POLYPHENOLS AND FLAVONOIDS: AN OVERVIEW

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1.1 INTRODUCTION

There has been an increase in pharmaceutical and biomedical therapeutic interest in natural products as reflected in sales of nutraceuticals and in the global therapeutic use of traditional medicines. Use of traditional medicines is based on knowledge, skills, and practices based on experiences and theories from different cultures that are used to prevent and maintain health, which may ultimately improve, and/or to treat physical and mental illnesses. The popularity of these products encompasses almost every aspect of our daily lives from health and beauty, dietary supplements, performance enhancement supplements, food and beverage to overall health and well-being products. It is apparent that this growing demand for phytotherapies could be very profitable for nutraceutical and pharmaceutical companies. Nutraceutical as well as pharmaceutical companies are interested in many of these naturally occurring compounds that can be extracted from plants and be further modified, synthesized, formulated, manufactured, marketed, and sold for their reported health benefits. Pharmaceutical companies are also using these natural compounds as lead drug candidates that can be modified and formulated to be potential new drug candidates. From drug discovery and development to marketing, between 15 and 20 years may lapse with billions of dollars spent on drug
development and research of pharmaceuticals. Consumers are looking for beneficial health-related products that have efficacy at a low cost to the consumer, while the nutraceutical industry is struggling to develop therapies at a low cost and to bring them to the market. Through scientific studies, natural products can be scrutinized using pharmaceutical approaches to develop and provide alternative or adjunctive therapies.

The drug discovery process is expensive and time-consuming. It has been estimated to take 10–15 years and $800 million to get a drug to the approval process. Part of this cost is due to advances in technology whereby drug manufacturers have adopted a target-based discovery paradigm with high throughput screening of compound libraries. This approach, although expected to have vast potential, has not necessarily proven itself. Reviews of new chemical entities have shown that natural products or derivatives of natural products are still the majority of newly developed drugs. For instance, 63% of the 974 new small molecule chemical entities developed between 1981 and 2006 were directly isolated from nature or semisynthetic derivatives of a natural product. This trend continues even into this century where approximately 50% of new small molecule chemical entities approved from 2000 to 2006 have a natural origin. It is apparent that natural products are important compounds to be explored in the drug discovery process. More importantly, however, there remains a multitude of bioactive compounds yet to be systematically characterized. It is estimated that of the 250,000–750,000 higher plant species, only 10–15% have been screened for potential therapeutic agents. Characterizing bioactive molecules in microbial and marine life is even more limited. Nonetheless, natural products remain a reservoir of potential therapeutic agents.

It has been reported that 5000–10,000 compounds are screened before a single drug makes it to the market, and on average, it takes 10–15 years to develop a single drug. Of the successfully developed drugs, 60% have a natural origin, either as modified or unmodified drug entities, or as a model for synthetic drugs—not all of them used for human diseases—and it is estimated that 5–15% of the approximately 250,000–750,000 species of higher plants have been systematically screened for bioactive compounds. Structure–activity relationship (SAR) programs are generally employed to improve the chances of phytochemicals being developed as drug entities. Further studies to develop more drugs of natural origin have been limited in part due to their structural complexity, which is sometimes incompatible with high throughput formats of drug discovery and high extraction costs. The potentially long resupply time and unforeseen political reasons such as warfare in developing nations also limit the development of plant-based drugs. As a result, plants remain and represent a virtually untouched reservoir of potential novel compounds. Nevertheless, the number of drugs developed each year based on natural products has remained constant over the last 22 years.

A class of molecules with well-documented therapeutic potential is the polyphenols. Polyphenols are small molecular weight (MW) compounds (MW 200–400 g/mol) that occur naturally. They are produced as secondary
metabolites that serve to protect the plant from bombardment of pathogens and ultraviolet (UV) radiation. Upon environmental threat, the plant host activates one of the synthesis pathways and polyphenol structures are produced and subsequently secreted. Which specific polyphenol is produced depends largely on its host, the region of origin, and the environmental stimuli. Many polyphenols are synthesized by the phenylpropanoid pathway. Several classes of polyphenols exist including flavonoids, stilbenes, isoflavonoids, and lignans. Polyphenols of all classes are found in a wide range of plants and plant by-products such as herbal supplements and beauty products.

1.2 SYNTHESIS

An understanding of the biosynthesis of natural compounds will enable researchers to further investigate possible therapeutic uses based on the activity of phytochemicals in plants. Plant chemicals are often given the moniker “phytochemicals” and can be classified either as primary or secondary metabolites. Primary metabolites are widely distributed in nature and are needed for physiological development in plants. On the other hand, secondary metabolites are derived from the primary metabolites, are limited in distribution in the plant kingdom, and are restricted to a particular taxonomic group (Fig. 1.1). Secondary metabolites usually play an ecological role; for example, they act as pollinator attractants, are involved in chemical defense, are often end products of other biochemical pathways, and can be used by humans as medicines, flavorings, and fragrances.

![Figure 1.1](#) Biosynthetic origin of some plant-derived compounds. Major groups of secondary metabolites are indicated by ovals.
products from chemical adaptations to environmental stresses, or are synthesized in specialized cell types at different developmental stages of plant development or during disease or are induced by sunlight.\textsuperscript{19}

Allelochemicals are phytotoxic compounds produced by higher plants that include flavonoids. Like other secondary metabolites, flavonoids have complex structures where multiple chiral centers are common.\textsuperscript{19} Flavonoids consist of a C\textsubscript{15} unit with two benzene rings A and B connected by a three-carbon chain (Fig. 1.2). This chain is closed in most flavonoids, forming the heterocyclic ring C; however, chalcones and dihydrochalcones present as an open ring system.\textsuperscript{20} Depending on the oxidation state of the C ring and on the connection of the B ring to the C ring,\textsuperscript{21} flavonoids can be classified into various subclasses. Flavonoids can undergo hydroxylation, methylation, glycosylation, acylation, prenylation, and sulfonation; these basic chemical metabolic substitutions generate the different subclasses: flavanols, flavanones, flavones, isoflavones, flavonols, dihydroflavonols, and anthocyanidins.\textsuperscript{20,21} Flavonoids in nature are naturally most often found as glycosides and other conjugates; likewise, many flavonoids are polymerized by plants themselves or as a result of food processing.\textsuperscript{21}

1.2.1 Synthesis of Flavonoids

In plants, primary metabolites such as sugar are associated with basic life functions including, but not limited to, cell division, growth, and reproduction.\textsuperscript{22} On the other hand secondary metabolites are involved in the adaptive necessity of plants to their environments, such as pigmentation, defense from toxins, and enzyme inhibition;\textsuperscript{23–25} additionally, these secondary metabolites can have pathogenic or symbiotic effects.\textsuperscript{26} Secondary metabolites including polyphenols have been associated with having many health benefits.\textsuperscript{27} The abundance of polyphenols in foodstuffs is apparent, although they often have not been adequately characterized; however, an assortment of polyphenols is prevalent in unprocessed and processed foods and beverages and nutraceuticals.\textsuperscript{28}
Structurally, polyphenols or phenolics have one or more aromatic rings with hydroxyl groups and can occur as simple and complex molecules. Polyphenols can be subdivided into two major groups: hydroxybenzoic acids and hydroxycinnamic acids (Fig. 1.3). Examples of hydroxybenzoic acids include gallic and vanillic acids. They are typically found in the bound form as a smaller entity of a ligand or tannin or are linked to a sugar or an organic acid in plant foods. Alternatively, hydroxycinnamic acid examples include p-coumaric and caffeic acids. These molecules are found esterified with small molecules, bound to cell walls, and/or proteins. A subcategory of p-coumaric acid derivatives is the flavonoids (flavanones, flavanones, flavonols, flavanols [proanthocyanidins, catechins, epicatechins, procyanidins, prodelphinidins], and anthocyanins) as these are the most abundant polyphenols in our diets (Fig. 1.4). Flavonones and isoflavones can be predominantly found in citrus fruits and soy products, respectively. Proanthocyanidins are complex polymeric flavanols found in conjunction with flavanol catechins from apples, pears, grape, and chocolate products; these flavonoids are primarily responsible for the astringency of foods. Anthocyanins are located in an assortment of fruits (cherries,
In addition to these polyphenol subclasses, in nature, flavonoids are also prevalent as a glycoside (parent compound or aglycone with a sugar moiety attached) as this sugar moiety helps to facilitate water solubility and transportability of the aglycone. Flavonoids are synthesized via the phenylpropanoid pathway and are derived from estrogen. The phenylalanine structure from phenolic compounds is transformed to cinnamate by the enzyme phenylalanine ammonia-lyase (PAL). The cinnamate 4-hydroxylase (C4H) converts cinnamate to plums, strawberries, raspberries, blackberries, and currants). In addition to these polyphenol subclasses, in nature, flavonoids are also prevalent as a glycoside (parent compound or aglycone with a sugar moiety attached) as this sugar moiety helps to facilitate water solubility and transportability of the aglycone. Another important factor to consider is that the distribution of polyphenols in plant tissues is heterogenous; thus, the seed, pericarp, flavedo, and albedo contain polyphenols in different proportions.

Flavonoids are synthesized via the phenylpropanoid pathway and are derived from estrogen. The phenylalanine structure from phenolic compounds is transformed to cinnamate by the enzyme phenylalanine ammonia-lyase (PAL). The cinnamate 4-hydroxylase (C4H) converts cinnamate to
p-coumarate, and then an acetyl-CoA group is added by the CoA ligase enzyme to yield cinnamoyl-CoA. Lastly, this product is transformed by chalcone synthase (CHS) to yield a general chalcone structure. Stilbenoids are synthesized in much the same fashion except for the C4H enzymatic step (Fig. 1.5).

The chalcone structure is further metabolized by the chalcone isomerase (CHI) to the general chiral flavanone structure. From the general chiral flavanone structure, the other derivatives, namely, dihydroflavonols, flavonols, flavones, flavan-3-ols, flavan-3,4-diols, isoflavonoids, and neoflavonoids, are further metabolized by a well-characterized enzymatically derived process (Fig. 1.6). Anthocyanidins and anthocyanins are derived from flavan-3,4-diols by leuocyanidin oxygenase (LO) and anthocyanidins-3-O-glucosyltransferase, respectively. Chromones are synthesized from isoflavonoids through the chromone synthase (ChS), while lignans and coumarins are derived from
neoflavonoids by lignan synthase (LS) and coumarin synthase (CS), respectively (Fig. 1.6).

In addition to flavanone, other small natural compounds found in a wide variety of food and plant sources exist. These compounds, namely, flavonoids, isoflavonoids, and lignans, have generated much scientific interest in their potential clinical applications in the possible dietary prevention of different diseases. Flavanones, stilbenes, lignans, isoflavonoids, and other flavonoid derivatives are similar in structure and provide host-protective purposes. They share the common parent compound, estrogen, in their synthesis and are differentiated based on key structural differences, specific plant hosts, and the environment (Fig. 1.7).

1.3 SOURCES

In 1936, Professor Szent-Györgyi reported the isolation of a substance that was a strong reducing agent acting as a cofactor in the reaction between peroxidase and ascorbic acid. This substance was initially given the name “vitamin P”; this substance has been subsequently categorized as the flavonoid rutin. Professor Szent-Györgyi’s seminal investigations identified rutin and reported its isolation from both lemons and red pepper.35 Since this time, more than other 4000 flavonoids have being identified and studied. Flavonoids are a group of polyphenolic compounds of low MW36 that present a common benzo-γ-pyrone structure.37 They are categorized into various subclasses
including flavones, flavonols, flavanones, isoflavonones, anthocyanidins, and catechins.

Consumption of polyphenols could be close to 1 g/day in our diet, making polyphenols the largest source of antioxidants. Dietary sources of polyphenols include fruits, vegetables, cereals, legumes, chocolate, and plant-based beverages such as juices, tea, and wine. Extensive biomedical evidence suggests that polyphenolic compounds no matter their class may contribute to the prevention of cardiovascular disease, cancer, osteoporosis, diabetes, and neurodegenerative diseases. As polyphenols are found in plant sources consumed regularly or that are used in traditional medicine, there is a necessity to study these potentially beneficial compounds. Additionally, potential health benefiting properties such as antiinflammatory, antiproliferative, and colon protection may call for development of these compounds into future therapeutic agents. The average human diet contains a considerable amount of flavonoids, the major dietary sources of which include fruits (i.e., orange, grapefruit, apple, and strawberry), vegetables (i.e., onion, broccoli, green pepper, and tomato), soybeans, and a variety of herbs. Due to the constant and significant intake of these compounds in our diet, the United States Department of Agriculture (USDA) has created a database that contains the reported average content of these compounds in different foodstuffs. Among the classes of flavonoids, flavanones have been defined as citrus flavonoids due to their almost unique presence in citrus fruits. However, flavanones have been also reported in tomatoes, peanuts, and some herbs such as mint, gaviota tarplant, yerba santa, and thyme. Flavonoids are consumed in the human diet; the calculated flavonoid intake varies among countries since cultural dietary habits, available flora, and weather influence what food is consumed and, therefore, the amount and subclasses of flavonoids ingested. However, in the Western diet, the overall amount of flavonoids consumed on a daily basis is likely in the milligram range. It has been determined that the consumption of selected subclasses of flavonoids may be more important in determining health benefits than the total flavonoid intake. The content of flavonoids is also potentially influenced by food processing and storage conditions, which can result in transformation of flavonoids, and loss of flavonoid content.

Flavonoids in general have been studied for more than 70 years in in vivo and in vitro systems. They have been shown to exert potent antioxidant activity in some instances, stronger than α-tocopherol (vitamin E). They have also been shown to exhibit beneficial effects on capillary permeability and fragility to have antiplatelet, hypolipidemic, antihypertensive, antimicrobial, antiviral, antiallergenic, antiulcerogenic, cytotoxic, antineoplastic, antiinflammatory, antiatherogenic, and antihepatotoxic activities. There are multiple chiral flavanones; however, they have been generally thought of as achiral entities and their chiral nature, in many cases, has not been recognized or denoted. Furthermore, the USDA database reports these
compounds as achiral entities and uses the aglycone terminology interchangeably with the glycosides. The importance of considering the chiral nature of naturally occurring compounds and xenobiotics has been previously reviewed by Yáñez et al. The chirality of flavonoids was initially examined by Krause and Galensa’s studies in the early 1980s. Chirality plays an important role in biological activity; disciplines like agriculture, nutrition, and pharmaceutical sciences have long recognized the existence of natural chiral compounds; however, developed methods of analysis have often failed to stereospecifically separate and discriminate compounds into their respective antipodes. The advantage of chiral separation methods includes a more thorough appreciation of the stereospecific disposition of natural compounds including flavonoids. Moreover, the lack of configurational stability is a common issue with chiral xenobiotics. Some chiral flavonoids have been reported to undergo nonenzymatic interconversion of one stereoisomer into another in isomerization processes such as racemization and enantiomerization. Racemization refers to the conversion of an enantioenriched substance into a mixture of enantiomers. Alternatively, enantiomerization refers to a reversible interconversion of enantiomers. The importance of isomerization in stereospecific chromatography as well as in the pharmaceutical manufacturing process has been described. Therefore, the development of chiral methodology to analyze this kind of xenobiotics is necessary.

The study of the stereochemistry of flavonoids comprises mainly C-2 and C-3; nevertheless, the majority of natural flavonoids possess only one stereochemical isomer at the C-2 position. C-2 and C-3 act as chiral centers of dihydroxyflavonols and are important in flavonoid metabolism. The nomenclature of flavonoids with two chiral centers remains a topic of debate since the use of symbolism (+/−) or 2,3-cis or -trans seems to be inadequate to describe four possible enantiomers. It is also argued that the R, S nomenclature for absolute configuration is confusing for flavonoids because the designation of R or S changes at C-2 depending on the priority of neighboring groups, even though the stereochemistry remains the same. An alternative nomenclature system was proposed by Hemingway et al. based on that used for carbohydrate chemistry. In this system, the prefix ent- has been used for the mirror images. However, scientific consensus has not been reached on stereochemical lexicon cognates, and, to date, all these systems of nomenclature still remain being used and appearing in the biomedical, biochemical, agricultural, and food science literature.

1.4 PHARMACOLOGICAL ACTIVITIES OF SELECTED FLAVONOIDS

Humans have utilized and/or consumed polyphenols for health benefits. For centuries, alternative medicine has been practiced in different countries as
exemplified by the use of plant extracts as traditional medicinal folk agents in the prevention and treatment of an assortment of ailments like menses, coughing, digestive problems, and so on. There are a variety of health benefits that can be attributed to the use/consumption of polyphenols including antioxidant, anticancer, antihyperlipidemic, antiallergenic, antibacterial, antiviral, and antiinflammatory. Conversely, there are also toxic effects associated with the use/consumption of polyphenols such as anemia due to the inhibited absorption of nutrients and minerals and inhibitory effects on cytochrome P450 enzymes (P450) resulting in potential drug–drug interactions. Current uses of polyphenols, in addition to their dietary health-related benefits and herbal remedies, are their use as dietary supplements and as pharmaceutical leads; thus, the reported intake of polyphenols is in the tens to hundreds of milligrams per day in human diets.

The World Health Organization (WHO), published a comprehensive study and analysis in September 2008 naming the leading causes of mortality in the world in 2004 to include cardiovascular and pulmonary ailments and cancer accounting for approximately 22.9 million deaths. These statistics remain consistent with the data published in 2007 with similar primary causes of mortality as seen in 2002. There appears to be evidence that suggests that the leading causes of death are often multifactorial and intertwined, for example, dyspnea, malignant pericardial effusion, malignant pleural effusion, and superior vena cava syndrome, all of which are cardiopulmonary and/or vascular problems. Biomedical literature suggests etiologies of cardiovascular and pulmonary ailments and cancer have been linked to diet and nutrition, environment, exercise, genetics, hormones, lifestyles, radiation, sex, and weight; however, direct correlations of the disease, etiologies, and pathogenic mechanisms have not been fully elucidated. Contemporary Western medicine provides a variety of options to prevent and treat cardiovascular and pulmonary ailments and cancer. It is becoming increasingly popular and apparent that there is a need for other effective means to prevent, treat, and develop newer drugs or alternatives to disease treatment for both the consumer and the nutraceutical and pharmaceutical industry at a lower cost.

There are a several assay methodologies to determine the total polyphenolic content of a sample through the use of the Folin–Denis and Folin–Ciocalteu reagents and complexation with aluminum III ion. The Folin–Denis or Folin–Ciocalteu reducing reagents are able to form phosphomolybdic–phosphotungstic–phenol complexes, which can be monitored at a visible wavelength of 760 nm via reduction–oxidation reaction. These assays may have some inherent falsely elevated values because of interference as there may be other components in the sample that are also reducing reagents. As previously mentioned, the total phenolic content of the sample can be quantified; thus, this method is a nonspecific measurement of polyphenol content. Alternatively, complexation of polyphenols with aluminum III ion can be used to determine the quantity of polyphenols in the sample monitored at a wavelength of 425 nm. This method is dependent upon the aluminum ion
complexing with the carbonyl and hydroxyl groups of the polyphenol. Again, these processes are not specific for a particular polyphenol; therefore, it is necessary to develop analytical methods to quantify individual polyphenols in a sample to enable determination of a correlation between the amount of a polyphenol in a sample and a health-related benefit.

1.4.1 Hesperidin and Hesperetin

Hesperidin (\((+/-)\) 3,5,7-trihydroxy-4’-methoxyflavanone 7-rhamnoglucoside) C\(_{28}\)H\(_ {34}\)O\(_ {15}\), MW 610.56 g/mol, experimental octanol to water partition coefficient (XLogP) value of -1.1 (Fig. 1.8), is a chiral flavanone-7-O-glycoside consumed in oranges and in other citrus fruits and herbal products. The rutinoside sugar moiety is rapidly cleaved off the parent compound to leave the aglycone bioflavonoid hesperetin (\((+/-)\) 3,5,7-trihydroxy-4’-methoxyflavanone) C\(_{16}\)H\(_ {14}\)O\(_ {6}\), MW 302.28 g/mol, XLogP value of 2.174 (Fig. 1.9), also a chiral flavonoid. There is current interest in the medical use of bioflavonoids, including hesperetin, in the treatment of a variety of cancers and vascular diseases.

1.4.1.1 Antifungal, Antibacterial, and Antiviral Activity

Hesperidin extracted from grapefruit (Citrus paradise Macf., Rutaceae) seed and pulp ethanolic extracts has been related to have antibacterial and antifungal activity against 20 bacterial and 10 yeast strains. The level of antimicrobial effects was assessed employing an in vitro agar assay and standard broth dilution susceptibility test. It was observed that hesperidin exhibits strong antimicrobial activity against Salmonella enteritidis (minimum inhibitory concentration [MIC] of 2.06% extract concentration—m/V), while its activity against other bacteria and yeasts ranged from 4.13% to 16.5% m/V. Furthermore, hesperi-
din has also been observed to have protective effects in infected mice with encephalomyocarditis (EMC) virus and *Staphylococcus aureus* that were administered with hesperidin before or coadministered with the lethal viral-bacterial dose.\(^{107}\)

In the case of the aglycone hesperetin, it has been shown to have MIC > 20 \(\mu g/mL\) against *Helicobacter pylori*. However, neither hesperetin nor other flavonoids and phenolic acids inhibited the urease activity of *H. pylori*.\(^{108}\)

Furthermore, hesperetin has shown to be an effective in vitro agent against severe acute respiratory syndrome (SARS) (or similar) coronavirus (CoV) infections.\(^{109}\) Hesperetin inhibits the SARS-CoV replication by interacting with the spike (S) glycoprotein (S1 domain) in the host cell receptor and fusing the S2 domain with the host cell membrane activating the replicase polyproteins by the virus-encoded proteases (3C-like cysteine protease [3CLpro] and papain-like cysteine protease) and other virus-encoded enzymes such as the NTPase/helicase and RNA-dependent RNA polymerase. The blocking of the S1 may play an important role in the immunoprophylaxis of SARS.\(^{109}\) Similar activities have also been observed for hesperetin against the replication of the neurovirulent Sindbis strain (NSV) having 50\% inhibitory doses (ID\(_{50}\)) of 20.5 \(\mu g/mL\). However, its glycoside, hesperidin, did not have inhibitory activity, indicating the possibility that the rutinose moiety of flavanones blocks the antiviral effect.\(^{110}\) Nevertheless, hesperetin has also been reported to be effective against the replication of herpes simplex virus type 1 (HSV-1), poliovirus type 1, parainfluenza virus type 3 (Pf-3), and respiratory syncytial virus (RSV) in in vitro cell culture monolayers employing the technique of viral plaque reduction.\(^{83}\)

### 1.4.1.2 Antiinflammatory Activity

The inflammatory process involves a series of events encompassed by numerous stimuli such as infectious agents, ischemia, antigen–antibody interactions, and chemical, thermal, or mechanical injury. The inflammatory responses have been characterized to occur in three distinct phases, each apparently mediated by different mechanisms: an acute phase characterized by local vasodilatation and increased capillary permeability, a subacute phase characterized by infiltration of leukocyte and phagocyte cells, and a chronic proliferative phase, in which tissue degeneration and fibrosis occur.\(^{111}\) Different animal models have been developed to study the different phases of an inflammatory response. In the case of testing acute inflammatory response, the carrageenan-induced paw edema in mice\(^{112}\) and the xylene-induced ear edema\(^{113}\) are widely employed. Methods to test the proliferative phase (granuloma formation) include the cotton pellet granuloma model.\(^{114}\) Another model that allows the assessment of acute and chronic inflammation is the adjuvant–carrageenan-induced inflammation (ACII) model to induce adjuvant arthritis.\(^{115}\) Hesperidin and hesperetin were tested under these models, and it was observed that only hesperetin had a positive effect in reducing the carrageenan-induced paw edema in mice by 48\% and 29\% after 3 and 7 hours postinflammatory insult.\(^{111}\) In the case of the
xylene-induced ear edema model, both hesperidin and hesperetin had a positive effect by reducing the edema by 45% and 44%, respectively. Similar observations were observed in the cotton pellet granuloma, whereas hesperidin and hesperetin inhibited granuloma formation by 30% and 28%, respectively. In the case of the ACII model, hesperidin exhibited activity in the acute phase (day 6) by causing a reduction in paw edema of 52% and exhibited a more moderate reduction in the chronic phase (7–21 days) by reducing the paw edema by 36%, 44%, 47%, 38%, and 31% at 7, 8, 10, 12, and 16 days postinflammatory insult, respectively. Different mechanisms to elucidate how hesperidin, hesperetin, and other polyphenols might carry their antiinflammatory activity have been proposed. Among these, it has been observed that after carrageenan injection, there is an initial release of histamine and serotonin during the first 1.5 hours with a posterior release of kinin between 1.5 and 2.5 hours, followed with a release of prostaglandins until 5 hours. Thus, it is believed that hesperidin and hesperetin might be involved with a variety of steps during the development of inflammation.

Other studies have reported that hesperidin downregulates the lipopolysaccharide (LPS)-induced expression of different proinflammatory (tumor necrosis factor-alpha [TNF-α], IL-1 beta, interleukin-6 [IL-6]) and antiinflammatory mediators (IL-12), cytokines as well as cytokines (KC, MCP-1 and MIP-2), while enhancing the production of other antiinflammatory cytokines (IL-4 and IL-10). In this study, mice were challenged with intratracheal LPS (100 µg) 30 minutes before treatment with hesperidin (200 mg/kg oral administration) or vehicle. After 4 and 24 hours, bronchoalveolar lavage fluid was collected, observing that hesperidin significantly reduced the total leukocyte counts, nitric oxide production, and inducible nitric oxide synthase (iNOS) expression. These results correlate with in vitro studies that have demonstrated that hesperidin suppresses the expression of IL-8 on A549 cells and THP-1 cells, the expression of TNF-α, IL-1 beta, and IL-6 on THP-1 cells, and the expression of intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) (responsible for cell adhesion) on A549 cells. The suppression of these inflammatory mediators is regulated by nuclear factor-kappa B (NF-κB) and AP-1, which are activated by IκB and mitogen-activated protein kinase (MAPK) pathways, indicating that hesperidin might interact within these pathways to exert its antiinflammatory activity.

1.4.1.3 Antioxidant Activity Hesperidin and its aglycone, hesperetin, have been assessed in various in vitro chemical antioxidant models (cell-free bioassay systems). It has been observed that both hesperidin and hesperetin exhibited similar patterns of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities. Similar results have been reported elsewhere for hesperidin, an antioxidant that was comparable in efficacy to Trolox® (positive control). Furthermore, hesperetin alone has been reported to effectively scavenge peroxynitrite (ONOO−) in a concentration-dependent manner. Peroxynitrite (ONOO−) is a reactive oxidant formed from superoxide (•O2−) and
nitric oxide (\textsuperscript{NO}), which can oxidize several cellular components, including essential protein, nonprotein thiols, DNA, low density lipoproteins (LDLs), and membrane phospholipids.\textsuperscript{122}

Both hesperidin and hesperetin have also been assessed for their antioxidant capacity \textit{in vivo}. It has been observed that hesperidin (25 mg/kg body weight [BW] p.o.) offers protection against lung damage induced by a subcutaneous injection of nicotine at a dosage of 2.5 mg/kg BW for 5 days a week. Hesperidin treatment resulted in a decreased level of all the marker enzymes, the recovery of the \textit{in vivo} antioxidant status back to near baseline level,\textsuperscript{123} and different matrix metalloproteinases (MMPs) were downregulated.\textsuperscript{124} Hesperidin (60 mg/kg BW/day p.o. for 9 days) has also been shown to increase the free SH-group concentration (SHC), hydrogen-donating ability (HDA), and natural scavenger capacity, and to decrease the hepatic malonaldehyde content and dien conjugate (DC) in male Wistar albino rats with alimentary-induced fatty livers.\textsuperscript{125} Furthermore, hesperidin in the same animal models has been reported to increase both the total scavenger capacity (TSC) and the activity of superoxide dismutase (SOD) in liver homogenates, and to induce slight changes in the Cu, Zn, Mn, and Fe contents of liver homogenates.\textsuperscript{126} Similar results were observed for hesperidin (100 and 200 mg/kg p.o. for 1 week) in CCl\textsubscript{4}-induced oxidative stressed rats, whereas the thiobarbituric acid-reactive substances (TBARSs) decreased and the glutathione (GSH) content, SOD, and catalase (CAT) levels increased in liver and kidney homogenates.\textsuperscript{127} In the case of hesperetin, it was observed to be a potent antioxidant, inhibiting lipid peroxidation initiated in rat brain homogenates by Fe$^{2+}$ and L-ascorbic acid. Hesperetin was found to protect primary cultured cortical cells against the oxidative neuronal damage induced by H$_2$O$_2$ or xanthine and xanthine oxidase (XO). In addition, it was shown to attenuate the excitotoxic neuronal damage induced by excess glutamate in the cortical cultures.\textsuperscript{120}

\subsection*{1.4.1.4 Anticancer Activity \textit{In vitro} tests have shown that hesperidin reduces the proliferation of many cancer cells.\textsuperscript{128} For instance, hesperidin (100 \textmu M) has been shown to reduce the cell viability (65 ± 0.05\%) of human colon cancer cells, SNU-C4 based in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.\textsuperscript{129} It was proposed that hesperidin treatment decreased the expression of B-cell CLL/lymphoma 2 (BCL2) mRNA and increased the expression of BCL2-associated X protein (BAX) and of the apoptotic factor caspase-3 (CASP3) inducing apoptosis.\textsuperscript{129} Another study, less mechanistic in nature, observed that hesperidin and hesperetin at smaller concentrations (1 \textmu M) inhibit the neoplastic transformation of C3H 10T1/2 murine fibroblasts induced by the carcinogen 3-methylcholanthrene.\textsuperscript{130} Hesperetin has been reported to affect the proliferation and growth of a human breast carcinoma cell line, MDA-MB-435, with an IC$_{50}$ of 22.5 \textmu g/mL and to exhibit low cytotoxicity (>500 \textmu g/mL for 50\% cell death).\textsuperscript{88} Furthermore, hesperetin has also been reported to significantly inhibit cell proliferation of MCF-7 cells in a concentration-dependent manner by causing cell cycle
arrest in the G1 phase. In the G1 phase, hesperetin downregulates the cyclin-dependent kinases (CDKs) and cyclins while upregulating p21(Cip1) and p27(Kip1) in MCF-7 cells. Hesperetin also decreases CDK2 and CDK4 together with cyclin D. In addition, hesperetin increases the binding of CDK4 with p21(Cip1) but not p27(Kip1) or p57(Kip2), indicating that the regulation of CDK4 and p21(Cip1)1 may participate in the anticancer activity pathway of hesperetin in MCF-7 cells.\textsuperscript{131}

The Apc\textsuperscript{Min/+} mouse model and the azoxymethane (AOM) rat model are the main animal models used to study the effect of dietary agents on colorectal cancer.\textsuperscript{132} Different chemopreventive agents in the AOM rat model have been analyzed,\textsuperscript{132,133} and it was observed that hesperidin and hesperetin-rich foods are able to suppress colon adenocarcinoma and/or consistently inhibit adenoma and aberrant crypt foci (ACF) in several independent rat studies.\textsuperscript{90,132,134–136} Other animal studies have reported that hesperidin has the capacity to inhibit tumor initiation and promotion in CD-1 mice skin. Subcutaneous application of hesperidin did not inhibit 7,12-dimethylbenz(a)anthracene-induced tumor initiation but did inhibit 12-O-tetradecanoyl-13-phorbol acetate-induced tumor promotion.\textsuperscript{137} Furthermore, male imprinting control (ICR) mice that were N-butyl-N-(4-hydroxybutyl)nitrosamine (OH-BBN) (500 µg/mL) induced for urinary bladder tumors were fed with hesperidin (1 mg/mL), diosmin (1 mg/mL), and combination (4.9 mg/mL diosmin and 0.1 mg/mL hesperidin) for 8 weeks. It was observed that hesperidin and diosmin alone or in combination significantly reduced the frequency of bladder carcinoma and preneoplasia. Also, a significant decrease in the incidence of bladder lesions and cell-proliferation activity estimated by enumeration of silver-stained nucleolar-organizer-region-associated proteins (AgNORs) and by the 5-bromodeoxyuridine (BUdR)-labeling index was observed.\textsuperscript{90} However, other research groups have observed that hesperidin (100 µg/mL) and diosmin (100 µg/mL) alone or in combination (900 µg/mL diosmin and 100 µg/mL hesperidin) provide no pathological alterations during the initiation and post-initiation phases of esophageal carcinogenesis initiation with N-methyl-N-amyl nitrosamine (MNAN) in male Wistar rats.\textsuperscript{138}

1.4.1.5 Cyclooxygenase-1 and -2 Inhibitory Activity Hesperidin has been assessed for its inhibitory effect on LPS-induced overexpression of cyclooxygenase-2 (COX-2), iNOS proteins, overproduction of prostaglandin E\textsubscript{2} (PGE\textsubscript{2}) and nitric oxide (NO) using mouse macrophage cells. Treatment with hesperidin suppressed production of PGE\textsubscript{2}, nitrogen dioxide (NO\textsubscript{2}), and expression of iNOS protein. In the case of COX-2, hesperidin did not affect the protein levels expressed. Thus, hesperidin has been reported to be a COX-2 and iNOS inhibitor, which may explain its antiinflammatory and antitumorigenic efficacies in vivo.\textsuperscript{139} Furthermore, hesperetin and hesperidin in the concentration range 250–500 µM have been shown to potently inhibit the LPS-induced expression of the COX-2 gene in RAW 264.7 cells, also
demonstrating the antiinflammatory activity of these compounds. The ability of hesperetin and hesperidin to suppress COX-2 gene expression has been suggested to possibly be a consequence of their antioxidant activity.\textsuperscript{140}

**1.4.1.6 Antiadipogenic Activity** Obesity is biologically characterized at the cellular level to be an increase in the number and size of adipocytes differentiated from fibroblastic preadipocytes in adipose tissue. It has been reported that hesperidin inhibits the formation of 3T3-L1 preadipocytes by 11.1\%. Apoptosis assays indicate that hesperidin increased apoptotic cells in a time- and concentration-dependent manner. Treatment of cells with hesperidin also decreased the mitochondrial membrane potential in a time- and dose-dependent manner. The cell apoptosis/necrosis assay demonstrated that hesperidin increased the number of apoptotic cells but not necrotic cells. Hesperidin treatment of cells caused a significant time- and concentration-dependent increase in the CASP3 activity. Western blot analysis indicated that treatment of hesperidin also markedly downregulated poly ADP-ribose polymerase (PARP) and Bel-2 proteins, and activated CASP3, Bax, and Bak proteins. These results indicate that hesperidin efficiently inhibits cell population growth and induction of apoptosis in 3T3-L1 preadipocytes.\textsuperscript{141} Furthermore, in the same in vitro, model hesperidin has been recently reported to inhibit intracellular triglyceride and glycerol-3-phosphate dehydrogenase (GPDH) activity by 40.2 ± 3.2\% and 37.9 ± 4.6\%, respectively.\textsuperscript{142}

**1.4.1.7 Other Reported Activities** Hesperidin and its aglycone, hesperetin, have been shown to have a very weak estrogenic effect, and its regular use can alleviate certain symptoms related with menopause and dysmenorrhea.\textsuperscript{143,144} For instance, in a controlled clinical study, 94 menopausal woman with hot flashes were given a daily formula for 1 month containing 900 mg hesperidin, 300 mg hesperidin methyl chalcone, and 1200 mg vitamin C. After 1 month of treatment, the symptoms of hot flashes were completely relieved in 53\% and reduced in 34\% of the women.\textsuperscript{145}

**1.4.2 Naringin and Naringenin**

Naringin ((+/-) 4’,5,7-trihydroxyflavanone 7-rhamnoglucone) C\textsubscript{27}H\textsubscript{32}O\textsubscript{14}, MW 580.53 g/mol, XLogP value of -1 (Fig. 1.10), is a chiral flavanone-7-O-glycoside present in citrus fruits, tomatoes, cherries, oregano, beans, and cocoa.\textsuperscript{146-151} After consumption, the neohesperidose sugar moiety is rapidly cleaved off the parent compound in the gastrointestinal tract and liver to leave the aglycone bioflavonoid naringenin ((+/-) 4’,5,7-trihydroxyflavanone) C\textsubscript{15}H\textsubscript{12}O\textsubscript{5}, MW 272.25 g/mol, XLogP value of 2.211 (Fig. 1.11). The ratio between the amount of naringenin and naringin varies among different food products. For instance, citrus fruits contain higher amounts of the glycoside naringin, while tomatoes have higher amounts of the aglycone naringenin.\textsuperscript{148}
Antifungal, Antibacterial, and Antiviral Activity  

Naringin present in grapefruit (C. paradise Macf., Rutaceae) seed and pulp ethanolic extracts has been related to have antibacterial and antifungal activity against multiple bacteria, fungi, and yeast strains.\textsuperscript{106,152} Naringin was assessed employing an \textit{in vitro} agar assay and standard broth dilution susceptibility test, and it was observed that it exhibited the strongest antimicrobial effect against \textit{S. enteritidis} (MIC of 2.06\% extract concentration—m/V) and an MIC ranging from 4.13\% to 16.5\% m/V for the other tested bacteria and yeasts.\textsuperscript{106} Similar results have been reported for naringin present in Argentine \textit{Tagetes} (Asteraceae)\textsuperscript{153} and in \textit{Drynaria quercifolia}.\textsuperscript{154}

Naringenin isolated from ethanol extracts of propolis from four different regions of Turkey and Brazil exhibited to have MIC values ranging from 4 to 512 \(\mu\)g/mL for all the analyzed bacterial strains. Death was observed within 4 hours of incubation for \textit{Peptostreptococcus anaerobius}, \textit{Peptostreptococcus micros}, \textit{Lactobacillus acidophilus}, and \textit{Actinomyces naeslundii}, while 8 hours for \textit{Prevotella oralis} and \textit{Prevotella melaninogenica} and \textit{Porphyromonas gingivalis}, 12 hours for \textit{Fusobacterium nucleatum}, and 16 hours for \textit{Veillonella parvula}.\textsuperscript{155} Similar results were found for naringenin-rich ethanol extracts of propolis having MIC values of 2 \(\mu\)g/mL for \textit{Streptococcus sobrinus} and \textit{Enterococcus faecalis}; 4 \(\mu\)g/mL for \textit{Micrococcus luteus}, \textit{Candida albicans}, and \textit{Candida krusei}; 8 \(\mu\)g/mL for \textit{Streptococcus mutans}, \textit{S. aureus}, \textit{Staphylococcus epidermidis}, and \textit{Enterobacter aerogenes}; 16 \(\mu\)g/mL for \textit{Escherichia coli} and \textit{Candida tropicalis}; and 32 \(\mu\)g/mL for \textit{Salmonella typhimurium} and
Pseudomonas aeruginosa. Similar MIC values have been observed for naringenin isolated from the capitula of Helichrysum compactum. Naringenin has also been shown to have MIC > 20 µg/mL against H. pylori. However, neither naringenin nor other flavonoids and phenolic acids inhibited the urease activity of H. pylori.

Naringenin has also been reported to have antiviral activity. For instance, naringenin exhibited an inhibitory effect on the replication of the NSV having a 50% inhibitory dose (ID₅₀) of 14.9 µg/mL. However, its glycoside, naringin, did not have inhibitory activity. Similar results were observed for naringin, which was also ineffective on the replication of HSV-1, poliovirus type 1, Pf-3, and RSV in in vitro cell culture monolayers employing the technique of viral plaque reduction. Furthermore, naringenin has demonstrated activity against HSV-1 and type 2 (HSV-2) infected Vero cells in a virus-induced cytopathic effect (CPE) inhibitory assay, plaque reduction assay, and yield reduction assay. However, both naringin and naringenin are ineffective in inhibiting poliovirus replication.

1.4.2.2 Antiinflammatory Activity Naringenin has been reported to have poor or no effect over different inflammatory mediators in vitro. For instance, naringenin was ineffective in inhibiting endothelial adhesion molecule expression or in attenuating expression of E-selectin and ICAM-1, VCAM-1, and TNF-α-induced adhesion molecule expression in human aortic endothelial cells. In another study, naringenin also exhibited virtually no effects on cytokines, metabolic activity, or on the number of cells in the studied cell populations of stimulated human peripheral blood mononuclear cells (PBMCs) by LPS. Furthermore, the lack of ability of naringenin to inhibit the activity of NOS-2 has been reported; however, the induction of NOS-2 protein in LPS-treated J774.2 cell was evident by Western blotting techniques.

However, naringin has been reported to regulate certain inflammatory mediators and to possess antiinflammatory activity. Naringin (10, 30, and 60 mg/kg intraperitoneal [i.p.]) dose dependently suppressed LPS-induced production of TNF-α in mice. To further examine the mechanism by which naringin suppresses LPS-induced endotoxin shock, an in vitro model, RAW 264.7 mouse macrophage cells, was utilized. Naringin (1 mM) suppressed LPS-induced production of NO and the expression of inflammatory gene products such as iNOS, TNF-α, inducible cyclooxygenase (COX-2), and IL-6 as determined by RT-PCR assay. Naringin was also found to have blocked the LPS-induced transcriptional activity of NF-κB in electrophoretic mobility shift assay and reporter assay. These findings suggest that suppression of the LPS-induced mortality and production of NO by naringin is due to inhibition of the activation of NF-κB.

Similarly, a separate study assessed the effect of naringin in an endotoxin shock model based on Salmonella infection. Intraperitoneal (i.p.) infection with 10 cfu S. typhimurium aroA caused lethal shock in LPS-responder but not in LPS-nonresponder mice. Administration of 1 mg naringin 3 hours
before infection resulted in protection from lethal shock, similar to LPS-
nonresponder mice. The protective effect of naringin was time- and dose
dependent. Treatment with naringin resulted not only in a significant decrease
in bacterial numbers in spleens and in livers, but also in a decrease in plasma
LPS levels. In addition, naringin markedly suppressed TNF-α and normalized
the activated states of blood coagulation factors such as prothrombin time,
fibrinogen concentration, and platelet numbers caused by infection. 164

1.4.2.3 Antioxidant Activity Different in vitro chemical and biological
assays have reported that naringin and naringenin have considerable antioxi-
dant properties. For instance, naringin has been reported to scavenge the
DPPH, 2,2′-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) and
nitric oxide (NO) radicals in vitro in a concentration-dependent manner. 165
Furthermore, naringin and naringenin have been assessed in the beta-carotene–
linoleic acid, DPPH, superoxide, and hamster LDL in vitro models to measure
their antioxidant activity. Using the beta-carotene–linoleate model, naringin
(10 µM) and naringenin (10 µM) exhibited an 8% and 9% inhibition, respec-
tively, whereas both compounds demonstrated negative free radical scaveng-
ing activity using the DPPH method and a 25% and 30% inhibition of
superoxide radicals for naringin and naringenin, respectively. Naringin and
naringenin increased the lag time of LDL oxidation to 150 minutes (a 32%
increase from baseline levels). Thus, indicating that both compounds have
significant in vitro antioxidant properties. 166 Furthermore, naringin has been
reported to have a positive effect in iron-induced oxidative stress and in a
variety of cellular processes like respiration and DNA synthesis. For this,
HepG2 cells were treated with 0.5, 1.0, 2.5, and 5.0 mM naringin 1 hour before
exposure to 0.1, 0.25, 0.5, and 1.0 mM ferric iron. Pretreatment of HepG2 cells
with naringin resulted in inhibition of lipid peroxidation, arrested the iron-
induced depletion in the GSH concentration, and increased various antioxi-
dant enzymes like glutathione peroxidase (GSHPx), CAT, and SOD. 167

Naringin has also demonstrated antioxidant properties in different in vivo
animal models. A comparison study between grapefruit juice and naringin
reported that the total antioxidant activity of a quantity of red grapefruit juice
was higher than that of naringin. Animals received a cholesterol-rich diet and
after administration of naringin (0.46–0.92 mg p.o.) or red grapefruit juice
(1.2 mL), it was observed that diets supplemented with red grapefruit juice
and, to a lesser degree, with naringin improved the plasma lipid levels and
increased the plasma antioxidant activity. 168

1.4.2.4 Anticancer Activity Naringin and naringenin have been reported
to have anticancer activities. For instance, naringenin has been reported to
induce cytotoxicity in cell lines derived from cancer of the breast (MCF-7,
MDA-MB-231), stomach (KATOIII, MKN-7), liver (HepG2, Hep3B, and
Huh7), cervix (Hela, Hela-TG), pancreas (PK-1), and colon (Caco-2), as
well as leukemia (HL60, NALM-6, Jurkat, and U937). Naringenin-induced
cytotoxicity was low in Caco-2 and high in leukemia cells compared to other
cell lines. Naringenin dose dependently induced apoptosis, with hypodiploid cells detected in both Caco-2 and HL60 by flow cytometric analysis. Furthermore, naringenin at concentrations higher than 0.71 mM has been reported to inhibit cell proliferation of HT29 colon cancer cells, while naringin has been reported to induce cytotoxicity via apoptosis in mouse leukemia P388 cells and to slightly increase the activities of the antioxidant enzymes, CAT, and GSHPx in these cells.

Naringin and naringenin have also been assessed for its effects on proliferation and growth of a human breast carcinoma cell line, MDA-MB-435. The concentration at which cell proliferation was inhibited by 50% (IC$_{50}$) was around 20 µg/mL for naringin and naringenin with low cytotoxicity (>500 µg/mL for 50% cell death). Two possible mechanisms that could modulate breast tumor growth have been proposed, one via inhibition of aromatase (CYP19) and the other via interaction with the estrogen receptor (ER). Multiple *in vitro* studies confirmed that naringin and naringenin act as aromatase inhibitors potentially reducing tumor growth. It is thought that in the *in vivo* situation, breast epithelial (tumor) cells communicate with surrounding connective tissue by means of cytokines, prostaglandins, and estradiol forming a complex feedback mechanism. It has been reported that naringenin affects MCF-7 proliferation with an EC$_{50}$ value of 287 nM and acts as an aromatase inhibitor with an IC$_{50}$ value of 2.2 µM. These results show that naringenin can induce cell proliferation or inhibit aromatase in the same concentration range (1–10 µM). The second proposed mechanism is related to the ER, and it has been observed that naringenin exerts an antiproliferative effect only in the presence of ER$\alpha$ or ER$\beta$. Moreover, naringenin stimulation induces the activation of p38/MAPK leading to the proapoptotic CASP3 activation and to the poly(ADP-ribose) polymerase cleavage in selected cancer cell lines. Notably, naringenin shows an antiestrogenic effect only in ER$\alpha$-containing cells, whereas in ER$\beta$-containing cells, naringenin mimics the 17beta-estradiol effects. Nevertheless, naringenin-mediated growth arrest in MCF-7 breast cancer cells has also been observed. Naringenin was found to inhibit the activity of phosphoinositide 3-kinase (PI3K), a key regulator of insulin-induced GLUT4 translocation, as shown by impaired phosphorylation of the downstream signaling molecule Akt. Naringenin also inhibited the phosphorylation of p44/p42 MAPK. Inhibition of the MAPK pathway with PD98059, a MAPK kinase inhibitor, reduced insulin-stimulated glucose uptake by approximately 60%. The MAPK pathway therefore appears to contribute significantly to insulin-stimulated glucose uptake in breast cancer cells.

In the case of human prostate cancer cells (PC3) stably transfected with activator protein 1 (AP-1) luciferase reporter gene, the maximum AP-1 luciferase induction is of about threefold over control after treatment with naringenin (20 µM). At higher concentrations, naringenin demonstrated inhibition of AP-1 activity. The MTS assay for cell viability at 24 hours demonstrated that even at a very high concentration (500 µM), cell death was minimal for naringenin. Furthermore, induction of phospho-C-Jun N-terminal kinase (JNK) and phospho-ERK activity was observed after a 2-hour incubation of
PC3-AP-1 cells with naringenin. However, no induction of phospho-p38 activity was observed. Furthermore, pretreating the cells with specific inhibitors of JNK reduced the AP-1 luciferase activity that was induced by naringenin, while pretreatment with MAPK (MEK) inhibitor did not affect the AP-1 luciferase activity. It was also observed that naringenin induced apoptosis of human promyeloleukemia HL60 cells by markedly promoting the activation of CASP3, and slightly promoting the activation of caspase-9, but with no observed effect on caspase-8. The apoptosis-induced mechanism of naringenin has also been linked with the activation of NF-κB and the degradation of IκBα, which has been observed in human promyeloleukemia HL60 cells, in human colon carcinoma HCT116 cells, and in human liver carcinoma HepG2 cells.

Neoangiogenesis is required for tumor development and progression. Many solid tumors induce vascular proliferation by production of angiogenic factors, prominently vascular endothelial growth factor (VEGF). It has been reported that naringin has a significant inhibitory activity against VEGF at 0.1 µM in MDA-MB-231 human breast cancer cells and that glioma cells were similarly sensitive, with U343 more active than U118. Inhibition of VEGF release by naringin in these models of neoplastic cells suggests a novel mechanism for mammory cancer prevention.

Animal models have also demonstrated that grapefruit juice as well as the isolated citrus compound naringin can protect against AOM-induced ACF by suppressing proliferation and elevating apoptosis through antiinflammatory activities. Grapefruit juice suppressed aberrant crypt formation and high multiplicity ACF (HMACF) formation and expansion of the proliferative zone that occurs in the AOM-injected rats consuming the control diet. Grapefruit juice also suppressed elevation of both iNOS and COX-2 levels observed in AOM-injected rats consuming the control diet. Naringin suppressed iNOS levels in AOM-injected rats; no effect was observed with respect to COX-2 levels. Thus, lower levels of iNOS and COX-2 are associated with suppression of proliferation and upregulation of apoptosis, which may have contributed to a decrease in the number of HMACF in rats provided with naringenin. These results suggest that consumption of grapefruit juice or naringin may help to suppress colon cancer development. Similar inhibition in tumor growth and formation in sarcoma S-180-implanted mice have been reported for naringenin.

1.4.2.5 Cyclooxygenase-1 and -2 Inhibitory Activity Naringenin has been assessed for its effects on nitric oxide (NO) and PGE₂ production induced by LPS in the macrophage cell line J774A.1. Naringenin (0.5–50.0 µM) was observed to be a significant inhibitor of NO production, and this effect was concentration dependent and significant at both 5 and 50 µM. A similar pattern was observed with the inhibitory effect of naringenin on LPS-induced PGE₂ release and COX-2 expression. Naringenin markedly decreased PGE₂ release and COX-2 expression in a concentration-dependent manner. Thus, narin-
genin inhibits iNOS and COX-2 expression and may be one of the important mechanisms responsible for their antiinflammatory effects.  

1.4.2.6 Antiadipogenic Activity A recent study has looked at the activity of naringin and naringenin and other flavonoids on preadipocyte cell population growth. The results demonstrated that the inhibition of naringin and naringenin on 3T3-L1 preadipocytes was 5.6% and 28.3%, respectively. Apoptosis assays demonstrated that naringin and naringenin increased apoptotic cells in a time- and concentration-dependent manner. Treatment of cells with naringin and naringenin also decreased the mitochondrial membrane potential in a time and dose-dependent manner. The cell apoptosis/necrosis assay demonstrated that both naringin and naringenin increased the number of apoptotic cells but not necrotic cells. Naringin and naringenin treatment of cells caused a significant time- and dose-dependent increase in the CASP3 activity. Western blot analysis indicated that treatment with both naringin and naringenin also markedly downregulated PARP and Bcl-2 proteins, and activated CASP3, Bax, and Bak proteins. These results suggest that the glycoside naringin and the aglycone naringenin efficiently inhibit cell population growth and induction of apoptosis in 3T3-L1 preadipocytes. Furthermore, in this same in vitro model, naringin and naringenin have been recently reported to inhibit intracellular triglyceride by 41.3 ± 8.4% and 39.4 ± 7.8%, respectively, and also to inhibit GPDH activity by 39.4 ± 5.6% and 35.7 ± 1.4%, respectively.

1.4.2.7 Cardioprotective Effects Naringin (10, 20, and 40 mg/kg, administered orally for 56 days) has been reported to decrease heart weight, blood glucose, serum uric acid, serum iron, levels of total proteins, and iron binding capacity, as well as to increase Na(+)/K(+) ATPase and to decrease the activities of Ca(2+) and Mg(2+) ATPase in the heart and the levels of glycoproteins in serum and in the heart in an isoproterenol (85 mg/kg sc) (ISO)-induced myocardial infarction (MI) animal model. Similar results have been observed for naringin reducing the levels of cardiac troponin T (cTnT), lactate dehydrogenase (LDH)-isoenzymes 1 and 2, cardiac marker enzymes, electrocardiographic (ECG) patterns and lysosomal hydrolases.

1.4.2.8 Effect on Cytochrome P450 Naringin and naringenin are the main flavanones present in grapefruit juice. These compounds have been shown to markedly augment the oral bioavailability of several drugs. This effect was originally based on an unexpected observation from an interaction study between the dihydropyridine calcium channel antagonist, felodipine, and ethanol in which grapefruit juice was used to mask the taste of the ethanol. Naringenin has been reported to competitively inhibit CYP3A4 altering the bioavailability of felodipine, most dihydropyrirdines, terfenadine, saquinavir, cyclosporin, midazolam, triazolam, quinine, verapamil, and one of
the verapamil metabolites, norverapamil,\textsuperscript{187} and this interaction may also occur with lovastatin, cisapride, and astemizole.\textsuperscript{188,189}

Grapefruit juice contains a variety of flavonoid molecules, such as naringin, naringenin, quercetin, and kaempferol, and some nonflavonoid molecules such as 6',7'-dihydroxybergamottin, which are known to inhibit CYP3A4 activity \textit{in vitro}.\textsuperscript{190} These polyphenolic compounds are electron-rich molecules and, therefore, are likely substrates for CYP3A4 and may inhibit the enzyme.\textsuperscript{190} These molecules are known to interfere with intestinal CYP3A4 and hepatic CYP2A6, thereby lowering the biotransformation of several drugs and increasing their bioavailability.\textsuperscript{191} Earlier efforts to identify the inhibitory substance(s) present in grapefruit juice largely focused on naringin and quercetin. However, when administered to humans, both compounds failed to reproduce the inhibition of dihydropyridine metabolism caused by grapefruit juice.\textsuperscript{192,193} Edwards and Bernier\textsuperscript{194} have suggested that naringin and naringenin are not the primary inhibitory compounds in grapefruit juice, although results from rat and human liver microsomes demonstrate that naringenin and other flavonoids in grapefruit juice can inhibit the metabolism of dihydropyridine calcium antagonists.\textsuperscript{195,196} In the continued quest to verify and identify the active inhibitor in grapefruit juice, 6',7'-dihydroxybergamottin, a furanocoumarin, was identified as a potent inhibitor of CYP3A4 activity.\textsuperscript{197} This study was followed by another study that confirmed the presence of 6',7'-dihydroxybergamottin as a major substance in grapefruit juice being responsible for enhanced oral availability of CYP3A4 substrates, although other furanocoumarins probably also contribute to this phenomena.\textsuperscript{198} These results have been corroborated by others\textsuperscript{199} that reported similar findings of altered bioavailability. It has been suggested that hydrophilic components other than flavonoids, probably coumarin derivatives, are also responsible for the inhibitory effect of grapefruit juice. In another recent study, it was found that naringin alone was ineffective in causing the inhibition of the metabolism of 1,2-benzopyrone (coumarin) in humans, thereby concluding that the inhibitory effect of grapefruit juice may be modulated by naringenin.\textsuperscript{191} In view of the existing literature, it is apparent that the inhibition of first-pass metabolism by grapefruit juice probably involves the flavonoid naringenin and also furanocoumarins. Recent reviews on drug interactions with grapefruit juice are available elsewhere.\textsuperscript{200–202} Concern regarding the mechanism of inhibition of CYP3A enzymes by grapefruit juice has now centered on protein expression studies. In a recent study, a selective 62% downregulation of CYP3A4 protein levels in small intestine epithelia (enterocytes) with no corresponding change in CYP3A4 mRNA levels was reported.\textsuperscript{203} In contrast, grapefruit juice did not alter hepatic CYP3A4 activity, colon levels of CYP3A5, or small bowel concentrations of P-glycoprotein, villin, CYP1A1, and CYP2D6. In another study, it was demonstrated that grapefruit juice induced a two- to fivefold increase in the ability of the P-glycoprotein pump to transport drugs such as vinblastine, cyclosporin, losartan, digoxin, and fexofenadine across intestinal cell monolayers \textit{in vitro}.\textsuperscript{204} However, drugs such as nifedipine and felodipine were not transported by P-glycoprotein in these cells, and their passage through the monolayer was unaffected by grapefruit juice.
juice since these drugs are not P-glycoprotein substrates. Orange juice is also known to inhibit the activity of CYP3A enzymes; however, there is a large difference between grapefruit and orange juice in their enzyme inhibition potencies. The difference in potency may be accountable in part to lack of detectable naringin and 6',7'-dihydroxybergamottin in orange juice. Perhaps this may partly explain why orange juice did not affect the bioavailability of orally administered nifedipine or pranidipine, whereas grapefruit juice significantly increased their bioavailability. Nevertheless, red wine, which also contains a complex mixture of flavonoids and other polyphenolic compounds, inhibits CYP3A4 activity in vitro. Interestingly, white wine and its components do not apparently inhibit CYP3A4 activity.

1.4.2.9 Other Reported Activities Naringin has also been reported to have antigenotoxic properties. Naringin was assessed in an in vitro biological model: bleomycin-induced genomic damage of cultured V79 cells. Exposure of V79 cells to bleomycin (50 µg/mL) induced a concentration-dependent elevation in the frequency of binucleate cells bearing micronuclei (MNBNC) and a maximum number of MNBNCs. Treatment of cells with 1 mM naringin before exposure to different concentrations of bleomycin arrested the bleomycin-induced decline in cell survival accompanied by a significant reduction in the frequency of micronuclei when compared with bleomycin treatment alone. The cell survival and micronuclei induction were found to be inversely correlated. The repair kinetics of DNA damage induced by bleomycin was evaluated by exposing the cells to 10 µg/mL bleomycin using single-cell gel electrophoresis. Treatment of V79 cells with bleomycin resulted in a continuous increase in DNA damage up to 6 hours postbleomycin treatment as evident by the migration of greater amounts of DNA into the tails (tail DNA) of the comets and a subsequent increase in olive tail moment (OTM), an index of DNA damage. Treatment of V79 cells with 1 mM naringin reduced bleomycin-induced DNA damage and accelerated DNA repair as indicated by a reduction in percent tail DNA and OTM with an increasing assessment time. A maximum reduction in the DNA damage was observed at 6 hours post bleomycin treatment, where it was five times lower than bleomycin alone.

Other reported effects of naringin include protection against radiation-induced chromosome damage. For this, naringin extracted from the ethyl acetate fraction of Aphananixis polystachya was investigated on the radiation-induced chromosome damage in the bone marrow cells of Swiss albino mice exposed to various doses of gamma radiation. The mice were divided into two groups: One group was exposed to 0, 1, 2, 3, 4, or 5 Gy of gamma radiation, while another group received 7.5-mg/kg BW of the ethyl acetate fraction of A. polystachya 1 hour before exposure to 0, 1, 2, 3, 4, or 5 Gy of gamma radiation. Various asymmetrical chromosome aberrations were studied in the bone marrow cells of mice at 12, 24, or 48 hours postirradiation. Irradiation of mice to various doses of gamma radiation caused a dose-dependent elevation in the frequency of aberrant cells and chromosome aberrations like chromatid
breaks, chromosome breaks, dicentrics, acentric fragments, and total aberrations at all the postirradiation times studied. The maximum asymmetrical aberrations were scored at 24 hours postirradiation except chromatid breaks that were highest at 12 hours postirradiation. A maximum number of polyploid and severely damaged cells (SDCs) were recorded at 24 hours postirradiation in the SPS plus irradiation group. Treatment of mice with 7.5 mg/kg BW of the naringin-rich ethyl acetate fraction of *A. polystachya* before exposure to 1–5 Gy of whole body gamma radiation significantly reduced the frequencies of aberrant cells and chromosomal aberrations like acentric fragments, chromatid and chromosome breaks, centric rings, dicentrics, and total aberrations at all postirradiation scoring times. It can be observed from this study that the naringin-rich ethyl acetate fraction of *A. polystachya* protects mouse bone marrow cells against radiation-induced chromosomal aberrations, and this reduction in radiation-induced chromosome damage may be due to free radical scavenging and reduction in lipid peroxidation. The radioprotection caused by the naringin-rich ethyl acetate fraction of *A. polystachya* is comparable to the protection demonstrated by naringin.

### 1.4.3 Eriocitrin and Eriodictyol

Eriocitrin ((+/−) -5,7,3′,4-tetrahydroxyflavanone 7-O-rutinoside) \( \text{C}_{27}\text{H}_{32}\text{O}_{15} \), MW 596.53 g/mol, XLogP value of −1.4 (Fig. 1.12) is a chiral flavanone-7-O-glycoside present in lemons, tamarinds, and other citrus fruits, as well as in mint, oregano, fennel thyme, and rose hip. After consumption, the neohesperidose sugar moiety is rapidly cleaved off the parent compound in the gastrointestinal tract and liver to leave the aglycone bioflavonoid eriodictyol ((+/−)-5,7,3′,4′-tetrahydroxyflavanone) \( \text{C}_{15}\text{H}_{12}\text{O}_{6} \), MW 288.25 g/mol, XLogP value of 1.837 (Fig. 1.13).

#### 1.4.3.1 Antibacterial Activity

Eriocitrin extracted from peppermint (*Mentha piperita* L.) leaves has been demonstrated to have antimicrobial
activities. Furthermore, eriodictyol extracted from the leaves of *Rhus retinorhoea* Steud, ex Olive has exhibited moderate antimalarial activity with an IC$_{50}$ of 0.98 µg/mL against *Plasmodium falciparum* (W2 clone) and weak activity against *P. falciparum* (D6 clone) with an IC$_{50}$ of 2.8 µg/mL, with no cytotoxic effects. However, eriodictyol isolated from *Gleditsia sinensis* Lam. spines demonstrated a lack of activity against *Xanthomonas vesicatoria* and *Bacillus subtilis*.

1.4.3.2 Antiinflammatory Activity Eriodictyol extracted from *Thymus broussonetii* Boiss (Labiatae) leaves, a herbal drug used in Moroccan traditional medicine, has been assessed using the croton oil ear test in mice and reported significant antiinflammatory properties. Furthermore, pretreatment of RAW 264.7 with eriodictyol inhibited TNF-α release in LPS-stimulated macrophages. The potency of eriodictyol in inhibiting cytokine production was reported with an IC$_{50}$ of less than 10 µM for TNF-α release. It was also observed that pretreatment of cells with eriodictyol decreased IκB-α phosphorylation and reduced the levels of IκB-α.

1.4.3.3 Antioxidant Activity Eriocitrin and eriodictyol isolated from lemon (*Citrus limon*) juice exhibited a potent radical scavenging activity for DPPH and superoxide. Eriocitrin and eriodictyol were found to significantly suppress the expression of ICAM-1 at 10 µM in human umbilical vein endothelial cells (HUVECs) induced by TNF-α. Eriocitrin obtained from peppermint leaves (*Menthae × piperitae* folium) (total eriocitrin 38%) exhibited a strong antiradical activity (determined as DPPH* scavenging features). Eriocitrin also exhibited a strong anti-H$_2$O$_2$ activity. Similarly, eriocitrin extracted from different Mentha species, varieties, hybrids, and cultivars was identified as the dominant radical scavenger in these extracts in an online high performance liquid chromatography-1,1-diphenyl-2-picrylhydrazyl (HPLC-DPPH*) method. Furthermore, eriocitrin was reported to play a role as antioxidant *in vivo* in streptozotocin-induced diabetic rats. Diabetic rats were provided a diet that contained 0.2% eriocitrin. After the 28-day feeding period, the concentration of the TBARS in the serum, liver, and kidney of diabetic rats administered eriocitrin significantly decreased as compared...
with that of the diabetic group. The levels of 8-hydroxydeoxyguanosine, which is exchanged from deoxyguanosine owing to oxidative stress, in the urine of diabetic rats administered eriocitrin significantly decreased as compared with that of the diabetic rat group. Eriocitrin also suppressed oxidative stress in the diabetic rats.\textsuperscript{221}

Eriodictyol isolated from the aerial parts from \textit{Eysenhardtia subcoriacea} was assessed using an antioxidant activity assay-guided chemical analysis, using a rat pancreas homogenate model. The isolated eriodictyol demonstrated moderate radical scavenging properties against DPPH radical\textsuperscript{222,223} and reduced the GSH levels in rat pancreatic homogenate.\textsuperscript{222} Furthermore, eriodictyol was assessed for its protective role against UV-induced apoptosis of human keratinocytes, the principal cell type of epidermis. The results demonstrated that eriodictyol had a positive effect on cell proliferation of human HaCaT keratinocytes. Treatment with eriodictyol, in particular, resulted in significant suppression of cell death induced by UV light, a major skin-damaging agent. It was also observed that eriodictyol treatment apparently reduced the percentage of apoptotic cells and the cleavage of poly(ADP-ribose) polymerase, concomitant with the repression of CASP3 activation and reactive oxygen species (ROS) generation. The antiapoptotic and antioxidant effects of eriodictyol were also confirmed in UV-induced cell death of normal human epidermal keratinocyte (NHEK) cells, suggesting that eriodictyol can be used to protect keratinocytes from UV-induced damage, implying the presence of a complex SAR in the differential apoptosis-modulating activities of eriodictyol and similar flavonoid compounds.\textsuperscript{224}

\subsection*{1.4.3.4 Anticancer Activity} Eriodictyol extracted from lemon fruit (\textit{C. limon} Burm. f.) altered the DNA fragmentation of HL60 cells when analyzed by flow cytometry. An apoptotic DNA ladder and chromatin condensation were observed in HL60 cells when treated with eriodictyol.\textsuperscript{225} Eriodictyol was also assessed for its protective role against UV-induced apoptosis of human keratinocytes, the principal cell type of the epidermis. The results demonstrated that eriodictyol had a positive effect on cell proliferation of human HaCaT keratinocytes. Treatment with eriodictyol in particular resulted in significant suppression of cell death induced by UV light, a major skin-damaging agent. Eriodictyol treatment apparently reduced the percentage of apoptotic cells and the cleavage of poly(ADP-ribose) polymerase, concomitant with the repression of CASP3 activation and ROS generation. The antiapoptotic and antioxidant effects of eriodictyol were also confirmed in UV-induced cell death of NHEK cells.\textsuperscript{224} Eriodictyol also protected L-929 cells from TNF-induced cell death. The magnitude of protection and potentiation by eriodictyol was concentration dependent, and these effects were not altered when eriodictyol was added as much as 2 hours after TNF treatment.\textsuperscript{226} Eriodictyol possess antiproliferative activities against several tumor and normal human cell lines. Eriodictyol has IC\textsubscript{50} of 12, 10, 8.3, and 6.2 \textmu M in human lung carcinoma (A549), melanin pigment producing mouse melanoma (B16 melanoma
4A5), human T-cell leukemia (CCRF-HSB-2), and metastasized lymph node (TGBK11TKB), respectively.\textsuperscript{227}

### 1.4.3.5 Cyclooxygenase-1 and -2 Inhibitory Activity

Eriodictyol extracted from the methanol fraction of the stem bark of *Populus davidiana* demonstrated moderate inhibition against COX-1 only and exhibited suppressive effects on XO.\textsuperscript{228}

### 1.4.3.6 Other Reported Activities

Eriodictyol also been reported to have antimutagenic activities in a model induced by tert-butyl hydroperoxide (BHP) or cumene hydroperoxide (CHP) in *S. typhimurium* TA102 (ID\textsubscript{50} < 1 \(\mu\)mol per plate). These effects correlated with the radical scavenging activities of eriodictyol against peroxyl radicals generated from 2,2′-azo-bis(2-amidinopropane)dihydrochloride (AAPH) as measured in the hemolysis test and confirmed that, in general, eriodictyol is an effective radical scavenger. From these results, it was concluded that in the *Salmonella*/reversion assay with strain TA102, the antimutagenic activities of eriodictyol against the peroxide mutagens CHP and BHP are mainly caused by radical scavenging effects.\textsuperscript{223}

### 1.4.4 Phloretin

Phloretin [3-(4-hydroxyphenyl)-1-(2,4,6-trihydroxyphenyl)propan-1-one] \(\text{C}_{15}\text{H}_{14}\text{O}_5\), MW 274.3 g/mol is a hydrophobic, polyphenolic compound (XLogP 2.6). Phloretin’s structure varies from the stilbenoid structure of pterostilbene and phloretin as it exists as a dihydrochalcone (Fig. 1.14). A dihydrochalcone is defined by the presence of two benzenoid rings connected by three carbons.

Phloretin has been identified in apples and in other natural sources including *Pieris japonica*, *Kalmia latifolia*, *Hoveniae lignum*, and *Loiseleuria procumbens*.\textsuperscript{229–234} Phloridzin is the glycoside of phloretin (phloretin-2′-glucose) (Fig. 1.15). Phloridzin has been identified in apples, strawberries, and in several other plants including *P. japonica* and *Lithocarpus pachyphyllus*.\textsuperscript{233,235–237} After consumption, it has been suggested that the glucose sugar moiety of phloridzin is rapidly cleaved off the parent compound in the gastrointestinal tract and liver to leave the aglycone, phloretin.\textsuperscript{235} It has been suggested that the aglycone

![Figure 1.14. Chemical structure of phloretin.](image)
form of polyphenols is pharmacologically a more active species than the glycoside form.\textsuperscript{238}

Phloretin and phloridzin show structural similarity to other dihydrochalcones specifically those isolated from rooibos (\textit{Aspalathus linearis}), a broom-like shrub commonly used as a caffeine-free tea in South Africa.\textsuperscript{239} Besides a strong antioxidant capacity, rooibos has shown numerous bioactivities including antimutagenic,\textsuperscript{240} hepatoprotective,\textsuperscript{241} and hypoglycemic properties.\textsuperscript{242} Two C-linked dihydrochalcone glucosides have been associated with the antioxidant properties of rooibos including aspalathin and nothofagin.\textsuperscript{243,244} Aspalathin itself has extensive antioxidant abilities, abilities to modulate blood sugars, and hepatoprotective properties.\textsuperscript{242,244,245} Nothofagin demonstrates slightly less potent antioxidant activities.\textsuperscript{243}

Research to characterize the pharmacological activities of both phloretin and phloridzin has shown these compounds reduce oxidative stress, induce apoptosis, and alter glucose transport.\textsuperscript{235,246–256} Most importantly, apples, which contain large amounts of phloretin and phloridzin, have been correlated with numerous health benefits including reduced risk of cardiovascular disease, asthma, some cancers, and diabetes.\textsuperscript{257} A summary of activities of phloretin and phloridzin follows below.

\textbf{1.4.4.1 Glucose Transport} Phloretin and phloridzin both are well-known inhibitors of glucose transport. Phloridzin is an inhibitor of SGLT1 and SGLT2, sodium-linked active glucose transporters found in the small intestine and in the proximal tubule of nephrons.\textsuperscript{235,256} Phloretin is an inhibitor of GLUT2, a facilitated glucose transporter found in the liver, pancreatic $\beta$-cells, and the basolateral surface of kidney and intestinal epithelia.\textsuperscript{255,256} GLUT2 has also been shown to traffic to the apical membrane of intestinal epithelium in response to high glucose concentrations during assimilation of a meal.\textsuperscript{258,259} This apical GLUT2 is a major pathway of sugar absorption.\textsuperscript{260} Polyphenols from strawberries and apples, specifically quercetin, have reported activities in inhibiting this intestinal GLUT2 in Caco-2 cells.\textsuperscript{261} It is thought that since
numerous polyphenols are poorly bioavailable and will attain their highest concentrations in the gastrointestinal tract lumen, they may be promising agents for obesity treatment.\textsuperscript{260}

\textbf{1.4.4.2 Antioxidant Activity} Phloretin possesses potent antioxidant activity that has been attributed to its unique dihydrochalcone structure. Phloretin has been shown to be a potent peroxynitrite, hydroxyl radical, and DPPH radical scavenger and to inhibit lipid peroxidation.\textsuperscript{246,247} In fact, phloretin shows greater antioxidant capacity than flavanones with corresponding structures and functional groups.\textsuperscript{247}

\textbf{1.4.4.3 Anticancer Activity} Numerous groups have examined the \textit{in vitro} and \textit{in vivo} anticancer activity of phloretin and/or phloridzin. This established activity may be attributed to two possible mechanisms. First, the ability of phloretin and phloridzin to inhibit glucose transport, and second, the ability of phloretin to inhibit P-glycoprotein. Additionally, phloretin may alter the expression of apoptosis regulatory proteins. It should be noted that phloretin has been shown to be nontoxic to HUVECs.\textsuperscript{262} Reports of phloretin’s and phloridzin’s anticancer activities are summarized below.

Phloretin and phloridzin have shown \textit{in vivo} activity in rat mammary adenocarcinoma and Fischer bladder cell carcinoma cell lines as determined by a decrease in tumor diameters in comparison to controls.\textsuperscript{248} This result supports the ability of phloretin and phloridzin to block glucose transport into tumor cells \textit{in vitro} and tumor tissues \textit{in vivo}.\textsuperscript{249} Kobori et al. found phloretin induced apoptosis in B16 mice melanoma 4A5 cells. These results were contributed to the inhibition of glucose transmembrane transport by phloretin and possible promotion of Bax (Bcl-associated X) protein expression and caspase activation.\textsuperscript{250} The same research group showed phloretin induces apoptosis in HL60 human leukemia cells that was attributed to inhibition of protein kinase C activity.\textsuperscript{263} Park et al. reported apoptosis in HT29 cells by phloretin through activation of caspases and promotion of Bax expression.\textsuperscript{264} Kim et al. found phloretin induces apoptosis in H-Ras-transformed MCF10A human breast epithelial (H-Ras MCF10A) cells though inducing JNK, p38, and caspase-3.\textsuperscript{265} Additionally, phloretin has been show to potentiate the anticancer actions of paclitaxel in both \textit{in vitro} grown HEP-G2 cells and \textit{in vivo} in xenografted mice. Phloretin was also shown to resensitize liver cancer cells to the effects of paclitaxel and to induce apoptosis.\textsuperscript{253}

Phloretin is suggested to be a P-glycoprotein inhibitor. Zhang et al. demonstrated an increase in accumulation of daunomycin in P-glycoprotein-overexpressing MCF-7/ADR cells incubated with phloretin.\textsuperscript{266} Phloretin has also been shown to inhibit multi-drug resistant (MDR) efflux pumps in human MDR1 gene-transfected mouse lymphoma cells and human breast cancer cells (MDA-MB-231) and Panc-1 cells expressing the MRP1 pump.\textsuperscript{251,252} Phloretin’s P-glycoprotein inhibition activity was further confirmed in MCF-7 and MDA435/LCC6 cell lines.\textsuperscript{267}
1.4.4.4 Antiinflammatory Activity Phloretin inhibited proinflammatory gene expression in studies by Jung et al. This study demonstrated phloretin represses NF-κB, IP-10-, IL-8-promoter-, and STAT1-dependent signal transduction in a dose-dependent manner. Phloretin has also been shown to inhibit mouse T-lymphocyte proliferation.

1.4.4.5 Other Activities Other pharmacological properties of phloretin are starting to be elucidated. Phloretin and phloridzin have been shown to act as phytoestrogens. Additionally, in a study by Stangl et al., phloretin was shown to possess antithrombotic properties. Phloretin suppressed the stimulation of three endothelial adhesion molecules including ICAM-1, VCAM-1, and endothelial leukocyte adhesion molecule-1 (E-selectin). Other studies have shown that apple juices were able to inhibit cytochrome P450 1A1 induction, which may confer reduced activation of certain chemical carcinogens.

1.4.5 Homoeriodictyol Homoeriodictyol (+/−3′-O-methyl-eriodictyol; +/−5,7,4′-trihydroxy-3′methoxy-flavanone; C_{16}H_{14}O_{6}; MW = 302.27 g/mol; XLogP = 1.1) is a chiral flavanone consumed in citrus fruits and herbal products. Homoeriodictyol and its glycosides have been successfully identified or extracted from several plants in a variety of botanical families including Anacardiaceae (Rhus), Asteraeae (Lychnophora), Hydrophyllaceae (Eriodictyon), Loranthaceae (Viscum–), Poaceae (Zea), and Rutaceae (Citrus).

Homoeriodictyol and its analogs have been commercially used as flavor modifiers. Products made from yerba santa have been used in the pharmaceutical industry as bitter remedies for several years. However, these products may not be suitable for many food or pharmaceutical applications because they are too aromatic. Homoeriodictyol, a constituent of yerba santa, and its sodium salt have been used in sensory studies and have been shown to significantly decrease the bitter taste of caffeine without interfering with the desired intrinsic flavors or taste characteristics. Moreover, homoeriodictyol sodium salt has been further investigated for its bitter masking properties in different chemical classes of bitter molecules.

Yerba santa (Eriodictyon glutinosum) is also commercially available. It has been used for the treatment of the common cold and asthma. In the late nineteenth century, alcoholic extracts of yerba santa were used as masking agents for quinine. Currently, yerba santa is being used to enhance the moisturizing and lubricating properties of cosmetic, medical, and dental products.

Biosynthesis of homoeriodictyol has been previously studied. McCormick first described homoeriodictyol as a precursor of the anthocyanin peonidin, a pigment found in both immature and mature seeds in mutant maize aleurone tissue. Subsequently, in 2003, Ibrahim et al. described the fungus Cunninghamella elegans as capable of converting 5,4′-dihydroxy-7,3′-
dimethoxyflavanone into both homoeriodictyol and homoeriodictyol-7-sulfate. The importance of this study was its contribution to the understanding of the possible similarities between mammalian and microbial systems in phase II conjugation reactions as a novel tool in metabolic drug investigations. *C. elegans* carried out C-7 demethylation, and sulfation of 5,4′-dihydroxy-7,3′-dimethoxyflavanone to successfully produce the flavanone homoeriodictyol and its sulfoconjugate. In addition, methylation reactions have also been described to be a part of the biosynthesis of flavonoids. For example, the flavonoids detected in *Catharanthus roseus* have a simple methylation pattern; methyl groups in positions 3′ and 5′ are introduced by an unusual O-methyltransferase that performs two consecutive methylations in the B-ring. A recently identified O-methyltransferase (CrOMT6) was described to methylate the B-ring at 4′ position and, in collaboration with dioxygenases, facilitates the conversion of flavanones into flavones, dihydroflavonols, and flavonols. Homoeriodictyol was reported to be the preferred substrate for CrOMT6, and depending on the acting dioxygenases, the corresponding flavone (flavone synthase), dihydroflavonol (flavanone 3β-hydroxylase), or flavonol (flavonol synthase [FLS], anthocyanidin synthase) resulted as a product.

### 1.4.5.1 Pharmacokinetic Studies

Homoeridictyol and its glucuro- and sulfoconjugates have been detected as metabolites in plasma and/or urine after the oral administration of flavanone, hesperidin or eriocitrin in rats and in humans. Flavanone glycosides or aglycones were administered to healthy male humans, and plasma was analyzed for metabolites using high performance liquid chromatography (HPLC); homoeriodictyol was detected only in samples of volunteers receiving flavanone glycosides but not in those who received flavanone aglycones. Similarly, hesperidin was orally administered to rats, and plasma was analyzed using liquid chromatography–mass spectrometry (LC-MS); homoeriodictyol was detected as a monoglucuronide and as a sulfate metabolite. In another study, eriocitrin was orally administered to rats, and plasma and urine were analyzed using HPLC and LC-MS; both homoeriodictyol and its glucuroconjugate were detected. To our knowledge, only one study has examined the pharmacokinetics of homoeridictyol in rats. Booth et al. administered racemic homoeridictyol at a dose of 150 mg/rat and used paper chromatography for the analysis of homoeridictyol metabolites in urine. Homoeridictyol and its glucuroconjugates, m-hydroxyphenylpropionic acid, m-coumaric acid, and dihydrofurelic acid, were detected in urine after oral administration of homoeridictyol. However, no stereospecific analysis or pharmacokinetic disposition parameters was reported. In another study, homoeridictyol-7-O-β-D-glucopyranoside (HEDT-Glu) was administered to male and female rats via intravenous (IV) injection, and urine and tissues were analyzed via HPLC or LC-MS. The previously developed analytical assays also detected homoeridictyol, but neither reported enantioseparation of homoeridictyol enantiomers.
Pharmacokinetic parameters and tissue distribution values were reported for HEDT-Glu and homoeriodictyol, but individual enantiomers were not analyzed. Plasma concentrations of HEDT-Glu in rats were detectable for at least 5 hours after IV administration; HEDT-Glu was cleared from the blood and was distributed mainly to the liver and small intestine; at 0.083 hours postdose, the concentrations of HEDT-Glu in these tissues were 0.65 ± 0.24 µg/g and 0.51 ± 0.07 µg/g, respectively. In comparison, homoeriodictyol was mainly detected in the kidney, reaching 10.93 ± 2.92 µg/g at 0.083 hours postdose.

1.4.5.2 Pharmacological Activity Homoeriodictyol and its glycosides have been described to possess antimicrobial, antioxidant, anticancer, antiinflammatory, antifungal, and antiosteoporotic activity. Homoeriodictyol was also described to increase coronary flow rate, decrease platelet aggregation, and act as a bitter masking or sweet enhancing agent.

1.4.6 Isosakuranetin

Isosakuranetin (+/- 4’-methylnaringenin; +/- 4’-methoxy-5,7-dihydroxyflavanone; +/- ponciretin; C₁₆H₁₄O₅; MW = 286.28 g/mol; XLogP = 2.3) is a flavanone flavonoid with two enantiomeric forms: 2S- and 2R-isosakuranetin (Fig. 1.16). This flavanone has been identified as an important component of propolis, and several plants found in divergent botanical families including Asteraceae (Baccharis, Combretaceae (Terminalia), Eupatorieae (Chromolaena and Eupatorium), and Rutaceae (Citrus). Didymin (2S-isosakuranetin-7-rutinoside) and poncirin (2S-isosakuranetin-7-neohesperidoside), two main glycosides of isosakuranetin (Fig. 1.17), have been reported exclusively in Rutaceae (Citrus and Poncirus).

Flavanones in nature are found mostly as glycosides, attached to β-neohesperidose or β-rutinoside sugars through the C-7 hydroxyl group. The flavanone neohesperidosides and rutinosides can be easily distinguished by their taste properties: The neohesperidosides are bitter, whereas the rutinosides are tasteless. Hot alkali on 7-β-neohesperidoside splits off the B-ring and carbon-2 to yield phloracetophenone 4’-β-neohesperidoside; however, 7-β-rutinosides do not display phloracetophenone 4’-β-rutinoside formation when exposed to hot alkali but instead generate a sugar–aglycone bond split.

Figure 1.16. Chemical structure of isosakuranetin enantiomers, 2S-isosakuranetin (left) and 2R-isosakuranetin (right).
Isosakuranetin has been included in its glycosylated form (isosakuranetin-7-β-rutinoside) in dietary supplements, vitamins, skin care products, energy drinks and so forth. Isosakuranetin is a major component of propolis. Propolis is a natural resinous substance made by honeybees from plant exudates and used to protect honeycombs against intruders. The composition of propolis depends on the plants in the region and the season in which it is collected by the bees. Propolis has been used in folk medicine and is currently studied for its biological activities. Currently, propolis is extensively incorporated in food and beverages as a dietary supplement.

Biosynthesis of isosakuranetin has been poorly studied. Preliminary evidence of the existence of a “flavanone synthase,” which converts chalcone glycosides into flavanone glycosides, was presented in 1956. The enzymatic activity of flavanone synthase responsible for poncirin chalcone’s conversion into poncirin was studied using various sources including Citrus, Poncirus, Cosmos, and Dahlia. Peel tissue from Citrus aurantium showed the highest flavanone synthase activity. Subsequently, Kim et al. demonstrated the existence of SOMT-2, a soybean (Glycine max, Fabaceae) O-methyltransferase expressed in E. coli capable of converting naringenin into isosakuranetin by methylation at the 4′-hydroxyl position. O-Methylation of flavonoids has been described to alter the chemical reactivity of their phenolic hydroxyl groups and to enhance their lipophilicity.
1.4.6.1 Pharmacokinetic Studies Isosakuranetin and its glucuroconjugates have been previously detected as metabolites in rats administered the flavonoid naringin. According to Silberberg et al., the methylation of 4′-hydroxyl in naringin produced isosakuranetin since aromatic flavonoid compounds can undergo methylation, hydroxylation, and demethylation reactions via bacterial metabolism in the large intestine. Both healthy rats and rats bearing Yoshida’s sarcoma cells produce isosakuranetin and its glucuronides in plasma, urine, liver, and kidney; however, lower concentrations were detected in tumor-bearing rats. A reduction in tumor concentration of flavonoids could be the result of multi-drug resistance-associated protein (MRP) activity, for which flavonoids may act as substrates.

Metabolism of flavonoids has been described to occur in intestinal microflora. Poncirin, for example, is converted to isosakuranetin, 4-hydroxybenzoic acid; 2,4-dihydroxyacetophenone; phloroglucinol; and pyrogallol by human intestinal microflora in vitro, in particular: Fusobacterium K-60, Eubacterium YK-4, and Bacteroides JY-6. Isosakuranetin was further converted to phenolic acid by Streptococcus S-1, Lactobacillus L-2, Bifidobacterium B-9, and Bacteroides JY-6. Shimuzu et al. demonstrated that isosakuranetin in propolis extracts can be incorporated into intestinal Caco-2 cells and transported from the apical to the basolateral side in vitro. These findings are valuable for studies related to intestinal cell function involved in absorption from the gastrointestinal tract. To our knowledge, there are no pharmacokinetic studies of isosakuranetin that acknowledge the importance of its chiral nature and disposition.

1.4.6.2 Pharmacological Activity Isosakuranetin has been previously described to have antimycobacterial, antifungal, antioxidant, antibacterial, neuroprotective, enteroprotective, anticancer, and anti-allergic properties. Poncirin and didymin were found to have numerous biological activities such as antiinflammatory, enteroprotective, antioxidant, and antiplatelet, antiatherogenic, and immunomodulatory properties. However, there is a lack of information regarding the stereospecific activity or disposition of isosakuranetin enantiomers in biological matrices like urine and serum. Achiral analysis of isosakuranetin may be misleading in that absorption, distribution, metabolism, and elimination may all be stereoselective processes. Measuring enantiomers may facilitate the establishment of more meaningful concentration effect relationships of chiral drugs. Separation of enantiomers in biological matrices is thus important to comprehensively understand the stereospecificity of action and disposition of isosakuranetin.

1.4.7 Taxifolin

Racemic taxifolin (+/− 3,5,7,3′,4′-pentahydroxyflavanone; +/− dihydroquercetin; C_{15}H_{12}O_{7}; MW = 304.25 g/mol; XLogP = 0.79–3.73), a dihydroflavonol, and its glycosides have been previously identified in plants included...
in a variety of botanical families including Alliaceae (Allium\textsuperscript{318}); Annonaceae (Cleistopholis\textsuperscript{319}); Apocynaceae (Trachelospermum\textsuperscript{320}); Asteraceae (Silybum,\textsuperscript{321,322} Tessaria,\textsuperscript{323} Centaurea,\textsuperscript{324} and Proustia\textsuperscript{325}); Cactaceae (Opuntia\textsuperscript{326}); Clusiaceae (Garcinia\textsuperscript{327} and Hypericum\textsuperscript{328}); Cupressaceae (Chamaecyparis\textsuperscript{329} and Thuja\textsuperscript{330}); Ericaceae (Rhododendron\textsuperscript{331}); Fabaceae (Acacia,\textsuperscript{332} Genista,\textsuperscript{333} and Trifolium\textsuperscript{334}); Juglandaceae (Englehardtia\textsuperscript{335}); Lamiaceae (Origanum\textsuperscript{147} and Thymus\textsuperscript{212}); Liliaceae (Rhyzoma\textsuperscript{336-340}); Loranthaceae (Taxillus\textsuperscript{341}); Ochnaceae (Ochna\textsuperscript{342}); Oleaceae (Olea\textsuperscript{343}); Pinaceae (Picea,\textsuperscript{344} Pinus,\textsuperscript{345-347} Larix,\textsuperscript{348,349} and Pseudotsuga\textsuperscript{349}); Poaceae (Fussia\textsuperscript{340}); Polygonaceae (Polygonum\textsuperscript{351}); Proteaceae (Helicia\textsuperscript{352}); Smilacaceae (Smilax\textsuperscript{340,353,354}); Solanaceae (Petunia\textsuperscript{355}); and Vitaceae (Ampelopsis\textsuperscript{356} and Vitis\textsuperscript{357}). Likewise, several cultivars of wine have been analyzed for their taxifolin content.\textsuperscript{358,359} The most widely studied of these plants is Rhizoma smilacis glabrae or tu fu ling, which has been used in traditional Chinese medicine to treat cancer and acquired immune deficiency syndrome (AIDS) patients.\textsuperscript{337} Clinically, taxifolin has been used to treat several illnesses including infection of the urinary system, leptospirosis, dermatitis, brucellosis, eczema, acute bacterial dysentery, acute and chronic nephritis, syphilis, arthritis, and folliculitis.\textsuperscript{336-340} Taxifolin has been successfully isolated from R. smilacis glabrae showing high extraction efficiency by sonication and use of hot solvents. Chen et al. did not accomplish total enantiomeric separation of the four taxifolin enantiomers using an HPLC method.\textsuperscript{337} Nevertheless, the separation and identification of the four glycosylated taxifolin enantiomers (neoastilbin, astilbin, neoisoastilbin, and isoastilbin; Fig. 1.18) and racemic taxifolin was attained in this study.\textsuperscript{337}

Taxifolin has been reported to be a potent antioxidant and has been used as a biological active supplement in the food industry.\textsuperscript{360} Taxifolin is commercially available as a food additive and is used in vegetable oils, milk powder, pastry, and so forth. Plants like French maritime pine bark (Pinus pinaster) and katsura (Cercidiphyllum japonicum), in which taxifolin is a major component, are currently being investigated. An extract of French maritime pine bark (P. pinaster), Pycnogenol\textsuperscript{10}, is being used in the treatment of attention deficit

Figure 1.18. Chemical structures of a taxifolin rhamnoside, astilbin.
hyperactivity disorder (ADHD) in Europe with positive results.\textsuperscript{347} Pycnogenol has been demonstrated to stimulate endothelial nitric oxide synthase. Increased production of nitric oxide (NO) may improve brain functions such as memory, learning, and modulation of wakefulness.\textsuperscript{347} Likewise, katsura (\textit{C. japonicum}) has been reported as an effective hair growth control agent.\textsuperscript{361} Polyphenolic compounds in katsura showed proliferation of mouse epithelial cells \textit{in vitro} that are currently being investigated as accelerators of hair regrowth.\textsuperscript{347}

Biosynthesis of taxifolin has been previously studied. Brignolas et al. reported the synthesis of taxifolin glycoside in a fungus-resistant clone of Norway spruce (\textit{Picea abies} Karst.) after inoculation with \textit{Ophiostoma polinicum} Siem, a pathogenic fungus associated with a bark beetle, \textit{Ips typographus} L., but not after sterile inoculation or in an unwounded clone. These findings suggest the flavonoid pathway may be involved in resistance to pathogenic fungi. In that report, CHS activity was described to be higher in the resistant clone than in a clone susceptible to the pathogenic fungus.\textsuperscript{344} Enzymes like CHS have been described to play a role in the biosynthesis of flavonoids. For example, production of rutin was described following administration of exogenous taxifolin (Fig. 1.19) in Satsuma mandarin (\textit{Citrus unshiu}) peel tissues, which demonstrated the ability of peel tissue to convert dihydroflavonols into flavonol glycosides. FLS increased in peel during maturation, unlike other enzymes involved in flavonoid biosynthesis including CHS, CHI, and flavanone 3-hydroxylase (F3H).\textsuperscript{362}

Taxifolin has been described as an intermediate in the biosynthesis of other flavonoids. In this matter, it has been reported that of the four phenolic hydroxyl groups, 7-OH is the most acidic and 5-OH the least acidic.\textsuperscript{323} These findings are important for the determination of the methylation pattern and the possible metabolic products of taxifolin methylation. In \textit{Centaurea maculosa} roots, for example, kaempferol was converted into taxifolin, which, in turn, was converted into catechin. These three flavonoids were described as phytotoxic root exudates produced by \textit{C. maculosa}.\textsuperscript{324} In comparison, Matsuda et al. described the biotransformation of catechin into taxifolin by \textit{Burkholderia} sp KTC-1. This biotransformation occurred in two steps: 4-hydroxylation, and dehydrogenation with the formation of leucocyanidin as an intermediate.\textsuperscript{363} (\textpm)-Catechin 4-hydroxylase and leucocyanidin 4-dehydrogenase were described to accumulate in the cytosol of the aerobic bacteria used in this study.

Only one study considers the isomerization of taxifolin.\textsuperscript{364} (2R3R)-Taxifolin was converted into (2S3R)-taxifolin with the opening of the heterocyclic ring and the formation of an intermediate quinine methide with heat <100°C. When acidic or basic methylation was used under heat >100°C, isomerization did not occur, and alphitonin was formed. Alphitonin (2-benzyl-2′,3′,4′,4′,6-pentahydroxy-3-coumaranone) is a by-product of taxifolin methylation.\textsuperscript{364}

\textbf{1.4.7.1 Pharmacokinetic Studies} There are a paucity of studies on the pharmacokinetics of taxifolin.\textsuperscript{365,366} A pharmacokinetic analysis of maritime
Figure 1.19. Biotransformation pathway of flavonoids in *Citrus unshiu*. (1) Naringenin chalcone; (2) (+/-)-naringenin; (3) (+/-)-dihydrokaempferol; (4) (+/-)-taxifolin; (5) quercetin; (6) rutin. The asterisks denote chiral centers. Adapted from Moriguchi et al.\textsuperscript{362}
pine bark extract demonstrated the presence of taxifolin in human plasma after single and multiple doses of the extract were administered orally (AUC_{0–t} = 2311.11 ± 85.98 ng/mL × h; C_{max} = 33.34 ± 12.54 ng/mL; t_{max} = 8.2 ± 2.5 hours). Likewise, after oral administration of Pycnogenol, the active constituent of maritime pine bark, taxifolin was detected in human urine. Flavonoid metabolism in humans is known to involve the intestinal microbiota, as well as liver enzymes. Among the microflora shown to participate in the metabolism of taxifolin, *Eubacterium ramulus* and *Clostridium orbiscindens* have been described to convert taxifolin into phenolic acids. *E. ramulus* is found in human feces and has been described to convert quercetin into taxifolin, which, in turn, is converted into its chalcone; following a series of additional conversions, taxifolin is finally converted into 3,4-dihydroxyphenylacetic acid (Fig. 1.20). Likewise, *C. orbiscindens* is found in human feces and has also been described to have the ability to convert taxifolin into 3,4-dihydroxyphenylacetic acid (Fig. 1.21). However, *C. orbiscindens* is an asaccharolytic organism that relies on the deglycosilation performed by human tissues (small intestine, liver) and bacteria such as *E. ramulus*, *Enterococcus casseliiflavus*, and *Bacteroides* sp. for flavonoid degradation. Flavonoid metabolism by liver enzymes has been studied by Nielsen et al.; in their study, cytochrome P450 activity did not appear to be involved in taxifolin metabolism in rat liver microsomes. Nielsen et al. described the structural characteristics and the tentative products of flavonoids metabolized by liver enzymes: (1) Flavonoids without a 4′-hydroxyl group in the B ring undergo hydroxylation by microsomal enzymes to catechol (3′,4′-dihydroxyl) structures; (2) flavonoids with a 4′-methoxyl group, but not those with a methoxyl in the 3′-position, undergo demethylation into the hydroxyl compound (i.e., isosakuranetin); and (3) flavonoids with two or more hydroxyl groups in the B ring or a 3′-methoxyl group are not metabolized by microsomal enzymes (i.e., homoeodictyol, taxifolin). In addition, cytochrome P450s involved in flavonoid metabolism have been described to exhibit stereoselectivity.

### 1.4.7.2 Pharmacological Activity

Several of the plants described to possess taxifolin are used in traditional and clinical medicine, some are ingested in the human diet, and others are being studied for their potential use in drug development.

Racemic taxifolin and its glycosides have been previously studied for their potent antioxidant properties. Taxifolin is a very common antioxidant additive in the food industry and has also been described to have antiinflammatory and analgesic properties, hepatoprotective capacity, free radical scavenger activity, and also demonstrates a protective role in plants against pathogens. (2R3R)-(2R3R)-(+) -Taxifolin, one of its four enantiomers, has been described to possess tyrosinase inhibitory capacity, and thus it is used in depigmentation drugs and whitening cosmetics, as well as a food additive and an insect control agent.
Figure 1.20. Metabolism of taxifolin by *Eubacterium ramulus*. (1) Quercetin; (2) (+/-)-taxifolin; (3) taxifolin chalcone; (4) hydrokaempferol chalcone; (5) phloroglucinol; (6) 2-keto-3-(3,4-dihydroxyphenyl)propionic acid; (7) 3,4-dihydroxyphenylacetaldehyde; (8) 3,4-dihydroxyphenylacetic acid. The asterisks denote chiral centers. Adapted from Schneider and Blaut.\textsuperscript{367}
Figure 1.21. Biotransformation of taxifolin by *Clostridium orbiscindens*.
1.4.8 Sakuranetin

Sakuranetin (Fig. 1.22), \(+/−\)-7-O-methylnaringenin, \(2,3\)-dihydro-5-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-4\(H\)-1-benzopyran-4-one, \(5\)-hydroxy-2-(4-hydroxyphenyl)-7-methoxy-2,3-dihydro-4\(H\)-chromen-4-one, or \(+/−\)-5,4′-dihydroxy-7-O-methoxyflavanone\) is a chiral flavanone that has been detected or isolated from numerous plants including rice \(\text{(Oryza sativa L.}\)), fingerroot \(\text{(Boesenbergia pandurata}\)), Dodonaea viscosa \(\text{(L.}\) Jacq. \(\text{(Sapindaceae}\)), yerba santa \(\text{(Eriodictyon californicum}\)), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron robinsonii)}\), Eucalyptus maculate \(\text{Hook}\), Larrea tridentata, Loranthaceae \(\text{(Phoradendron rob}

Induction of sakuranetin was achieved by UV light, blast infection, copper chloride, amino acid conjugates of jasmonic acid, methionine, coronatine, and chitosan oligomers in rice leaves. This induction of sakuranetin could be counteracted by tiron, kinetin, and zeatin. Production of sakuranetin in rice cells and rice leaves was accomplished by endogenously applying jasmonic acid, ethylene, and ethephon. Sakuranetin has been synthesized, induced, and produced in a variety of ways. Synthesis of sakuranetin derived from naringenin involves naringenin 7-O-methyltransferase in plants.

1.4.9 Gallic Acid

There has been an increased interest in both the study and consumption of natural products as evidenced by the increase in nutraceutical sales and the practice of alternative medicine worldwide. Use of these products is
substantial through health and beauty, dietary supplement, performance enhancement supplements, food and beverage, to overall health and well-being products. These xenobiotics can be extracted from plants or can act as precursors to drugs that can be further modified, synthesized, formulated, manufactured, and subsequently sold for their reported health benefits. Through evidence-based pharmaceutical and medical research, a better understanding of how or whether natural products can be used as therapeutics can be attained.

Secondary plant metabolites such as tannins are well known and typically have important roles in plant–plant and plant–animal interaction roles, more specifically in adaptation and esthetics. Tannins are appreciated by beer and wine connoisseurs due to their abundance in these beverages. The popularity of wine, particularly in France, has led to extensive studies of wine content. The phenomena widely known as the “French paradox” is the supposition that a regular intake of red wine in the diet may, because of its constituent phytochemicals and their protective effects, allow for the consumption of saturated fats without a high mortality from coronary heart disease (CHD). The exact mechanism of the French paradox has not been established; however, research thus far has indicated correlations with reactive oxygen and reactive nitrogen species scavenging consistent with polyphenol consumption.

Hydrolyzable tannins, as opposed to condensed tannins, are a subsection of plant tannins that can be described as esters of gallic acid. β-Glucogallin is also referred to as [(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl] 3,4,5-trihydroxybenzoate, 1-galloylglucose, 1-galloyl-β-glucose, 1-galloyl-glucose, and 1-O-galloyl-β-D-glucose, while gallic acid is identified as gallate and 3,4,5 trihydroxybenzoic acid (Fig. 1.23). β-Glucogallin and/or gallic acid have been detected and/or isolated from a variety of botanicals; however, the coelution of deleterious matter are often present in these studies. Amla, also known as the Indian gooseberry, is widely consumed as a fruit in India and is highly marketed as the major constituent of numerous health and beauty products. As β-glucogallin is thought to be a major component of amla

![Figure 1.23. Structure of (A) β-glucogallin and (B) gallic acid.](image)
and as the popularity of nutraceuticals has increased, there is a need to characterize the pharmacokinetics of these compounds.

Sources of β-glucogallin and gallic acid have been utilized in traditional folk medicines as skin lighteners and other beauty esthetics, in the treatment for skin disorders, and for termination of hemorrhages. Gallic acid is readily used in the pharmaceutical industry as an indicator of total phenol content and is a starting molecule for the synthesis of gallates.

1.5 CONCLUSIONS

Flavonoids are a large family of compounds that, as presented, follow a complex biosynthesis in plants that depend on host, the region of origin, and the environmental stimuli. Furthermore, it has been presented that these compounds have been studied for over 80 years, and multiple pharmacological activities have been identified in vitro and preclinically. These activities make them very attractive to pharmaceutical and nutraceutical companies that can use them by modifying their structures to improve efficacy and specificity. However, many of the studies have overlooked the fact that many of these flavonoids are chiral, which warrants the need for more studies that could allow these compounds to eventually move into the clinic and be able to obtain plant-derived drugs in the market.

REFERENCES


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