Tarun Belwal
Milen I. Georgiev
Jameel M Al-Khayri *Editors*

# Nutraceuticals Production from Plant Cell Factory

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Editors

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*Editors*
Tarun Belwal
Biosystems Engineering and Food Science
Zhejiang University
Hangzhou, China

Milen I. Georgiev
Institute of Microbiology
Bulgarian Academy of Sciences
Plovdiv, Bulgaria

Jameel M Al-Khayri
Agricultural Biotechnology
King Faisal University
Al-Hassa, Saudi Arabia

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# Preface

Plant cells have been effectively utilized over the past few decades to produce valuable natural bioactive compounds under artificial conditions. Nutraceutical compounds, which encompass nutrients and pharmaceuticals, are gaining higher market demands due to their health-promoting properties, added value to food products, and mitigation potential of various diseases. Considering their high demand and the limitations of natural resources, biotechnological tools based on cell culture techniques provide effective means of scaling up the production of these natural products. However, considering the complexity of cell types, genetic factors, and targeted nutraceutical compounds, the optimum cell culture conditions vary and hence require empirical determination.

The purpose of this book is to highlight the in vitro techniques, current status, and challenges of producing nutraceutical compounds. In addition, it provides an overview of different biosynthesis pathways and their modulation through cell culture technique for the production of nutraceutical compounds in high quantity and quality. The book also emphasizes assessment of the factors influencing production and advances in cell culture techniques, including scale-up approach using bioreactors. Overall, this book will provide the current status, methods, research, advances, and challenges of in vitro production of nutraceutical compounds along with recommendations for future research.

The book comprises different parts, namely theory and technology, in vitro production of nutraceutical compounds, and strategic advances and challenges. The theory and technology part covers the general description of nutraceutical compounds, plant cell culture technology, bioreactors, and factors affecting in vitro production of nutraceuticals. The in vitro production of nutraceutical compounds part mainly deals with the in vitro production of important nutraceutical compounds, namely polyphenols, alkaloids, coumarins, terpenoids, anthocyanins, carotenoids, saponins, steroids, tocopherols, phytosterols, and quinones. The last part of the book covers the strategic advances and challenges, comprises chapters dealing with optimization strategies for in vitro nutraceutical production, genetic engineering, and microbial cell factory for nutraceutical production, and highlights the challenges of in vitro nutraceutical production.

The book is an excellent reference source for researchers working in the area of in vitro biosynthesis of nutraceutical compounds, food science, plant biotechnology, nutraceutical research, and pharmacological activities. Also, it will be useful for industries working on plant biotechnology, especially the in vitro biosynthesis of nutraceutical compounds.

The editors appreciate chapter authors for their contributions towards the success and quality of this book, which represents the efforts of 56 scientists from five countries. We are also grateful to Springer for giving us an opportunity to compile this book.

Hangzhou, China — Tarun Belwal
Plovdiv, Bulgaria — Milen I. Georgiev
Al-Ahsa, Saudi Arabia — Jameel M. Al-Khayri

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# Part I

# Theory and Technology

# Nutraceutical Compounds, Classification, Biosynthesis, and Function

1

Hari Prasad Devkota

**Abstract**

In recent years, there is an increasing interest on the plant-based nutraceuticals, functional foods, and food supplements as potential agents for the maintenance of good health and the prevention and treatment of diseases. Phytochemicals, especially the polyphenols including flavonoids, phenolic acids, curcuminoids, and stilbinoids, are widely studied for their health beneficial properties. Among many other issues, one important issue is the continuous supply of active components in nutraceuticals to meet the market demand. As many phytochemicals present in nutraceuticals are specific to certain plant species, the conservation, cultivation, and sustainable utilization are equally important. Newer biotechnological tools such as tissue and cell culture have potential to provide the necessary amount of the specific nutraceutical compounds in future. For wider application, understanding their chemical classification, biosynthetic routes, potential health beneficial activities, and market trends must be well understood. This chapter focuses mainly on the classification of these compounds, their biosynthesis in plants and role in human health.

**Keywords**

Nutraceuticals · Functional foods · Phytochemicals · Polyphenols · Classification · Bioactivity

H. P. Devkota (✉)
Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan
e-mail: devkotah@kumamoto-u.ac.jp



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## 1.1 Introduction

Medicinal plants have been used in primary healthcare by more than 80% of the world's population (Fitzgerald et al. 2019; Devkota and Watanabe 2020), and plant-derived natural products have also played an important role in the discovery and development of modern drugs (Atanasov et al. 2015; Newman and Cragg 2020). Besides that, medicinal plants are also widely used as foods, spices, food supplements, food preservatives, food colorants, and sweeteners and also in cosmetics, aroma, and perfumes (Pawar et al. 2013; Sarkic and Stappen 2018; Negi 2012; Voon et al. 2012). In recent years, there is an ever-increasing interest on plant-based nutraceuticals, functional foods, and food supplements as potential agents in the maintenance of good health and the prevention and treatment of diseases (Gul et al. 2016; Martin et al. 2011; Tsao 2010). Phytochemicals, especially the polyphenols including flavonoids, phenolic acids, curcuminoids, and stilbinoids, are widely studied for their health beneficial properties (Tsao 2010; Lin et al. 2018; Shahidi and Ambigaipalan 2015; Yeung et al. 2019).

Various epidemiological studies have shown the important role of diet in the maintenance of healthy state of body and mind (Shahidi 2004). Foods rich in plant-based products including fruits and vegetables are well known for their health benefits. Evidences show that their high intake reduces the risk of metabolic diseases, cardiovascular problems, and cancers, which has contributed to the increased scientific and public interest in diet and their impact on health and diseases (Shahidi 2004; Day et al. 2009). Foods, nowadays, are consumed not only to satisfy hunger and to get nutrients but also to maintain and protect physical and mental health. Growing public awareness toward healthy lifestyle, expensive healthcare cost, increasing life expectancy, and the desire for improved health and better lifestyle by older people, all these factors have increased the market demand for health beneficial foods such as nutraceuticals and functional foods (Bigliardi and Galati 2013). Considering the already known evidences about the relationship between food and human health, researchers including food and nutrition scientists, health professionals, and food manufacturing companies are now focusing on the development of nutraceuticals that can fulfill the market demand (Bigliardi and Galati 2013).

In recent years, there are many nutraceuticals and related products available in the market, which are intended to use for the improvement of the health; however, the long-term goal of these products is the prevention of chronic diseases and hence increasing the healthy life span. Nutraceuticals are the food or food-derived products with health beneficial properties and are often formulated as capsules, tablet, powder, and solution. The term "nutraceutical" was defined by Stephen DeFelice, in 1989, and consists of two words "nutrition" and "pharmaceutical." Hence, nutraceutical can be defined as the products obtained from food or food itself which can be used for the treatment as well as for the prevention of diseases (Shahidi 2004; Andrew and Izzo 2017; Tapas et al. 2008). Nutraceuticals cover a wide range of natural products such as fortified products, functional foods, food supplements, etc. (Andrew and Izzo 2017). On the other hand, functional foods are defined as foods with additional health promoting functions and provide health beneficial effects beside nutrition and calories only (Day et al. 2009). The term "functional food" was first used in Japan in the 1980s to define the food products fortified with special constituents that contain advantageous physiological effects (Siró et al. 2008). There are now hundreds of functional foods in market that can be categorized under different groups, such as food to promote gut health, food to promote tooth and gum health, food to facilitate mineral absorption, food to promote bone strength, food for those who are concerned about hypertension, food for those who are concerned about blood sugar level, food for those who are concerned about blood cholesterol level, food for those who are concerned about body fat accumulation, etc. Most of the functional foods available in the market are probiotics, prebiotics, functional drinks, functional cereals, oat bran fiber, bakery products, spreads, functional meat, soy protein, fish oil fatty acids, and functional eggs (Day et al. 2009; Bigliardi and Galati 2013; Siró et al. 2008). Some examples of emerging nutraceuticals and functional foods from plants source are tea, flaxseed, tomato, soybean, citrus fruits, berries, garlic, grapes and wines, cruciferous vegetables, and their active constituents. Phytochemicals and other functional components from plant source are the potential candidates in the field of nutraceuticals. They provide high opportunity of minimizing the borderline between the food and medicine.

There is continuous rise in the development and launching of nutraceuticals in the market; however, there are many challenges to overcome. One of the most important issues is the scientific evidences for the claimed health benefits of these products, where the actual pharmacokinetic and pharmacodynamics properties of the active compounds in nutraceuticals are not well defined in many cases. Other important issues are the consumers' acceptance, the changing market trend, and the increasing trend of aging population. The long-term effects of these nutraceuticals are not always well studied. As nutraceuticals incorporate various bioactive ingredients, the processing and stability of the formulations is very important (Day et al. 2009). Among many other issues for raw materials, one important issue is the continuous supply of active components in nutraceuticals to meet the market demand. As many phytochemicals present in nutraceuticals are specific to certain plant species, the conservation, cultivation, and sustainable utilization are equally important. Newer biotechnological tools such as tissue and cell culture have the potential to provide the necessary amount of specific nutraceutical compounds in the future. For wider application, understanding their chemical classification, biosynthetic routes, potential health beneficial activities, and market trends is necessary. This chapter focuses mainly on the classification of these compounds, their biosynthesis in plants, and their role in human health.

## 1.2 Current Trend of Nutraceutical Research and Nutraceutical Market

Nutritional supplement provides adequate amounts of essential nutrients vital for the proper functioning of human bodies. They include vitamins, proteins, herbs, meal supplements, sports nutrition, and other related products. They are consumed to maintain health and provide support to the immune system and to reduce the risk of illness. The global nutritional supplement market is expected to expand at a CAGR of 10.01%, leading to global revenue of USD 245.43 Bn by 2023. Asia-Pacific is the fastest growing region for the nutritional supplement market due to the growing demand in China, Japan, and India (Research and Markets 2018). Another research showed that the global nutraceuticals market was valued at 252,535.4 million USD in 2018 and is projected to grow to 465,709.8 million USD by 2027 having CAGR of 7.1% (Nutraceuticals Market 2019). North America was reported to be the largest market which was mainly driven due to increased prevalence of lifestyle-related diseases (Nutraceuticals Market 2019). Similarly, the global market of raw or processed foods rich in bioactive phytochemicals such as dried fruit was valued at USD 8.94 billion in 2019 and is expected to witness significant growth in the forthcoming years. There is growing market for nutritious packaged food and wide application of dried fruit in the dairy, bakery, snack, and confectionery industries (Market Research 2020). With increasing life expectancy, aging population, and change in lifestyles, the market of nutraceuticals will grow more in the future.

## 1.3 Classification of Plant-derived Nutraceuticals

Most of the nutraceutical components in the market are derived from plants and their active constituents such as polyphenols, amino acids, peptides and derivatives, carotenoids, alkaloids, phytosteroids, triterpenoids and related compounds, non-starchy carbohydrates, etc. (Abuajah et al. 2015). In many cases, these compounds are present in various forms such as glycosylated, esterified, thiolated, and hydroxylated derivatives. They can be classified into various classes based on its chemical structure and functional activities. Some of the major classes of nutraceutical compounds are explained in following sections.

### 1.3.1 Polyphenols

Polyphenolic compounds are among the largest groups of plant secondary metabolites that are made of one or more aromatic rings with one or more hydroxyl groups. They are also among the most widely studied phytochemicals for their various pharmacological activities. Polyphenolic compounds can be classified into different groups such as phenolic acids, flavonoids, tannins, coumarins, stilbenes, curcuminoids, xanthones, and lignans, among others, based on their structures. The basic classification of polyphenolic compounds is represented in Fig. 1.1.

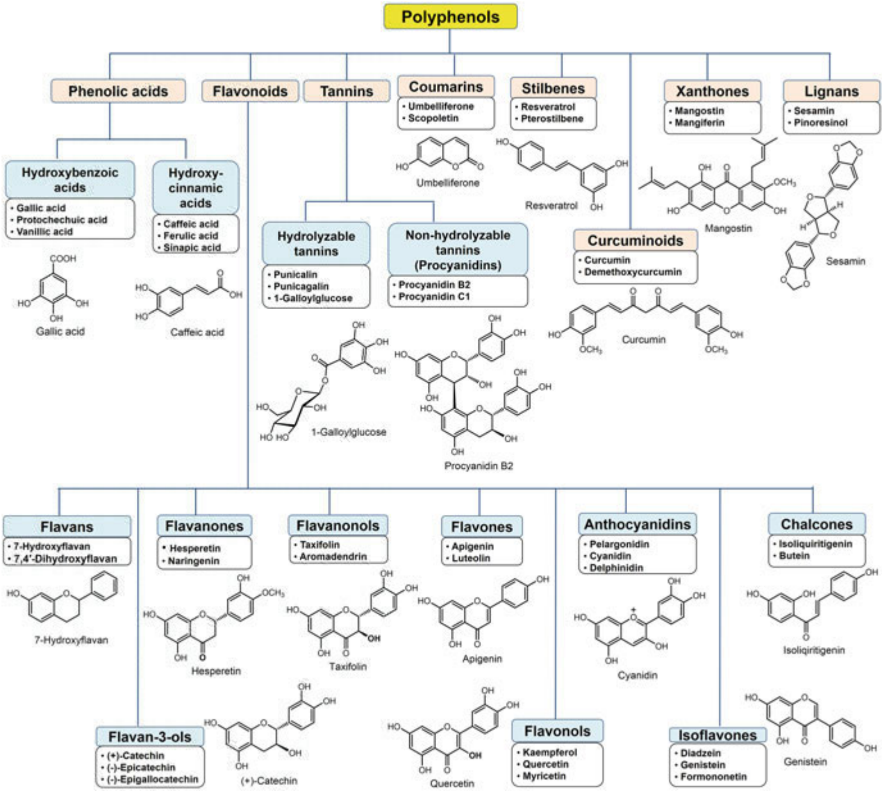



**Fig. 1.1** Basic classification of polyphenols based on their structures

#### 1.3.1.1 Phenolic Acids

Phenolic acids belong to the class of polyphenolic compounds and are commonly found in various plant species and abundant in vegetables, berries, fruits, and beverages (Zadernowski et al. 2005; Barros et al. 2009; Mattila et al. 2006; Nile and Park 2014). Phenolic acids are synthesized in plants during normal growth and development, as well as in response to stress conditions and against adverse factors such as drought, infections or physical damage, UV radiation, wounding, etc. (Dietrich 2004; Carl 2000). Based on their chemical structures, phenolic acids can be broadly divided into two groups, i.e., benzoic acid derivatives and hydroxycinnamic acid derivatives. The most common benzoic acid derivatives are gallic acid, protocatechuic acid, vanillic acid, and syringic acid (Fig. 1.2). These compounds mainly occur in plants either as free or as conjugated (bound) forms such as hydrolyzable tannins and lignins. Some of these hydroxybenzoic acid derivatives are also bound to flavonoid aglycones or flavonoid glycosides such as in case of epigallocatechin gallate (EGCG) or galloyl derivatives of flavonoid glycosides. The most common hydroxybenzoic acid derivatives are *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid, etc. (Fig. 1.2) (Barros et al. 2009; Mattila et al. 2006).

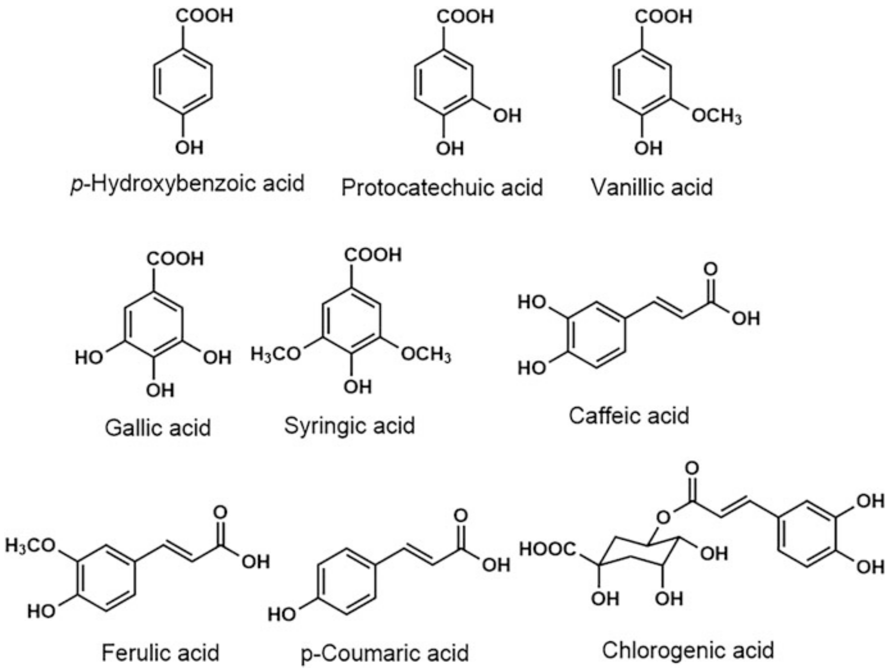



**Fig. 1.2** Structures of common phenolic acids

Hydroxycinnamic acids are usually found in bound form with quinic acid, tartaric acid, or sugars. The most common examples are mono-caffeoylquinic acid, dicaffeoylquinic acids, and tricaffeoylquinic acid. Due to their common natural abundance, phenolic acids are widely studied for their antioxidant, anticancer, anti-inflammatory, and other pharmacological activities.

#### 1.3.1.2 Flavonoids

Flavonoids are another class of polyphenolic compounds widely studied for their abundance and biological activities. Flavonoids contain two aromatic rings connected by a three-carbon bridge ($C_6$-$C_3$-$C_6$), and they are further divided into different classes based on the presence of double bond between 2 and 3 position carbons and the presence of hydroxyl group in position 3 and ketone in position 4. These classes are like flavans, flavanols, flavanones, flavanonols, flavones, flavonols, anthocyanidins, isoflavones, and chalcones (Fig. 1.1). Flavonoids are widely found in fruits, vegetables, legumes, wine, tea, and other beverages. These flavonoids usually present either in free form or their methylated, acylated, or glycosylated forms. The number of hydroxylation of the flavonoids is what makes them a diverse group of polyphenolic compounds.

Flavans (Fig. 1.3) do not have double bond in between carbon position 2 and 3 and also do not possess the hydroxyl or carbonyl group in position 3 and 4. They exist either in free form or glycosylated forms.

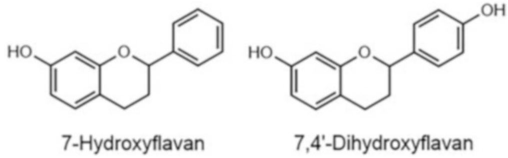



**Fig. 1.3** Structures of common flavans

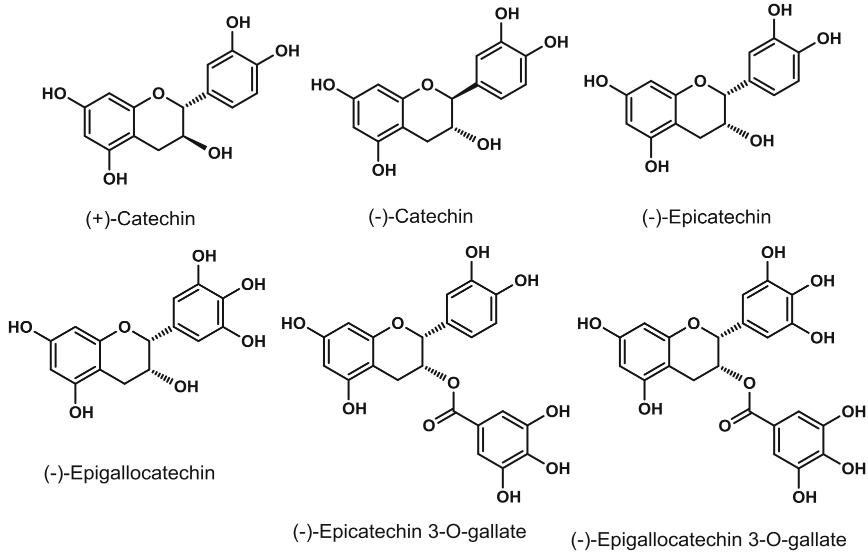



**Fig. 1.4** Structures of common flavan-3-ols

Flavan-3-ols, also known as catechins, are one of the main constituents in tea and other common functional food materials (Carloni et al. 2013). They belong to one of the most widely studied plant natural products for chemical and pharmacological aspects such as antioxidant, cancer chemopreventive, anti-inflammatory, immunomodulatory activities (Khan et al. 2019; Wai et al. 2018). Structures of most common flavan-3-ols are given in Fig. 1.4.

Flavanones, such as hesperetin, naringenin, and their glycosylated derivatives such as hesperidin, naringin, and neohesperidin (Fig. 1.5), are commonly found in *Citrus* plants and commonly known as potent antioxidant and anti-inflammatory compounds (Adhikari-Devkota et al. 2019). Most of the flavanones are hydroxylated in B-ring; however, some B-ring non-hydroxylated flavanones such as pinocembrin and pinostrobin are also found in different plant families (Rasul et al. 2013).

Flavanonols, also known as dihydroflavones, are found in many plants. Structures of common members of this group are represented in Fig. 1.6. Silibinin, a flavanolignan derivative isolated from milk thistle (*Silybum marianum* (L.) Gaertn.), is another common derivative of this group.

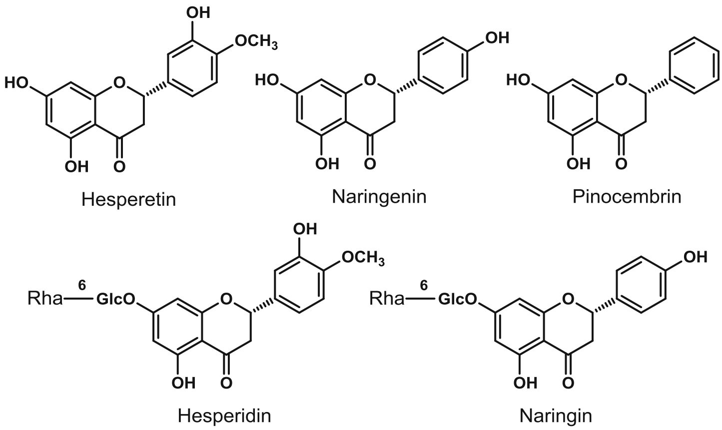



**Fig. 1.5** Structures of common flavanones and their glycosides

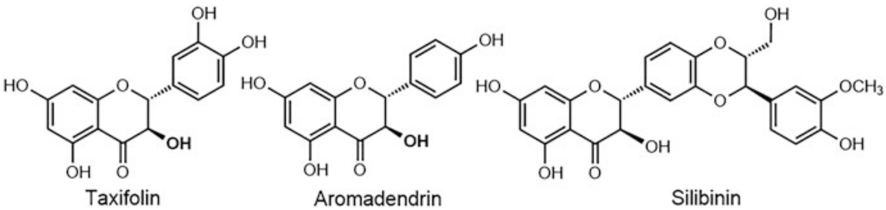



**Fig. 1.6** Structures of common flavanonols

Flavones are another class of common flavonoids such as apigenin, luteolin, and their derivatives. Other derivatives, widely found in *Citrus* plants, are polymethoxyflavones such as nobiletin, tangeretin (Fig. 1.7), and their derivatives which are potent anti-inflammatory agents.

Flavanols such as quercetin, kaempferol, myricetin, and their derivatives such as rutin, nicotiflorin, quercitrin, myricitrin (Fig. 1.8) are common in many leafy vegetables and flowers having potent bioactivities (Li et al. 2016; Wang et al. 2016; Boots et al. 2008).

Anthocyanidins (e.g., cyaniding, pelargonidin, delphinidin) (Fig. 1.9) are aglycones of anthocyanins, which are responsible for the beautiful colors of many colorful flowers, fruits, and vegetables (Iwashina 2015).

Isoflavones differ from other common flavonoids having the B-ring linked in C-3 position. Genistein, daidzein, puerarin, and their glycosylated derivatives (Fig. 1.10) are commonly found in soybean and many other plants such as fava bean, lupine, kudzu and psoralea, etc. (Odegaard et al. 2011).

Chalcones, are another class of flavonoids having open C-ring (Fig. 1.11). Various chalcones are reported from nature having diverse chemical characteristics and

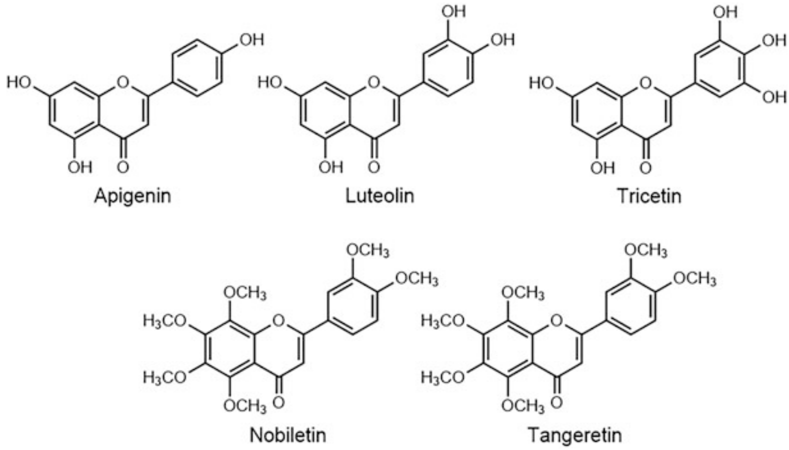



**Fig. 1.7** Structures of common flavones

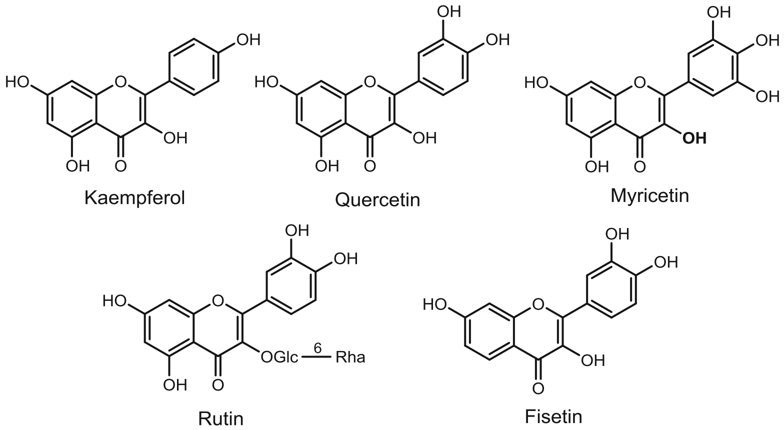



**Fig. 1.8** Structures of common flavonols

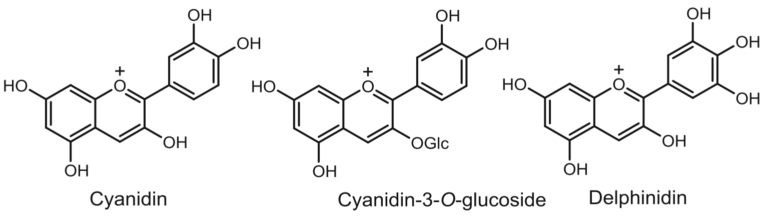



**Fig. 1.9** Structures of common anthocyanidins

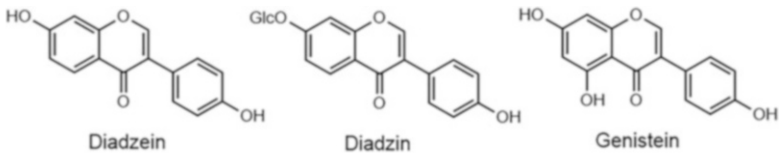



**Fig. 1.10** Structures of common isoflavones

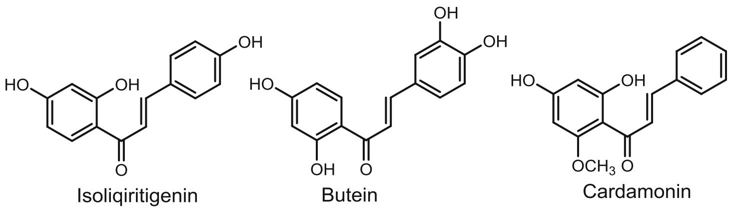



**Fig. 1.11** Structures of common chalcones

pharmacological activities. They usually exist as glycosides or prenylated derivatives.

#### 1.3.1.3 Tannins

Tannins are another class of polyphenolic compounds. They are mainly divided into two groups, i.e., hydrolyzable tannins and non-hydrolyzable tannins or condensed tannins (procyanidins). Hydrolyzable tannins are usually the glucosyl ester of gallic acids, and condensed tannins are polymers of catechins (Fig. 1.12). They are abundant in many plants and fruits.

#### 1.3.1.4 Coumarins

Coumarins are another class of polyphenolic compounds having benzopyran-2-one or chromen-2-one rings (Fig. 1.13). As in the case of flavonoids, they also exist as their dimers or methylated, acylated, or glucosylated derivatives.

#### 1.3.1.5 Stilbenes

Stilbenes or stilbinoids are another class of widely studied polyphenols mostly present in blueberries, grapes, raspberries, mulberries, pistachios, and peanuts. Resveratrol, oxyresveratrol, and pterostilbene (Fig. 1.14) are common monomers; however, they also exist as dimers, trimers, tetramers, or their respective glycosylated derivatives.

#### 1.3.1.6 Curcuminoids

Curcuminoids are a group of phenolic compounds present in the rhizomes of turmeric (*Curcuma longa* L.) and other plants of Zingiberaceae family. The most common member of this group, curcumin, also known as diferuloylmethane, is a member of the diarylheptanoid class of chemical compounds having two aryl

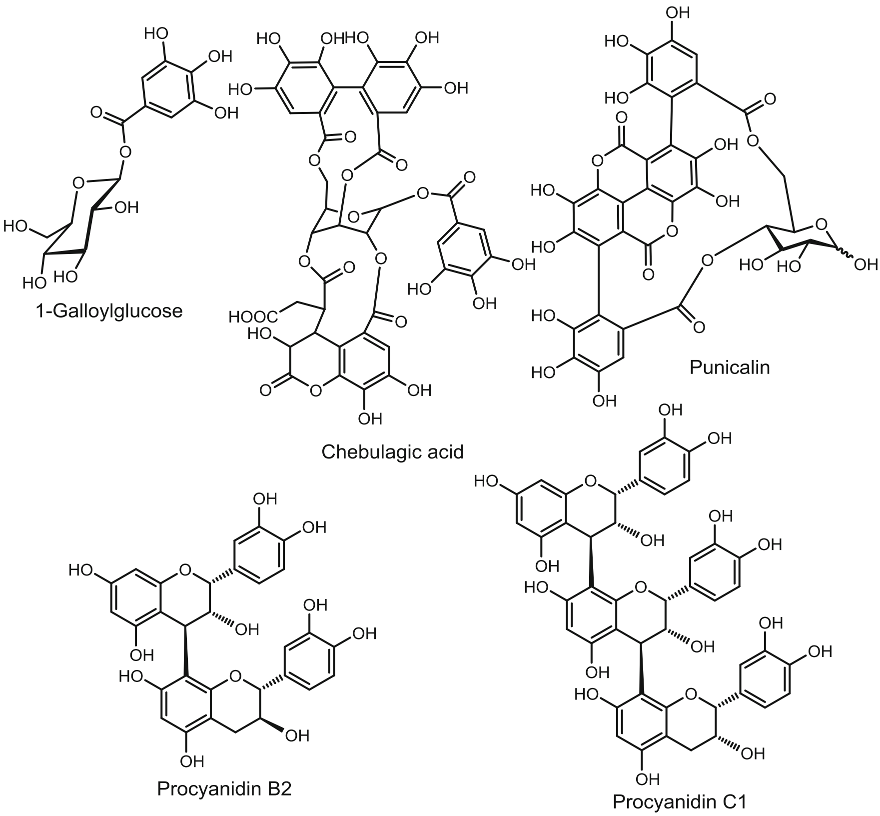



**Fig. 1.12** Structures of common tannins

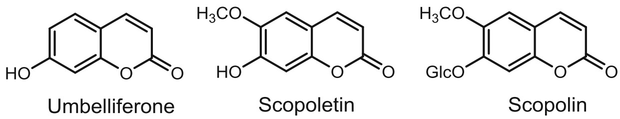



**Fig. 1.13** Structures of common coumarins

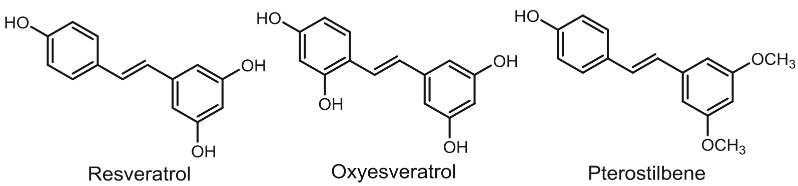



**Fig. 1.14** Structures of common stilbenes

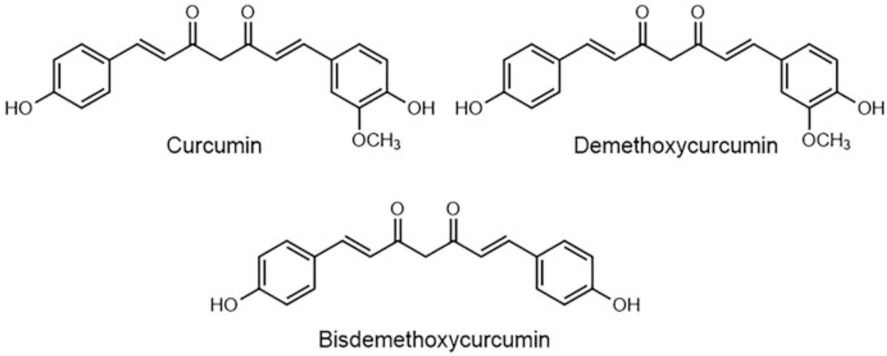
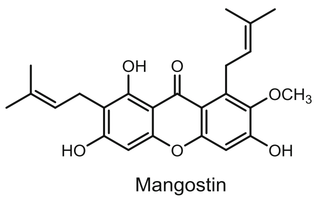



**Fig. 1.15** Structures of common curcuminoids

**Fig. 1.16** Structures of mangostin




(phenyl) rings attached to heptane derivative. Demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) (Fig. 1.15) are other two common natural curcuminoids. The content of curcuminoids in dried turmeric rhizomes is reported to be around 1–5% and is also responsible for the yellow color of the rhizome (Li et al. 2019; Prasad et al. 2014; The Ministry of Health Labor and Welfare of Japan 2016).

#### 1.3.1.7 Xanthones

Various xanthones are also reported as nutraceutical compounds. The most common example is mangostin (Fig. 1.16) obtained from mangosteen (*Garcinia mangostana* L.). Xanthones are widely reported as antioxidant and anticancer compounds.

#### 1.3.1.8 Lignans

Lignans are another class of polyphenolic compounds formed by the conjugation of two phenylpropanoid units. Sesamin and sesaminol (Fig. 1.17) found in sesame oil are widely reported as potent antioxidant, anti-inflammatory, and anticancer lignans. Based on the chemical structures, lignans are further classified into neolignans, lignan glucosides, and other derivatives.

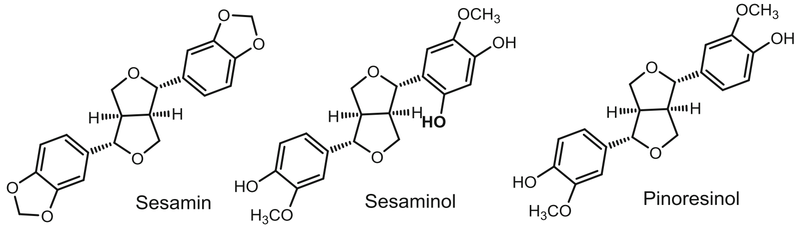



**Fig. 1.17** Structures of common lignans

### 1.3.2 Carotenoids

Carotenoids are another class of common nutraceuticals which are responsible for various health beneficial effects in humans as potent antioxidants, anticancer compounds, and precursors of vitamin A (Khoo et al. 2011; Sereno et al. 2018). They are derivatives of isoprenoids usually having 40 carbons with polyene chains (Tanaka et al. 2008). They play an essential role in plant physiology such as photoprotection during photosynthesis, providing substrates for biosynthesis of plant hormones, etc. They are also responsible for providing colors to many fruits and flowers (Tanaka et al. 2008). Various acylated and glycosylated derivatives are also abundant in nature. Carotenoids such as α-carotene, β-carotene, and lycopene (Fig. 1.18) are widely used as nutraceuticals along with their plant sources such as tomatoes and carrots. Many carotenoids are also used as food colorants. Crocin, a glycoside of carotenoid, crocetin, found in saffron and gardenia is widely used as nutraceutical compound and food colorant.

### 1.3.3 Amino Acids and Related Compounds

Various branch chain amino acids such as isoleucine, leucine, and valine are also used as nutraceuticals. Many short chain peptides obtained from the hydrolysis of food proteins obtained from soybeans, wheat, vegetables, etc. are also marketed as nutraceuticals (Andrew and Izzo 2017).

### 1.3.4 Alkaloids

Alkaloids are another group of phytochemicals that contain nitrogen atom in ring structure and are found in various plants where they play an important role in plant protection from various insects and other organisms. Alkaloids are divided into several groups: pyrrolidine, pyrrolizidine, pyridine, quinoline, isoquinoline, imidazole, etc. Various plant-derived alkaloids such as vinblastine, vincristine, taxol, etc. are further developed as drugs in anticancer therapy. Many plant-derived alkaloids are also used as nutraceutical agents. Structures of some of the common alkaloids used in nutraceutical market are represented in Fig. 1.19. Berberine, a yellow-

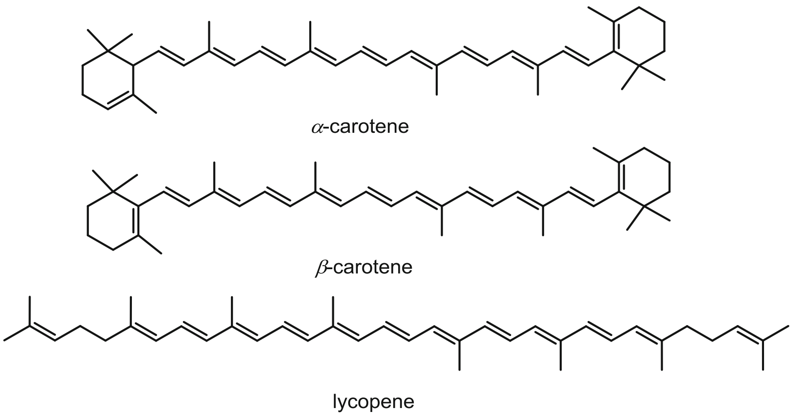



**Fig. 1.18** Structures of common carotenoids

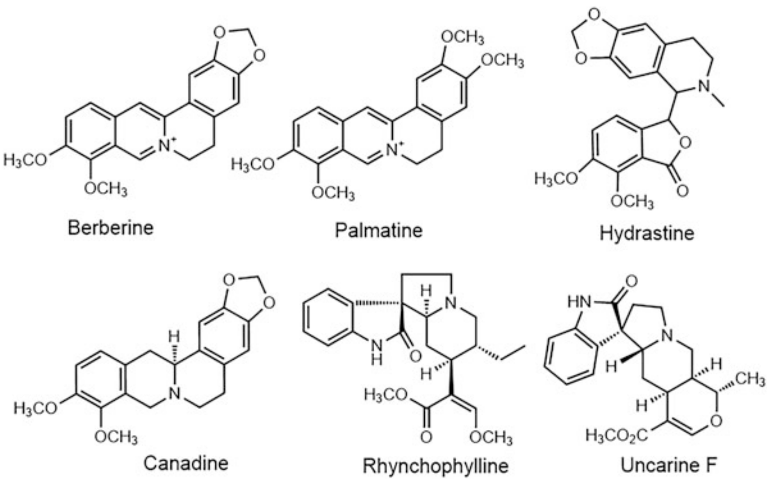



**Fig. 1.19** Structures of common alkaloids

colored isoquinolone alkaloid present in many medicinal plants such as *Berberis* spp*.*, *Coptidis* spp*.*, *Hydrastis spp.*, *Mahonia* spp*.*, *Phellodendron amurense, Tinospora cordifolia*, and many others (Belwal et al. 2020a; Yeung et al. 2020a; Mandal et al. 2020), is used widely as a nutraceutical compound. Berberine and palmatine-rich extracts from *Berberis* plants (Belwal et al. 2020a), berberine and hydrastine-rich goldenseal (*Hydrastis canadensis* L.) (Mandal et al. 2020), and rhynchophylline and uncarine F-rich extracts of cat's claw (*Uncaria tomentosa* (Willd. ex Schult.) DC.) (Batiha et al. 2020) are widely marketed as potent nutraceuticals.

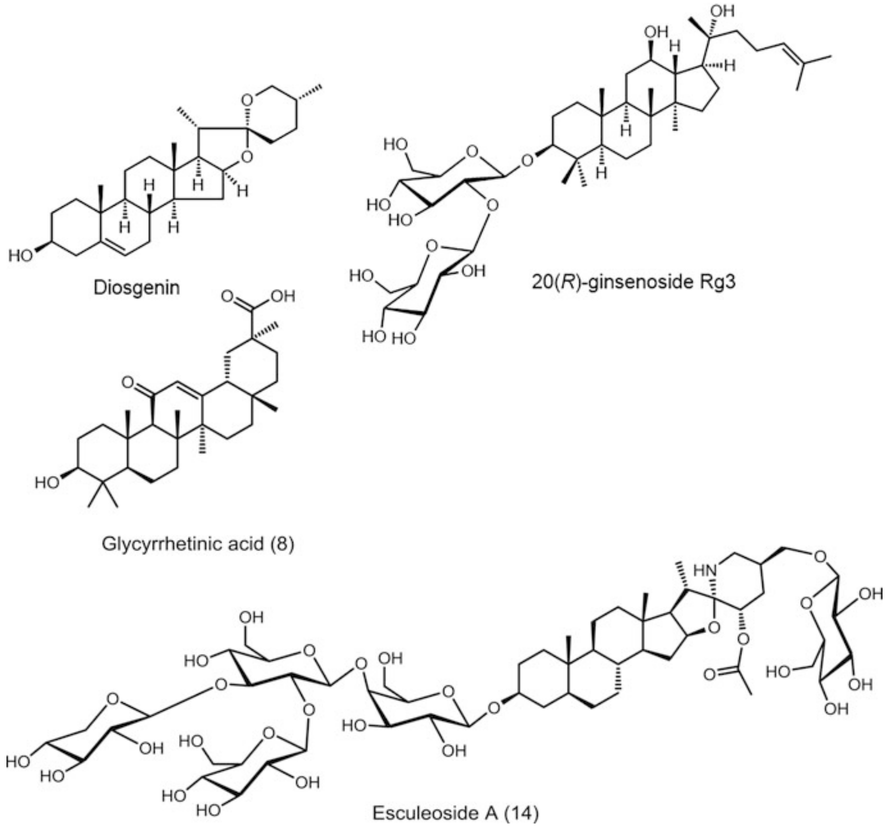



**Fig. 1.20** Structures of common steroid and triterpenoid derivatives

### 1.3.5 Steroids, Triterpenoids, and Saponins

Phytosterols and triterpenoids and their glycosides (saponins) are among the most abundant chemical compounds in plants (Mozos et al. 2018; Piironen et al. 2003). Various phytosteroids, triterpenoids, and their glycosides (saponins) are marketed as cancer chemopreventive and cholesterol-lowering agents. Some examples of these compounds are presented in Fig. 1.20.

## 1.4 Biosynthesis of Nutraceuticals

Biosynthetic pathways of several phytochemicals have received attention in recent years as biotechnological targets for the production of leads for drug discovery and nutraceuticals. In this section, the biosynthesis of polyphenols is discussed in brief.

In plants, polyphenols such as phenolic acids and flavonoids play an important role in plant physiology as antioxidants, defense regulators, and signaling molecules

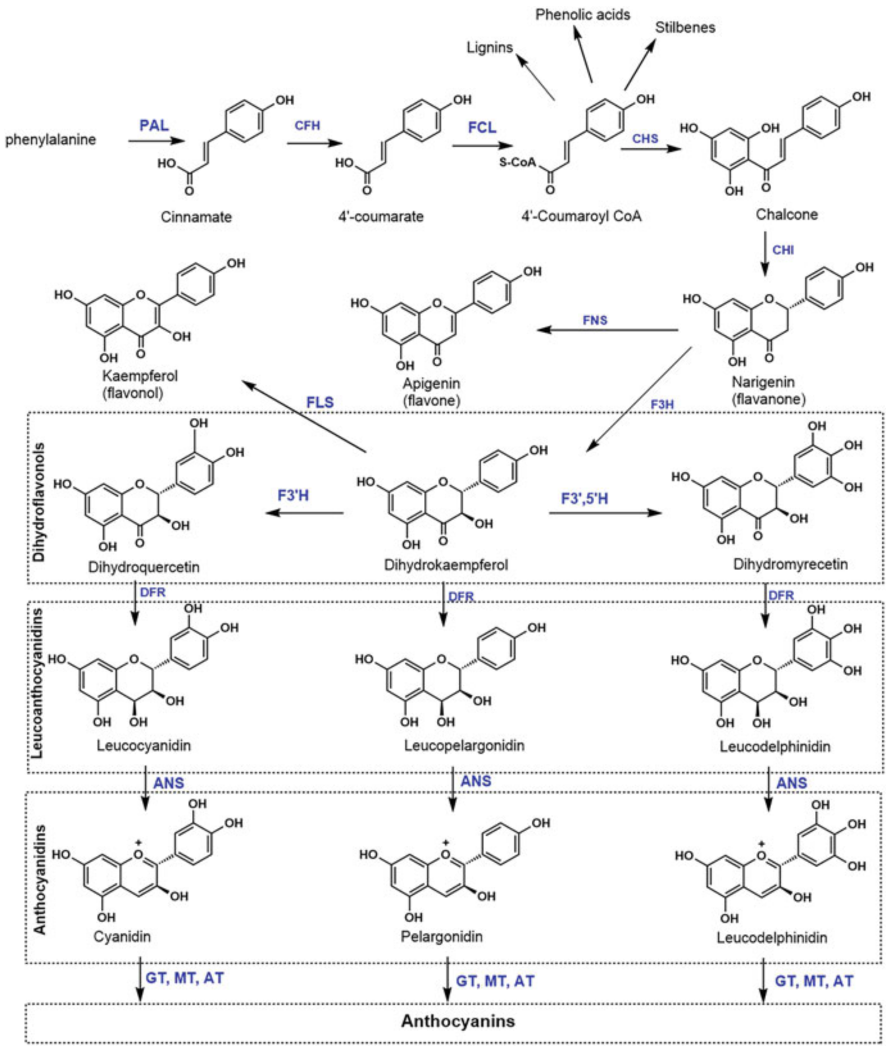



**Fig. 1.21** Biosynthesis of flavonoids including anthocyanidins and anthocyanins. PAL: phenylalanine ammonia lyase, CFH: cinnamate-4-hydroxylase, FCL: 4-coumarate CoA ligase, CHS: chalcone synthase, CHI: chalcone isomerase, FNS: flavone synthase, F3′H: flavonoid 3′-hydroxylase, F3′5′H: flavonoid 3′,5′-hydroxylase, DFR: dihydroflavonol-4-reductase, ANS: anthocyanidin synthase, GT: glucosyltransferase, MT: malonyltransferase, AT: acyltransferase (Tanaka et al. 2008)

(Agati et al. 2012; Brown et al. 2001; Harborne and Williams 2000; Khalid et al. 2019; Liu and Murray 2016; Nakabayashi and Saito 2015; Weston and Mathesius 2013); thus, many plants synthesize these compounds.

The schematic representation of synthesis of flavonoids and other polyphenolic compounds is provided in Fig. 1.21 (Tanaka et al. 2008; Forkmann 1991). It is one of the most widely studied phytochemical synthesis pathway. Flavonoids are synthesized in cytosol where various enzyme interactions occur. Formation of 4-′-coumaroyl coenzyme A (CoA) from phenylalanine through multistep enzymatic catalysis is the initial step in the synthesis of various phenolic compounds such as phenolic acids, lignins, stilbenes, and flavonoids. Chalcone synthase converts 4-′-coumaroyl CoA to tetrahydroxychalcone (THC) using three molecules of malonyl CoA (Tanaka et al. 2008). Thus, formed THC is then isomerized to naringenin which further is converted to dihydrokaempferol, a flavanonol, or dihydroflavonol. Different specific hydroxylation enzymes convert dihydrokaempferol to dihydroquercetin and dihydromyricetin. These dihydroflavonols are converted to respective leucocyanidins by dihydroflavonol-4-reductase. These leucocyanidins are then converted to anthocyanidins by anthocyanidin synthase. Various UDP-glucosyltransferases then conjugate glucose and other sugars to anthocyanidins resulting in the synthesis of anthocyanins.

## 1.5 Biological Functions and Pharmaceutical Activities of Nutraceuticals

Many epidemiological studies in vivo and clinical studies have demonstrated that these nutraceutical phytochemicals can be effective for the prevention of various chronic diseases. Most of the research related to the health-promoting activities of nutraceuticals were conducted after 2000, and there is a vast growth in these studies after 2010. Nutraceuticals have been widely studied for their antioxidant activities or anticancer and antiaging activities in general. A simple Scopus search (www.Scopus.com, December 25, 2020) with the keyword “nutraceutical OR nutraceuticals AND immunomodulatory” and similar activity words (as given in Fig. 1.22) showed that antioxidant and cancer-activities are two of the most widely studied activity areas in nutraceutical bioactivity. As shown in Fig. 1.22, antioxidant and cancer-related activities are most studied ones.

Tea catechins are among the widely studied nutraceuticals well studied for their health beneficial activities such as antioxidant, antihyperlipidemic, antiagenins, antidiabetic, antiobesity, and cancer chemopreventive activities (Zaveri 2006). Tea is not only used as a beverage but also in various forms as functional food (Kurauchi et al. 2019; Namal Senanayake 2013; Hara 2011; Sanna et al. 2015). Many review articles are also published in these aspects of tea catechins (Khan and Mukhtar 2010; Yang and Wang 2016; Boehm et al. 2009). Various review articles have been already published which extensively cover these activities such as antioxidant activity (Gramza and Korczak 2005; Higdon and Frei 2003); potential use for prevention and treatment of obesity, diabetes, and other metabolic diseases (Zaveri 2006; Gramza and Korczak 2005; Higdon and Frei 2003; Park et al. 2009; Masterjohn and Bruno 2012; Kao et al. 2006; Legeay et al. 2015); cardiovascular diseases (Hodgson and Croft 2010); cognitive functions (Weinreb et al. 2004; Da Silva 2013; Pervin et al. 2018); and antimicrobial activities (Reygaert 2014; Taylor et al. 2005).

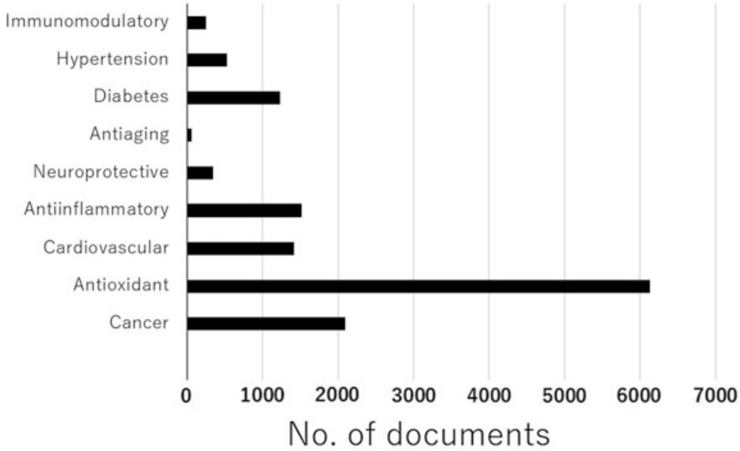



**Fig. 1.22** No. of studies related to different biological activities of nutraceuticals

Naringenin, hesperidin, nobiletin, and other flavonoids obtained mainly from *Citrus* fruits are highly bioactive and multifunctional compounds with potent anti-inflammatory, antioxidant, anti-adipogenic, and cardioprotective activities (Salehi et al. 2019; Tapas et al. 2008; Zaidun et al. 2018; Yang et al. 2011; Adhikari-Devkota et al. 2019). Nobiletin and other polymethoxyflavonoids have received great attention as a potent antioxidanst (Wang et al. 2018), anticancer (Chen et al. 2014), and anti-inflammatory agents (Zhang et al. 2016) and as potential compounds to prevent neurodegenerative diseases (Nakajima et al. 2014). Many epidemiological studies have also reported the potential health beneficial and disease preventive and therapeutic effects of the dietary intake of these flavonoids (Pan et al. 2010). Some studies have also reported that the intake of flavonoids may help in delaying the progression of Alzheimer's disease (AD) and other neurodegenerative disorders (Williams and Spencer 2012; Ayaz et al. 2019).

Silibinin, a derivative of flavanonol moiety, is also known for its hepatoprotective activities. Mixture of silibinin with other stereoisomers (known as silymarin) has been widely used in treatment of liver diseases and also reported to be a potent cancer chemopreventive agent (Zhao and Agarwal 1999; Bosch-Barrera and Menendez 2015; Tyagi et al. 2002; Lu et al. 2012).

Flavonols such as quercetin, kaempferol, and fisetin found in many fruits and vegetables are reported as potent anti-inflammatory, antioxidant, cancer chemopreventive, and neuroprotective agents (Li et al. 2016; Wang et al. 2016; Boots et al. 2008; Khan et al. 2013; Donado et al. 2011; Park et al. 2007; Ahmad et al. 2017).

Genistein, a isoflavone, is linked to various pharmacological activities, and the high consumption of genistein-rich soy products by Asians has been linked with lower incidences of diabetes mellitus (Odegaard et al. 2011).

Resveratrol, a stilbenoid, is well known for its antioxidant, anti-inflammatory, and anticancer activities (Yeung et al. 2020b) along with potential benefits in modulation of lung inflammatory diseases (Vargas et al. 2016).

Similarly, curcumin and other curcuminoids are reported as potent antioxidant, anti-inflammatory, anti-cancer, antimicrobial, hepatoprotective, and neuroprotective agent (Li et al. 2019; Prasad et al. 2014; Anand et al. 2008; Gupta et al. 2012; Lee et al. 2013; Maheshwari et al. 2006; Singh and Khar 2008; Ohori et al. 2006; Weber et al. 2005; Basnet and Skalko-Basnet 2011).

Berberine, an alkaloid found in various plant species, is reported for its potent anti-inflammatory activity, anti-diabetic activity, and cardioprotective and immuno-modulatory activities (Belwal et al. 2020a; Neag et al. 2018).

## 1.6 Pharmacokinetics and Advanced Drug Delivery Systems Related to Nutraceuticals

Plant-derived phytonutrients have shown various pharmacological activities in in vitro systems; however, the poor water solubility, low bioavailability, and extensive metabolism have been one of the main limiting factors for the therapeutic use of these phytochemicals (Selby-Pham et al. 2017; Aqil et al. 2013; Karaś et al. 2017). Studies in animals and humans have shown that the most of the ingested polyphenols are absorbed in the intestines in very low quantity (about 5%), and the remaining portion is passed unchanged to the large intestine.

For example, although EGCG has shown promising bioactivities in in vitro systems, poor bioavailability is of great concern for clinical effectiveness (Cai et al. 2018) as less than 1% of EGCG was measured in blood after oral administration in rats (Chen et al. 1997). Similar reports of low bioavailability and extensive metabolism of nutraceuticals are commonly reported for curcumin (Anand et al. 2008; Basnet and Skalko-Basnet 2011), resveratrol, and many other compounds.

In recent years, various novel drug delivery systems including nano-formulations such as liposomes, micelles, solid-liquid nanoparticles, nanoemulsions, etc. have been designed, developed, and evaluated for the effective delivery of these phytochemicals (Wang et al. 2014; Li et al. 2015a,b; Mangal et al. 2017; Bonferoni et al. 2017). These nanoparticles and advanced drug delivery formulations are aimed to increase the pharmaceutical properties of phytochemicals such as solubility and improve the bioavailability and targeted delivery (Davatgaran-Taghipour et al. 2017). High-quality research in the coming years in these areas can greatly improve the issues related to the bioavailability and bioactivity of nutraceuticals.

## 1.7 Research Trends, Natural Abundance, Sustainable Utilization, and New Technologies for the Production of Nutraceuticals

In recent years, there is a growing interest in the area of nutraceuticals research due to high market and industrial demand which need development of novel technologies in identification, estimation, and bioactivity evaluation of nutraceuticals. A simple Scopus search (www.Scopus.com, December 25, 2020) with the keyword "nutraceutical OR nutraceuticals" resulted into a total of 17,189 documents (search filed:

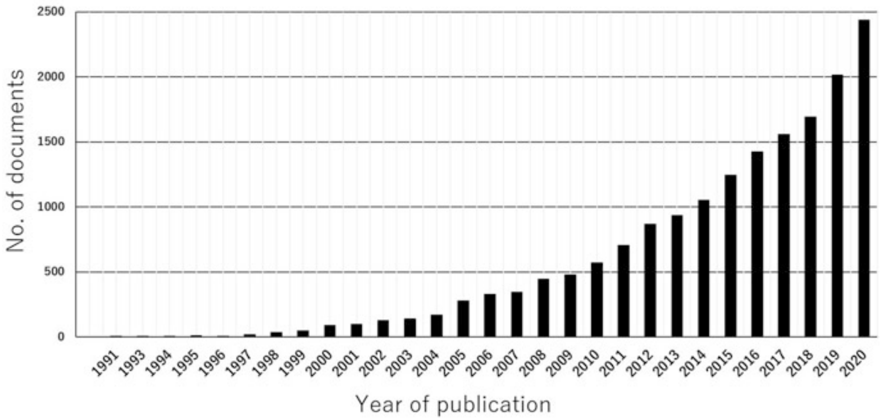



**Fig. 1.23** Number of publications related to nutraceuticals

Abstract, Title, and Keywords). Based on author's affiliations, the United States, Italy, India, China, Canada, Spain, South Korea, the United Kingdom, Brazil, and Mexico were the leading countries on nutraceutical research. More than half of these studies are reported after 2010 (Fig. 1.23).

In recent years, there are many advanced techniques for the extraction and purification of phytochemicals that can be used as nutraceuticals (Belwal et al. 2018, 2020b). Similarly, many techniques related to harvesting and their processing have been developed and introduced in various fields. However, current cultivation practices may not fulfill the increasing demand of plant-based nutraceuticals, and extensive use of natural resources may result in extension of important plant species. Thus, sustainable methods for the natural resources use are very important. Tissue culture and cell culture methods have high potential to fulfill these demands as these methods can be easily manipulated to biosynthesize and produce target nutraceutical.

## 1.8 Conclusions and Future Recommendations

Nutraceuticals are the products that are consumed for their potential health beneficial effects which makes them different from normal food products. The market of nutraceuticals and related functional foods is growing worldwide, and there is an increasing demand. Most of these nutraceuticals are derived from plants such as polyphenols, alkaloids, carotenoids, etc. and are widely studied for their chemistry and pharmacological activities. However, in many cases, there are not much strong scientific evidences, and some reports also raise the concern of no benefits from the consumption of these products (Aronson 2017). Polypharmacy, drug interaction, side effects, and adulteration are also some other challenges in nutraceutical products. Future research studies should try to fulfill these research gaps by providing sufficient sources for production of these nutraceuticals. Furthermore, extensive studies related to their clinical efficacy and possible toxicity are necessary.

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