Naringenin Dihydrobinetin	Grapefruit juice, lemon juice, orange juice
Flavanols	Pinobanksin Silibinin Silymarin Taxifolin	Chocolates, cocoa beverages, cocoa
Catechins (proanthocyanidins)	(–) Epicatechin (+) Catechin Epicatechin-3-gallate Epigallocatechin Epigallocatechin-3-gallate Gallocatechin	Apricot, beans, black tea, blackberry, cherry, chocolate, cider, grapes, green tea, peach, red wine
Isoflavones	Daidzein Genistein Glycitein	Soy bean, soy cheese, soy flour, tofu
Anthocyanins	Cyanidin Delphinidin Malvidin Pelargonidin Peonidin Petunidin	Black grapes, blackcurrant, blue berry, cherry, plum, red cabbage, red wine, rhubarb, strawberry

### 3.1.2 Quercetin

Quercetin (3,3,4,5,7-pentahydroxy flavanone) is a unique bioflavonoid found abundantly in fruits and vegetables such as grapes, tomatoes, *Brassica* vegetables, onions, and tea (Häkkinen et al. 1999; USDA 2011). Reports suggest that quercetin in combination with many naturally occurring compounds such as luteolin



**Fig. 9.2** Illustration of multifarious biological activities of flavonoids

derivatives, resveratrol, 2-methoxyestradiol, ellagic acid, and synthetic drugs like cisplatin and doxorubicin resulted in synergistic anticancer activity (Akagi et al. 1995; Mertens-Talcott and Percival 2005; Nessa et al. 2011; Wang et al. 2012; Yang et al. 2015).

Quercetin modulates the cell signaling by inhibiting the high-mobility group box protein 1 (HMGB1)-induced TNF- $\alpha$  and IL-1 $\beta$  expression, which further regulates activity of various pro-inflammatory cytokines (Degryse et al. 2001). Furthermore, quercetin considerably inhibits the degradation of I $\kappa$ B $\alpha$  and nuclear translocation of NF- $\kappa$ B which is important for cytokine expression (Park et al. 2004; Korkkola et al. 2005). Quercetin has been demonstrated to prevent metastasis of breast cancer cells through suppression of matrix metalloproteinase-9 (MMP-9) in 12-O-tetradecanoyl phorbol-13-acetate (TPA)-treated MCF-7 cells (Lin et al. 2008). Oral administration of quercetin encapsulated with tamoxifen in PLGA nanoparticles significantly increased its bioavailability and attenuated breast cancer cell growth through induction of apoptosis (Jain et al. 2013).

### 3.1.3 Resveratrol

Resveratrol (3,5,4'-trihydroxystilbene) is a naturally occurring polyphenol belonging to stilbenes. It is found mostly in peanuts, berries, grapes, and plant sources and also in red wine (Bielsalski 2007). In plants, resveratrol exists in two isomeric

forms, i.e., *trans*-resveratrol and *cis*-resveratrol, and their glucosides, *trans*-piceid and *cis*-piceid. The anticancer potential of resveratrol was first published in 1997 (Jang et al. 1997).

Research reports suggest that resveratrol downregulates the  $K_{ras}$  expression, prevents the formation and progression of colorectal tumors, and increases the expression of miR-96 (Saud et al. 2014). Furthermore, it also modulates the mitomycin C-mediated effects of colorectal cancer by inhibiting cell growth and upregulating p21 which blocks cell cycle at G0/G1 and G2/M phases (Ali and Braun 2014). It further regulates the metabolism of glucose and regulates GLUT1 in ovarian cancer cell lines. Resveratrol suppresses glucose uptake and inhibits plasma membrane GLUT1 localization linked with the inhibition of the activity of Akt in ovarian cancer cell lines (Gwak et al. 2015).

In a clinical trial study, Patel and his colleagues demonstrated that in colon cancer patients, resveratrol at dose levels of 0.5 and 1.0 g reduces tumor cell proliferation by 5% (Patel et al. 2010). In an another study, Brown et al. showed that resveratrol causes a decrease in circulating insulin-like growth factor (IGF)-I and IGF-binding proteins (IGFBP)-3 in healthy volunteers (Brown et al. 2010). This study demonstrated that resveratrol may affect the IGF axis probably by direct effect on IGF-I and IGFBP-3. These proteins may also serve as potential markers in chemopreventive efficacy in human clinical trials (Jogie-Brahim et al. 2009) (Fig. 9.3).

## 3.2 Flavones

### 3.2.1 Luteolin

Luteolin or 3',4',5,7-tetrahydroxyflavone is a flavonoid present in various fruits, vegetables, as well as medicinal herbs. Traditional Chinese medicine has used luteolin-rich herbs as anti-inflammatory and anticancer agent. These biological effects of luteolin are attributed to antioxidant or pro-oxidant activity (Lin et al. 2008). Luteolin has been noted to kill various types of cancer cells including leukemia, pancreatic tumor, hepatoma, and lung carcinoma (Huang et al. 1999; Lee et al. 2002, 2005; Cheng et al. 2005). Luteolin has been demonstrated to serve as anticancer agent by inhibiting cell proliferation, metastasis, angiogenesis, and induction of

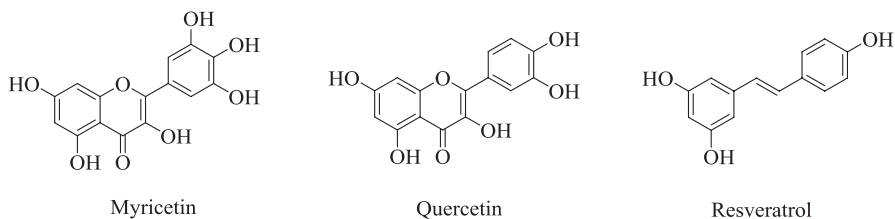


Fig. 9.3 Chemical structure

apoptosis. Luteolin promotes cytotoxicity in cancer cells by suppressing survival mechanisms such as PI3K/Akt pathway and stimulating the tumor suppressor p53 signaling (Han et al. 2002). Luteolin suppressed the cancer stem cell properties and their metastatic potential in prostate cancer cells (Tsai et al. 2016). Luteolin inhibits the human cytochrome P450 (CYP) 1 enzymes including CYP1A1, CYP1A2, and CYP1B1, which further suppress the activation of carcinogens (Kim et al. 2005). In vascular smooth muscle cells, luteolin inhibited the PDGF-mediated proliferation of endothelial cells and consequently inhibited the PDGF-induced activation of ERK, PI3K/Akt, and PLC-1 along with reduction of c-fos gene expression (Kim et al. 2005). Moreover, luteolin promotes JNK-mediated apoptosis by modulating bad or p53 pathways (Yu et al. 2004; Ju et al. 2007). Notably, JNK-mediated p53 activation governs the expression of Bax, which further regulates apoptosis (Yu et al. 2004). Luteolin has been demonstrated to induce endoplasmic reticulum stress and mitochondrial dysfunction which leads to apoptosis in glioblastoma (Wang et al. 2017). Luteolin treatment induced G<sub>0</sub>/G<sub>1</sub> phase arrest in SMMC-7721 hepatocarcinoma cell line. Luteolin promoted autophagy by increasing number of intracellular autophagosomes in cancer cells. Interestingly, chloroquine, an autophagy inhibitor, attenuated the anticancer effect of luteolin in hepatocarcinoma cell line (Cao et al. 2017). Another novel mechanism suggested for anticancer potential of luteolin is blockage of ribosomal S6 kinase (RSK). RSK is ERK regulated and is responsible for cell growth and its survival. Luteolin treatment blocked RSK-1 and demonstrated marked anticancer potential in MOLM-13 and Kasumi-1 leukemic cells (Deng et al. 2017). Luteolin inhibited the incidence rate of tumors and decreased tumor volume in 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary carcinogenesis in rats (Samy et al. 2006). In another study, luteolin reduced DMBA-induced lung carcinogenesis in mice (Kasala et al. 2016). An early phase I clinical trial is under process to examine whether luteolin and nano-luteolin exert an inhibitory effect on tongue squamous cell carcinoma cell lines by inducing apoptosis and to assess if nano-luteolin has more efficient apoptotic activity than luteolin on tongue squamous cell carcinoma cell line (<https://clinicaltrials.gov/ct2/show/NCT03288298>).

### 3.2.2 Diosmetin

Diosmetin (3',5,7-trihydroxy-4'-methoxyflavone) is the aglycone part of the flavonoid glycoside diosmin (3', 5, 7-trihydroxy-4'-methoxyflavone-7-aminoglycoside) which occurs naturally in the genus *Teucrium* (Lamiaceae) and in Portuguese olive leaves (Meirinhos et al. 2005; Macedonia 2005; Spanakis et al. 2009). Intestinal microflora enzymes hydrolyze diosmin to its aglycone diosmetin before its absorption into the body (Kanaze et al. 2004). Pharmacologically, it has been established that diosmetin possesses different medicinal properties such as antimicrobial, antioxidant, anti-inflammatory, as well as anticancer activities (Chandler et al. 2010; Domínguez et al. 2011; Zhao et al. 2011). In a study, diosmetin is identified as a CYP1 substrate (Androutsopoulos et al. 2009a). CYP1A1 is one of the cytochrome

P450 enzymes, which has been extensively examined for its capacity to activate compounds having carcinogenic potential. The exposure to environmental carcinogens is noted to increase the level of CYP1A1 expression through aryl hydrocarbon receptors. Diosmetin treatment inhibited cell proliferation of the human breast adenocarcinoma MCF-7 cells which were pre-induced with the potent CYP1 inducer 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Androutsopoulos et al. 2009a). Diosmetin inhibited the proliferation and progression of cell cycle in MDA-MB 468 cells by affecting CYP1 enzyme, whereas it had no aversive effect on normal breast cell lines MCF-10A. This shows its safety of use over other synthetic drugs. Diosmetin is also reported to induce G1 arrest in MDA-MB-468 cell lines. Interestingly, it is proclaimed that the diosmetin is metabolized to similar flavone luteolin in MDA-MB-468 breast cancer cell lines selectively through aromatic demethylation of the B ring by CYP1A1, CYP1B1, and the hepatic enzyme CYP1A2, which is not seen in MCF-7A cells (Androutsopoulos et al. 2009b).

### 3.2.3 Nobiletin

Nobiletin (5,6,7,8,3', 4'-hexamethoxyflavone) is a major component of *Citrus depressa* and is noted to exhibit anticancer activity in various in vitro and in vivo studies. Literature reveals that nobiletin inhibits the proliferation of skin, breast, prostate, and colon carcinoma cell lines (Kandaswami et al. 1991). It inhibits the production of matrix metalloproteinases which results in antiproliferative activity (Ishiwa et al. 2000). Nobiletin inhibits the invasion of human fibrosarcoma HT-1080 cells by suppressing the metalloproteinases and activating TIMP-1 production. Nobiletin inhibits the phosphorylation of mitogen-activated protein/extracellular signal-regulated kinase-1/2 (MEK-1/2). Notably, U0126, a MEK1/2 inhibitor, imitated the nobiletin's action to reduce ability to decrease 12-O-tetradecanoyl phorbol-13-acetate (TPA)-stimulated production of proMMPs-1 and proMMPs-9 in human fibrosarcoma HT-1080 cells (Miyata et al. 2004). Moreover, in TPA-treated HT-1080 cells, nobiletin assisted the phosphorylation of c-Jun NH<sub>2</sub>-terminal kinase (JNK), which is an important downstream signal factor of the PI3K/Akt pathway. Among 40 different flavonoids, nobiletin showed the maximum antiproliferative activity in six human cancer cell lines (Murakami et al. 2000; Manthey and Guthrie 2002). It suppresses the prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) production and cyclooxygenase-2 expression in in vitro studies (Kohno et al. 2001). Studies demonstrated that administration of nobiletin-rich *C. Reticulata* peel extract for 1 year exhibits preventive effects on the progression of the cognitive impairment in donepezil-pre-administered Alzheimer disease patients without any side effects. Unfortunately, the research on nobiletin clinical application is quite limited, which might be due to the uncertainty of molecular targets. More clinical trials of nobiletin and its metabolites are still needed.



### 3.3 Flavanones

#### 3.3.1 Hesperidin

Hesperidin (5,7,3'-trihydroxy-4'-methoxy-flavanone 7-rhamnoglucoside) belongs to the class of flavonoids called flavanones and is predominantly found in citrus fruits. Hesperidin has been noted to possess a diverse range of pharmacological activity attributing to its anti-inflammatory and antioxidant potential. In the last few years, hesperidin has gained attention of cancer biologists, and it has been screened extensively *in vitro* and *in vivo* for its antimutagenic and anticancer properties. In the endometrial cancer cells, hesperidin induced apoptosis by increasing Bax and decreasing Bcl<sub>2</sub> and promoted cell death by downregulating estrogen receptor I (Cincin et al. 2018). Hesperidin has been noted to mitigate the migration and invasion of A549 cancer cells by inhibiting SDF-1/CXCR-4 cascade (Xia et al. 2018b). Hesperidin has been demonstrated to suppress azoxymethane-induced colon carcinogenesis in rats (Tanaka et al. 1997). Interestingly, hesperidin administration along with doxorubicin has been reported to increase later's anticancer activity along with reduction in its side effects in Ehrlich ascites carcinoma-bearing mice (Donia et al. 2018). Hesperidin treatment demonstrated anticancer activity by inducing endoplasmic reticulum stress and G<sub>0</sub>/G<sub>1</sub> arrest in ovarian cancer cell line and A549 lung cancer cell line, respectively (Zhao et al. 2017; Xia et al. 2018a). Hesperidin attenuated diethylnitrosamine/carbon tetrachloride-induced hepatocarcinogenesis in rats through activation of PPAR- $\gamma$  and Nrf-2/ARE/HO-1 signaling (Mahmoud et al. 2017). Notably, hesperidin demonstrated better cytotoxic activity against human hepatic cancer HepG2 cell line than other flavonoids such as neohesperidin, naringin, and naringenin. Moreover, hesperidin has been noted to induce apoptosis in HepG2 cells through mitochondrial as well as death receptor pathway (Banjerdpongchai et al. 2016). Hesperidin has been noted to upregulate tumor suppressor phosphatase and tensin homologue (PTEN) and reduce the expression of PI3K/Akt survival pathway in azoxymethane-induced colon carcinoma in mouse. Moreover, hesperidin-mediated restoration of glycogen beta-synthase-3 attenuated the proto-oncogenes such as c-jun, c-myc, and  $\beta$ -catenin, thereby resulting in anti-cancer activity in colon cells (Saiprasad et al. 2014) (Figs. 9.4 and 9.5).

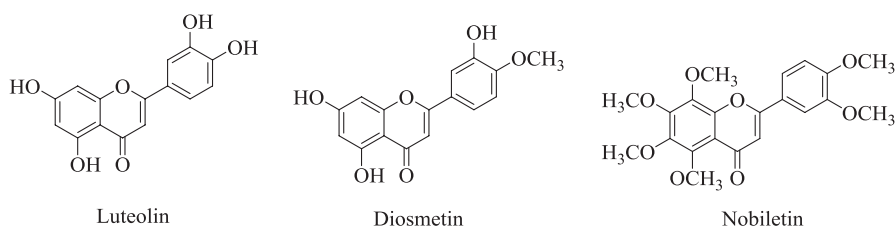
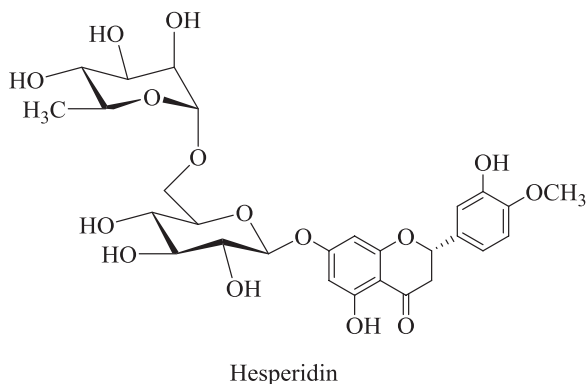


Fig. 9.4 Chemical structure

**Fig. 9.5** Chemical structure



### 3.4 Flavan-3-Ols

#### 3.4.1 Epigallocatechin-3-Gallate

Green tea is extracted from the leaves of evergreen shrub *Camellia sinensis* and is almost consumed all over the world (Yang et al. 2009). Green tea mainly contains polyphenols and catechins such as epigallocatechin-3-gallate (EGCG), epicatechin-3-gallate, epigallocatechin (EGC), and epicatechin (EC). Among all the above catechins, epigallocatechin-3-gallate (EGCG) possesses powerful anticancer activity due to its antioxidative potential (Katiyar and Mukhtar 1996). EGCG is the ester form of epigallocatechin and gallic acid. EGCG has also been reported to have beneficial effects in the treatment of neurodegenerative diseases (Hügel and Jackson 2012), cardiovascular diseases (Tipoe et al. 2007), cancer (Schramm 2013), diabetes (Thielecke and Boschmann 2009), and liver diseases (Xiao et al. 2014).

EGCG inhibits tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced lung tumorigenesis by inhibiting 8-hydroxydeoxyguanosine formation (Xu et al. 1992). Furthermore, it inhibits dimethylarsinic acid and cisplatin-induced lung tumorigenesis in rodent models (Mimoto et al. 2000; An et al. 2008) and diethylnitrosamine-induced liver tumorigenesis by inhibiting insulin-like growth factor signaling in diabetic and obese C57BL/KsJ-db/db mice (Shimizu et al. 2011). EGCG inhibits angiogenesis and tumor growth in human pancreatic cancer and breast cancer by downregulating VEGF expression both in serum-deprived HT29 human colon cancer cells and in vivo (Jung et al. 2011; Shankar et al. 2013; Braicu et al. 2013). Moreover, EGCG inhibits invasion and metastasis in hypopharyngeal carcinoma cells by downregulating hepatocyte growth factor (HGF)-induced MMP-9 as well as activation of urokinase-type plasminogen activator (uPA) (Lim et al. 2008).

The consumption of green tea exerts beneficial effects even after a single dose. The levels of prostaglandin E2 (stimulates colorectal carcinogenesis) in tissue were reduced in normal subjects after 4 h of green tea consumption (August et al. 1999). The derivatives of green tea have shown effectiveness against various malignancies such as cervical, hepatic, and prostate, without toxicity in patients with premalignant conditions (Ahn et al. 2003; Bettuzzi et al. 2006; Luo et al. 2006). However, in a phase

2 clinical trial of China, there is no marked effect observed in biomarkers of esophageal squamous carcinogenesis from decaffeinated green tea (Wang et al. 2002).

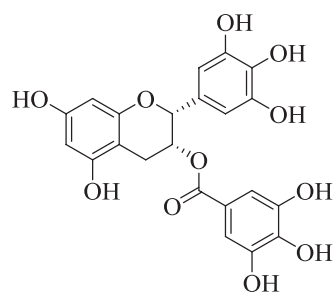
In a single-arm trial testing, the effectiveness of EGCG against radiation-induced dermatitis in the patients of breast cancer demonstrated promising results. Topical application of EGCG reduces the pain in 85.7%, itching in 87.8%, tenderness in 79.6%, and burning feel in 89.8% patients, who underwent radiotherapy (Zhu et al. 2016). In a phase II clinical trial, EGCG treatment ameliorated the acute radiation-induced esophagitis (ARIE) in patients with stage III lung cancer. ARIE is one of the dose-dependent toxicities complicated by thoracic radiotherapy (Zhao et al. 2015). The supplementation of green extract having high concentration of EGCG for 12 months showed no observed change in the mammographic density in all post-menopausal women, but a marked reduction in percent mammographic density (PMD) was observed in 50–55-year-old women suggesting the effectiveness of green tea supplementation in preventing breast cancer (Samavat et al. 2017). The use of EGCG in bladder cancer in patients has demonstrated prosperous result in phase II clinical trial (Gee et al. 2017) (Fig. 9.6).

### 3.4.2 Pomegranate-Derived Polyphenols

*Punica granatum* is a small tree of family Punicaceae, commonly known as pomegranate. The fruits of the plant are used in many cultures. Of note, the name *Punica* has been derived from the Roman name of city Carthage, where best pomegranates have been known to grow. This tree is native to Persia but is now cultivated in America, Mediterranean area, and Asia. A class of tannins known as punicalagins unique to pomegranates has been demonstrated to possess excellent free radical scavenging properties (Gil et al. 2000; Noda et al. 2002).

Scientific reports suggest the potential role of pomegranate in the prevention as well as treating various types of cancer such as skin cancer, lung cancer, breast cancer, and prostate cancer because of its antioxidant nutrients. It slows down the propagation of cancer cells and accelerates their death. It also diminishes the blood supply to tumors and makes them smaller by starvation (Adhami et al. 2009).

**Fig. 9.6** Chemical structure



Epigallocatechin-3-gallate

Polyphenols obtained from the fermented juice and pericarp of pomegranate inhibit the proliferation and invasion of cells by inhibiting the secretory phospholipase (Lansky et al. 2005; Seeram et al. 2007; Espín et al. 2007). Standardized pomegranate extract having ellagitannins and ellagic acid suppresses the expression of androgen receptor through the inhibition of androgen-synthesizing enzymes. Moreover, pomegranate juice or extract inhibits the CYP enzyme, induces apoptosis and inhibits tumor growth, and decreases the serum PSA levels (Malik et al. 2005; Espín et al. 2007; Rettig et al. 2008; Paller et al. 2013). In skin cancer, it protects the fibroblasts from cell death and facilitates the skin repair (Aslam et al. 2006; Pacheco-Palencia et al. 2008; Hayouni et al. 2011). It also inhibits the skin edema, hyperplasia, and leukocytic infiltration induced by UV-B (Afaq et al. 2010; Khan et al. 2011).

In a human study, drinking of 8 oz. pomegranate juice per day increased the amount of time it took for their prostate-specific antigen (PSA) to double in patients who had surgery or radiation therapy for treating prostate cancer. Notably, the patients doubling PSA levels a short period of time have more risk of getting prostate cancer. Daily consumption of pomegranate juice increased the time of PSA levels to double from about 15 months to 54 months (Hajleh and Al-Dujali 2016).

## 3.5 Isoflavones

### 3.5.1 Genistein

Genistein (4',5,7-trihydroxyisoflavone) was originally isolated from *Genista tinctoria* Linn. (Dyer's broom) in 1899. It is predominant isoflavone of soy products (Perkin and Newbury 1899). Genistein structurally resembles estrogen, and therefore isoflavones have also been known as phytoestrogens. Genistein can thus bind to estrogen receptors due to structural similarity (Kuiper et al. 1997). It is documented that genistein can inhibit the growth of various cell lines such as prostate, leukemia, lymphoma, breast, lung, head, and neck cancer cells both in vitro and in vivo (Taylor et al. 2009). Various studies have reported the role of genistein as anticancer in every step of tumor progression. Genistein has been noted to attenuate the growth of cancer cells by inhibiting PTK-mediated signaling pathways (Akiyama et al. 1987; Sakla et al. 2007). It also exerts its inhibitory effect on all steps of cancer progress through apoptosis and cell cycle arrest, regulating the AKT/IKK/NF- $\kappa$ B, androgen mediated and other signaling pathways in the development of carcinogenesis. Studies showed that genistein modulates the expression of genes that regulates cell cycle and growth and thereby inhibits progression of cancer (Pavese et al. 2010).

Genistein is an isoflavone, which means its B ring is attached to the heterocyclic ring at the C3 position instead of C2 (Jacob, Hagai and Soliman 2011). It is a prominently found in soy products, (Herman et al. 1995; Barnes 1995). It inhibits cancer cell growth and induces apoptosis by modulating the expression of genes related to apoptotic pathways and inhibits Akt activation and NF- $\kappa$ B in cancer cells (Li et al. 1999; Davis et al. 1999). Genistein inhibits the invasive potential of human prostate cancer cell lines which suggest that it could inhibit the metastatic growth of

prostate cancer (Santibanez et al. 1997). The *in vitro* studies using microarray shown that the genistein regulates the expression of genes involved in angiogenesis, cell cycle, cell growth, cell signal transduction, metastasis, and tumor cell invasion (Li and Sarkar 2002). In targeting the breast cancer, genistein possesses higher affinity toward ER $\beta$  subunit of estrogen receptor (ER) comparable to other isoflavones. This is attributed to the presence of a phenolic hydroxyl group, which is required for the formation of an intramolecular hydrogen bonding. The low concentrations of genistein (EC<sub>50</sub> 4 nM) overexpress gene expression and reduce proliferation more efficiently when ER $\beta$  is present. At higher doses, it stimulates the proliferation of MCF7/ER $\alpha$  cells which is counted as bad effects. At the end of 30-day clinical trial on adults, early-stage breast cancer patients (mainly HER2-negative and ER-positive), those with soy supplementation and high plasma genistein, had overexpression of tyrosine kinase receptor FGFR2 and other genes regulating proliferation pathways and cell cycle (Shike et al. 2014).

Genistein inhibited the HER2 expression, phosphorylation, and promoter activity through ER-independent manner (Sakla et al. 2007). MDA-MB-231 cell lines treated with varying concentrations (5–10–20  $\mu$ M) of genistein demonstrated induction of apoptosis and G2/M cell cycle arrest in a dose as well as time-dependent manner. This effect is due to genistein inhibition of NF $\kappa$ B through NOTCH-1 signaling, which affects Bcl-2 and Bcl-xl expression as a consequence of NF $\kappa$ B inhibition (Pan et al. 2012). In a phase 2 chemoprevention trial on bladder cancer, the daily oral dose of genistein (300 mg/day and 600 mg/day) for 14–21 days before the surgery targets p-EGFR (endothelial growth factor receptor). The difference between p-EGFR staining of placebo arm and genistein arm is significantly different in 300 mg/day group but not in 600 mg/day (Messing et al. 2012). Genistein displayed a possible bimodal effect on bladder cancer tissue EGFR phosphorylation. Phase 2 studies of genistein were conducted in patients with prostate cancer. In this study, before undergoing radical prostatectomy for localized prostate cancer, patients were randomized to treatment with 2 mg genistein per kg of body weight versus no treatment (Xu et al. 2009). Normal prostate epithelial cells were excised selectively from prostate tissue by laser capture microdissection after the treatment, and these cells represent an “at-risk” target-type cells which are decent target for compound which arrests the conversion to an invasive phenotype. The qRT-PCR used to measure levels of MMT-2 transcript demonstrated that genistein reduced the MMT-2 gene expression to 24% of the level observed in control subjects. This study establishes the possibility of inhibiting prometastatic processes through a targeted therapeutic intervention in human subjects (Xu et al. 2009). In another phase 2 trial, patients having progressive prostate cancer when treated with soy milk for 12 months 3 times a day reduced the rise in PSA antigen as compared to its increase in patients before entering the study. Moreover, in a third phase 2 trial of genistein, men with prostate cancer were administered soy extract for 6 months, and it was concluded that the therapy was well tolerated with less than 10% patients experiencing mild diarrhea, and in 17% of patients, there was reduction of PSA levels (deVere White et al. 2004; Pendleton et al. 2008). Administration of genistein has been noted to influence various genes responsible for cell proliferation in randomized double-blind clinical trial of patients with localized prostate cancer (Bilir et al. 2017).

Moreover, treatment with AXP107-11 (the crystalline form of genistein) in phase I trial of pancreatic cancer patients in combination with gemcitabine demonstrated a favorable pharmacokinetic profile along with its increased bioavailability without any toxicity (Lohr et al. 2016) (Fig. 9.7).

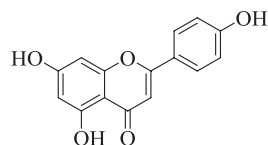
## 3.6 Anthocyanins

### 3.6.1 Cyanidin

Anthocyanins are widely distributed in human diets and are used for food color, suggesting that we ingest the considerable amount of anthocyanins from plant-based daily diets. In six different tumor cell lines (K562, PC3, HT-29, M-14, MCF-7, and DU145), it effectively halted the growth of cancer cells at lower  $GI_{50}$  concentrations than quercetin (Murphy et al. 2003). Cyanidin significantly attenuated zymosan-mediated inflammation in rodents. It suppressed the peritoneal exudates, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) interleukin-1  $\beta$  (IL-1 $\beta$ ) and IL-6, and cytokine-induced neutrophil chemoattractant-1 protein (CINC-1) levels (Tsuda et al. 2002). The zymosan elevated serum- $\alpha_2$ -macroglobulin, and decrease in serum albumin and transferrin level was corrected by cyanidin in vivo. Ingestion of cyanidin-3-glucoside (C3G) in Apc<sup>Min</sup> mice reduced the intestinal adenomas in a dose-dependent manner (Cooke et al. 2006). Total C3G concentration in mice was 43 ng/g and 8.1  $\mu$ g/g tissue, respectively, in the intestinal mucosa and 7.2 and 12.3  $\mu$ g/ml in the urine (Cooke et al. 2006). In a <sup>13</sup>C-tracer clinical trial, total eight participants consumed 500 mg isotopically labeled C3G (6,8,10,3',5'-<sup>13</sup>C<sub>5</sub>-C3G). The maximal elimination rate of C3G is seen after 6–24 h in feces while minimal in blood after 30 min. Although several studies have been done with cyanidin, yet very few have been conducted for anticancer activities (Fig. 9.8).

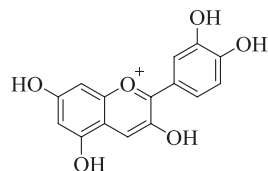
The data of various flavonoids which are clinically tested in various types of cancer patients are discussed in Table 9.2.

**Fig. 9.7** Chemical structure



Genistein

**Fig. 9.8** Chemical structure



Cyanidin

**Table 9.2** Clinically tested flavonoids in cancer patients

Flavonoid	Patient	Study design	Intervention	Effect	References
Resveratrol	11 (colon cancer)	Phase 1	Plant-derived resveratrol tablet at a dose of 80 mg/kg and 20 mg/kg and grape powder at a dose of 120 mg/kg and 80 mg/kg	Inhibition of Wnt signaling pathway	Nguyen et al. (2009)
Epigallocatechin-3-gallate	50 (prostate cancer)	Phase 1	Dietary supplementation of defined green tea catechin extract	Favorable changes in serum prostate-specific antigen, serum insulin-like growth factor axis, and oxidative DNA damage in blood leukocytes	Nguyen et al. (2011)
Epigallocatechin-3-gallate	98 (cervical intraepithelial neoplasia)	Phase 2	Dietary supplementation of defined green tea catechin extract	Safe and acceptable for long exposure but no positive response in treated groups	Garcia et al. (2014)
Genistein	60 (bladder cancer)	Phase 2	300 mg/day and 600 mg/day oral dose of genistein for 30 days	Lower doses suppress EGFR phosphorylation	Messing et al. (2012)
Genistein	30 (healthy postmenopausal women)	Phase 1	600 mg/day genistein capsules	Safer for postmenopausal women up to 900 mg/day	Pop et al. (2008)
Cyanidine-3-glucoside	8 (healthy volunteers)	Interventional	Isotopically labeled 500 mg of cyanidine-3-glucoside	Are more bioavailable than previously perceived	Czank et al. (2013)
Resveratrol	40 (healthy volunteers)	Phase 1	0.5, 1.0, 2.5, and 5.0 g daily for 29 days	Suppress insulin-like growth factor and insulin-like growth factor binding protein-3	Brown et al. (2010)
Lycopene	18 (prostatic intraepithelial neoplasia)	Phase 1	Tomato extract containing 30 mg/day lycopene	No any difference between treatment and placebo groups was seen	Gann et al. (2015)
Silibin	12 (prostate cancer)	Phase 2	13 g/daily in three divided doses	Low half-life of silibin in blood, silibin lacks tissue penetration	Flaig et al. (2010)

## 4 Conclusion

So far, tremendous information has been gathered by various studies exploring the role of flavonoids as potential anticancer agents in laboratories. The prosperous findings of cell lines and preclinical studies compelled the clinicians to further take up the flavonoids in clinical trials. In human trials, the flavonoids have demonstrated prosperous results. In addition, their supplementation reduced chemotherapy- and radiotherapy-induced complications in cancer patients. However, most of these trials are single centric and enrolled relatively small number of patients. The validity of flavonoids as potential anticancer agents is yet to be proven in multicentric trials involving large number of patients. In conclusion, the outcome of clinical studies is promising and presents flavonoids as potential anticancer agents.

**Conflict of Interest** The authors state no conflict of interest.

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