**Bioavailability and Recent Advances in the Bioactivity of Flavonoid and Stilbene Compounds**

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**ABSTRACT:**
Polyphenols such as flavonoids and stilbenes are abundant in our daily diet, and their roles in the protection and prevention of various diseases are substantial. However, the bioavailability varies among polyphenols and the actual compounds acting on the designated tissues are often not the native molecules consumed in the diet. Hereby, we review the bioavailability of the main classes of stilbenes and flavonoids, namely flavanones, flavones, isoflavones, flavanols, flavonols and anthocyanins, emphasizing on their absorption, distribution, metabolism and excretion (ADME). The bioavailability summary can be useful for future experimental design, especially the emphasis on the bioactivity on targeted tissues and organs. In addition, we review the bioefficacy of the polyphenols, emphasizing recent advances on health benefits both *in vivo* and *in vitro*. Other issues of importance, such as structure, food source and synthesis methods, are also considered.

**KEYWORDS:** Polyphenols, flavonoids, resveratrol, bioavailability, absorption, distribution, metabolism, excretion, bioactivity

**INTRODUCTION**

Flavonoids are plant secondary metabolites found in the plant kingdom and the largest phenolic group in nature. They are common in our daily diet such as fruits, vegetables, herbs, red wine and tea. A major part of their function in plant is related to their interactions with extreme environments. For example, flavonoids accumulated in epidermal cells by UV induction can act as a UV protectant by absorbing the radiation. Upon wounding or infection by pathogens, flavonoids also act as toxin and fungal pathogens as well as antimicrobial compounds. The high concentration and wide variety of flavonoids found in seed coats is attributed to the antifungal and antimicrobial properties of flavonoids to protect the seeds and indirectly facilitate plant reproduction[1]. Microbial is not necessary harmful to plants, especially in the case with leguminous plant, when they are in their nutrient-limited condition or more specific nitrate-deficient state. The plants induce flavonoids accumulation to attract nitrogen-fixing bacteria, *Rhizobium*[2], which convert nitrogen from the atmosphere into plant usable ammonium in the nodule. In return, the plant provides microaerobic environment and offers organic acids necessary for the bacteria as carbon sources[3]. This form of relationship between the plant and the bacteria is known as legume-*Rhizobium* symbiosis. Other proposed functions of flavonoids in plants include reproductive tissues protection, pollinator attraction, seed dispersal, coloration, feed deterrent and enzyme inhibition. Flavonoids are also involved in photosynthesis, morphogenesis and sex determination[1, 2, 4]. From all the benefits flavonoids possess, even though these compounds are not essential for cell survival, they serve a key role in giving the organism an evolutionary advantage to survive and reproduce[5], both in terms of physiological function and biochemical properties.

Stilbenes do not belong to the flavonoids but they share high resemblance to flavonoids in both functions in plant and chemical structure. Stilbenes are synthesized naturally by distinct plants and they are synthesized in response to infection by pathogens (phytoalexins) and ultraviolet light exposure, and are also involved in bacterial root nodulation and coloration[6, 7].

**Chemical Structure**

Flavonoids share a common three-ring structure but different subclasses, including chalcones, flavanones, flavones, flavonols, and anthocyanidins, differ mainly at the middle heterocycle ring (C-ring) where two benzene rings are linked. In the case of isoflavones, in addition to the difference in the C’ ring, there is also a position shift on the phenyl ring B. All structures are shown in Fig.(1) and detailed in the Table (1) below.

<table>
<thead>
<tr>
<th>Polyphenol</th>
<th>Structure Description (A- and B-ring at position 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavanones</td>
<td>Linked by a tetrahydropyrene</td>
</tr>
<tr>
<td>Isoflavones</td>
<td>Linked by a pyrone, but ring B is substituted at position 3</td>
</tr>
<tr>
<td>Flavones</td>
<td>Linked by a pyrone</td>
</tr>
<tr>
<td>Flavanols</td>
<td>Linked by a tetrahydropyrene hydroxylated at position 3</td>
</tr>
<tr>
<td>Anthocyanins</td>
<td>Linked by a pyrylim hydroxylated at position 3</td>
</tr>
<tr>
<td>Stilbenes</td>
<td>Linked by a methylene bridge</td>
</tr>
</tbody>
</table>

Table 1: Structure description of individual flavonoids and stilbenes

In addition to the structure of flavonoids discussed, subsequent substituent can occur with the attachment of organic acids, sugar group and hydroxyl group. On the A ring, typical flavonoids are hydroxylated at the 5 and 7 positions but modification in the A ring can exist, for instance, isoflavone hydroxylated at both 5, 7 positions is known as genistein while the other counterpart with only one hydroxyl group at position 7 is named daidzein. On the B ring, hydroxyl and methoxyl groups substitution usually occur on the 3’, 4’ or 5’ position. At last, C ring substitution is the uncommon one to flavonoids except for catechins and anthocyanidins, in which both the
Flavonoids and stilbene distribution in common foods[8-11].

<table>
<thead>
<tr>
<th>Category</th>
<th>Source</th>
<th>Polyphenol (content in mg/kg or mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fruits</strong></td>
<td></td>
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</tr>
<tr>
<td>Orange</td>
<td>Flavanones (400-600)</td>
<td>Flavonols (0-50) Flavones (0-15)</td>
</tr>
<tr>
<td>Lemon</td>
<td>Flavanones (150-250)</td>
<td>Flavonols (0-28) Flavones (0-15)</td>
</tr>
<tr>
<td>Lime</td>
<td>Flavanones (450)</td>
<td>Flavonols (5) Flavones (0-15)</td>
</tr>
<tr>
<td>Blackcurrant</td>
<td>Anthocyanins (1300-4000)</td>
<td>Flavonols (30-130) Flavonols (10)</td>
</tr>
<tr>
<td>Strawberry</td>
<td>Anthocyanins (150-750)</td>
<td>Flavonols (40) Flavonols (10-20)</td>
</tr>
<tr>
<td>Olives</td>
<td>Flavones (80-200)</td>
<td>Flavonols (60-120) Flavones (0-15)</td>
</tr>
<tr>
<td>Avocado</td>
<td>Flavanols (0.2-5)</td>
<td>Flavonols (0.5) Flavonols (5)</td>
</tr>
<tr>
<td>Plum</td>
<td>Anthocyanins (20-250)</td>
<td>Flavonols (50-100) Flavonols (10-15)</td>
</tr>
<tr>
<td>Black Grape</td>
<td>Anthocyanins (300-7500)</td>
<td>Flavonols (30-175) Flavonols (5-40)</td>
</tr>
<tr>
<td><strong>Vegetables</strong></td>
<td></td>
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<tr>
<td>Soybean</td>
<td>Isoflavones (200-900)</td>
<td>Flavonols (5-35) Flavones (0-15)</td>
</tr>
<tr>
<td>Celery</td>
<td>Flavones (20-140)</td>
<td>Flavones (0-15) Flavonols (5)</td>
</tr>
<tr>
<td>Broccoli</td>
<td>Flavanols (50-100)</td>
<td>Flavonols (0.5-10) Flavones (0-15)</td>
</tr>
<tr>
<td>Peanut</td>
<td>Stilbenes (0.4-5-1)</td>
<td>Flavonols (0-10) Flavonols (0-15)</td>
</tr>
<tr>
<td>Onions</td>
<td>Flavonols (300-1500)</td>
<td>Flavonols (0-10) Flavones (0-15)</td>
</tr>
<tr>
<td>Kale</td>
<td>Flavonols (300-600)</td>
<td>Flavonols (0.5-10) Flavones (0-15)</td>
</tr>
<tr>
<td>Tomato</td>
<td>Flavonols (3-20)</td>
<td>Flavonols (6-15) Flavonols (0-15)</td>
</tr>
<tr>
<td>Cabbage</td>
<td>Anthocyanins (250)</td>
<td>Flavonols (5) Flavones (0-15)</td>
</tr>
<tr>
<td><strong>Beverages</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green Tea</td>
<td>Flavanols (100-800)</td>
<td>Flavonols (40) Flavones (0-15)</td>
</tr>
<tr>
<td>Black Tea</td>
<td>Flavanols (60-500)</td>
<td>Flavonols (10-60) Flavones (0-15)</td>
</tr>
<tr>
<td>Beer</td>
<td>Flavanols (1-10)</td>
<td>Flavonols (0-10) Flavones (0-15)</td>
</tr>
<tr>
<td>Cider</td>
<td>Flavanols (40)</td>
<td>Flavonols (0.5-10) Flavones (0-15)</td>
</tr>
<tr>
<td>Soy milk</td>
<td>Flavonols (30-175)</td>
<td>Flavonols (0.5-10) Flavones (0-15)</td>
</tr>
<tr>
<td>Red Wine</td>
<td>Anthocyanins (200-350)</td>
<td>Flavonols (80-300) Flavonols (2-30)</td>
</tr>
<tr>
<td><strong>Miscellaneous Foods</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chocolate</td>
<td>Flavanols (35-600)</td>
<td>Flavonols (5-30) Flavones (0-15)</td>
</tr>
<tr>
<td>Honey</td>
<td>Flavonols (1-60)</td>
<td>Flavonols (3-50) Flavones (0-15)</td>
</tr>
<tr>
<td>Fruit Jams</td>
<td>Flavanols (1-380)</td>
<td>Flavonols (10-100) Flavones (0-15)</td>
</tr>
<tr>
<td>Rosemary</td>
<td>Flavonols (10)</td>
<td>Flavonols (0-10) Flavones (0-15)</td>
</tr>
<tr>
<td>Parsley</td>
<td>Flavonols (200-2400)</td>
<td>Flavonols (5-10) Flavones (0-15)</td>
</tr>
</tbody>
</table>

Presence in food
Flavonoids and stilbenes are presented in an abundant amount in our daily diet. Table (2) presents the polyphenols distributions in a few common foods.

Flavanones
Flavanones main dietary sources are restricted to citrus products such as orange (50-60mg/100g), grapefruit (30-55mg/100g), lemon (17-26mg /100g) and lime (17-47mg/100g)[8-10]. The main aglycones of flavanones are naringenin and hesperetin, but they are more commonly found in glycosides[11].

Isoflavones
In human diet, isoflavones are primarily found in leguminous plant. Soy bean is a major source of isoflavones, comprise of genistein, daidzein and their glucosides, genistin, daidzin. In both soybean and soybean related products like miso, soy milk and soy flour, isoflavones exist mostly in glucosides rather than the aglycone[12]. In miso, the concentration of genistein is higher than other soy product due to the microbes in the fermentation process which help convert glucosides to the unconjugated form.

Flavonols
Flavonols are widely distributed in human diet and similar to flavones, flavonols usually occur in diet as glycosides[13]. The major aglycones of flavonols are kaempferol and quercetin. Flavonols found in vegetables, fruits and beverages are generally low except for kale (300-450mg/kg), onion (300-1300mg/kg), broccoli, leeks, beans, apple, red wine and tea[11, 13].

Flavanones
Unlike most of flavonoids, the presence of flavanol glucosides are rare[13]. The most abundant flavanols in plant are catechins, gallo catechins and catechin gallates. Catechins can be found in a variety of fruits, red wine, tea and chocolate. Apricot can be reported to have the highest amount of flavanols among fruits (240-1850mg/kg and 20-950mg/kg respectively)[10, 11].

Anthocyanins
Due to the fact that anthocyanins are responsible for most of the red, blue and purple colors of different parts of plant, there is no surprise that anthocyanin are abundant in berries, grapes, grains and red wine. The richest source of anthocyanins is in berries, in which their content can top 10mg per 100g of edible portion of fruits[10]. Red wine also contain anthocyanin but in a minute amount. The reason the glycoside forms, anthocyanins are presented in this review instead of the aglycones, anthocyanidin is because the aglycones are highly unstable and rarely found in the plant. In recent years, anthocyaninss have drawn huge interest in their function as natural food colorants, much preferred than synthentic dyes. As a result, small amount of anthocyanins could also be consumed through healthy food colorant.
Stilbenes

One of the most recognized stilbene, resveratrol is found in tiny quantities in human diet and its primary dietary sources are peanuts (0.02-1.92mg/kg), cranberries and grapes (0.16-3.54 mg/kg)[14]. Therefore, there is no surprise that resveratrol is also found in wine (0.1-14.3mg/L)[9]. Red wine possesses a higher concentration of resveratrol compared to white because red wine is fermented together with the grape skin. This revealed that resveratrol is accumulated mostly in the skin and is consistent with the “red-better-than-white” hypothesis[15]. Generally, stilbene does not exist as single compound but as a mixture of multiple isomers or metabolites[7]. Besides trans-resveratrol, its cis isomer, resveratrol glycosides: trans- and cis-piceid also exist.

Biosynthesis in Nature

Individual pathways for the biosynthesis of flavonoids and stilbene are shown in Fig. (1). In plant, hydroxycinnamates-CoA ester, particularly p-coumaroyl-CoA serves as precursor for the biosynthesis of various plant secondary products, such as stilbenes and flavonoids. The ester is synthesized from amino acids building blocks, phenylalanine or tyrosine. First, the enzyme phenylalanine ammonia lyase (PAL) or tyrosine ammonia lyase (TAL) converts the amino acids into phenylpropanoic acids followed by another pivotal enzyme 4-coumaroyl-CoA ligase (4CL) that converts the phenylpropanoic acids to their CoA-esters.

Stilbenes and Chalcones

To synthesize both stilbenes and chalcones, the CoA-esters are extended by three rounds of stepwise decarboxylative condensation with malonyl-CoA to form a tetraketide intermediate. This is where two closely related enzymes, stilbene synthase (STS) and chalcone synthase (CHS) take place. Austin et al. published an excellent article that clarifies the intramolecular condensation mechanisms of the two highly similar sequence enzymes, CHS and STS which lead to the fabrication of two different products[16]. CHS mediates the claisen cyclization of the tetraketide intermediate (carbon 6 to carbon 1) and offloading naringenin chalcone, which is the first flavonoid compound formed. On the other hand, stilbene synthase (STS) is responsible for the aldol-type cyclization (carbon 2 to carbon 7) and decarboxylation to form trans-3, 5, 4′-trihydroxystilbene or resveratrol[17].

Flavanones

Flavanone synthase (IFS) is responsible for the oxidative attack and the ring migration of the flavanone precursor[18]. IFS, a type II P450 enzyme requires binding to the intracellular membrane and association with another membrane bound protein CPR that provides an electron to IFS via flavin mononucleotide (FMN) and flavin adenine dinucleotide FAD. As a result, 2-hydroxyflavonones are formed with 3-hydroxyflavanones as the byproduct. Subsequently, dehydration occurs spontaneously to form the final product, isoflavone. This 1,2-elimination of a water can be facilitated by a third enzyme, namely2-hydroxyisoflavanone dehydratase (HID)[19].

Figure 1 | Flavonoids and stilbene biosynthetic pathway. Enzyme abbreviations in text are as follows: PAL: phenylalanine ammonia-lyase; C4H: cinnamate 4-hydroxylase; TAL: tyrosine ammonia-lyase; 4CL: 4-coumaroyl:coenzyme A ligase; STS: stilbene synthase; CHS: chalcone synthase; CHI: chalcone isomerase; IFS: isoflavones synthase; FSI: flavone synthase; FHT: flavanone 3β-hydroxylase; FLS: flavonol synthase; DFR: dihydroflavonol 4-reductase; LAR: leucoanthocyanidin reductase; ANS: anthocyanidin synthase; 3GT: UDPG-flavonoid
Flavones are another subgroup of flavonoids that utilize flavanones as the precursors. In plants, the flavones biosynthesis is governed by two distinct enzymes: flavone synthase I (FSI), which is found only in plants belonging to the Apiceae family, and flavone synthase II (FSII), a membrane-bound protein ubiquitous in plants [18].

Flavonols

Two vital enzymes are required to produce flavonols. First, flavanones are converted to dihydroflavonols (DHF) by flavanone 3β-hydroxylase (F3H or FHT). DHFs are then used as the substrate for the second enzyme, flavonol synthase (FLS) to synthesize flavonols. Both the enzymes are 2-oxoglutarate-dependent enzymes.

Flavanols and Anthocyanins

The further down the molecules in the flavonoid biosynthesis pathway, the more tedious it is to synthesize. This is the case for anthocyanins in which five enzymes are required prior to the formation of flavanone (FHT, DFR, LAR, ANS and 3GT). Both anthocyanin and flavanol are grouped into the same section because flavanol can be one of the intermediates for anthocyanin biosynthesis. Similar to the first step towards producing flavonols, the biosynthesis of anthocyanidins utilize FHT to form DHKs. DHKs are used as substrates for dihydroflavonol 4-reductase (DFR) to produce leucoanthocyanidins. Subsequently, catechins are formed using leucoanthocyanidin reductase (LAR), in which it removes the hydroxyl group at position 4 from leucoanthocyanidins. Next, anthocyanidin synthase (ANS) takes place and this enzyme catalyzes the reduction of either catechins or leucoanthocyanidins to form anthocyanidins. At last, anthocyanidins are converted to anthocyanins with UDPG-flavonoid 3-O-glucosyl transferase (3GT).

Biosynthesis Methods

In the past few decades, different attempts have been made to synthesize flavonoids and stilbenes with the purposes of utilizing them as valuable nutraceuticals and colorants. Chemical synthesis or to be more precise organic synthesis is one of the more common approaches for polyphenolics biosynthesis and often, it takes multiple steps to synthesize the product of interest. However, this approach has a few limitations: first, being the starting materials for some of the reactions are cost prohibitive for large scale synthesis and second, in some instances, products are formed as racemic mixtures, which are highly unfavorable for purification. By-products of each synthesis steps can also be problematic and they have to be removed to prevent contamination of the final products. On the other hand, the main benefit of this method is it offers diverse structure modification to bioactive polyphenols. By using the polyphenols as a lead, this approach offers huge potentials in fabricating more desirable molecules, potentially with a better potency and more desirable traits, for instance higher binding affinity, less toxicity, altered lipophilicity and higher oral bioavailability. Biddle et al. recently reported the discovery of a bifunctional quinoline-derived thiourea catalyst that effectively regulates the chemical outcome and produces either a left-handed molecule or a right-handed molecule, not a one-to-one mixture of both. This catalyst was used in flavanones and chromanones synthesis and high yields were reported with excellent enantioselectivity, in addition to the advantages of further modification capability [20]. Apart from chemical synthesis, different polyphenolics biosynthesis approaches with each offers advantages and disadvantages are available, for example plant extract, plant cell cultures, and recombinant microorganisms.

Plant extracts
Plant kingdom is the origin of many potent drugs, and plant extract remains one of the most abundant and classic way to produce natural compounds. The disadvantage of using plant extract is the hefty amount of plant tissues required to produce a significant amount of individual flavonoid. This method can be very costly and time consuming. In addition, the “browning effect” of flavonoids extract remains one of the biggest obstacles in the field[18].

Plant cell cultures
Plant cell cultures can be one good alternative to plant extracts as there is no heavy depletion of environmental components; instead, plant cells are mass produced in the bioreactor. The major challenge of this production method is with the plant cells. They tend to form cells aggregates mainly due to the affinity of the cells not to separate after cell division[21]. This issue leads to a fraction of the cells within the aggregates not uniformly exposed to lightning. In the nature of plant cells, a few enzymes along the flavonoid and stilbene biosynthetic pathway are induced primarily by UV[22, 23], and it had been shown that the intensity of lightning improved the overall flavonoids productivity[24]. Without uniform lightning, there are limited induction and subsequently low protein expression within the cells, which will directly affect the output of the bioactive compounds. Until this issue is solved, this method remains economically non-feasible.

Recombinant microorganisms
Microbial production of polyphenols is one of the more popular methods which can involve both bacteria and yeast. Fig. (2A) demonstrates the essential steps of embedding a gene of interest isolated from plant or animal cells, cloning the gene into a functional plasmid, transforming the whole plasmid into the microorganisms and subsequently subjecting the microorganisms for fermentation. Microbial production possesses a few distinctive advantages. For one, microbial does not form aggregates and has a significant shorter doubling time compared to the plant counterpart. Secondly, plant biosynthetic pathways cloned have to be under the respective microbial promoters, which indeed exclude the dependent of UV induction exist in plants. By using this method the end products generated are not only limited to natural polyphenols, but also a diverse library of high-valued unnatural compounds. Fig. (2B) shows the potential of microbial to create diverse natural and unnatural dihydroflavonol through combinatorial mutasynthesis and subsequently generate a plethora of different colored anthocyanidin [Chemler et al. unpublished]. The only drawback using microbial for natural compound productions is the complication involved in embedding the biosynthetic pathways and to functionally express individual pathway in the microorganism. Escherichia coli strains have been engineered to produce natural compounds primarily due to the ease of gene manipulation and their rapid cell division. One major disadvantage of using the bacteria platform is the absence of intracellular membrane or endoplasmic reticulum that lead to the non-fuctionality of many key enzymes, for example the membrane bound cytochrome P450 family, which in some cases requires the association of another membrane bound protein or redox partner, namely cytochrome P450 reductases (CPRs). Efiendi et al. successfully designed an artificial P450 system that is functionally expressed in E. coli by mimicking the architecture of the bacterial P450BM-3[25, 26]. Other than the slight lengthy doubling time, yeast or Saccharomyces cerevisiae can be advantageous over bacteria especially of its capability to support the challenge of P450 enzymes with the existence of its endoplasmic reticulum. It also contains endogenous CPR that can facilitate electron transfer to the cytochrome P450 enzymes.

Both flavonoids and stilbenes have been shown to possess a range of beneficial effects across distant species and disease models. Oxidation in the body is detrimental to the health and it has been proved to be responsible for a few major degenerative diseases, including arteriosclerosis, cancer and heart disease. Polyphenols are known to prevent many of the oxidative processes by acting as potent metal chelators, free radical scavengers and chain-breaking antioxidant[4]. The number of flavonoids antioxidant activities related publications have increased exponentially in these past two decades covering activities in vitro, ex vivo and also in vivo[27]. Since the polyphenols’ antioxidant properties have been extensively studied, only the ones with higher antioxidant activity will be discussed in this review.

Flavonoids and stilbenes associated health benefits are apparent but there are still a lot of inquiries on how these small molecules proceed in the body once ingested, followed by promoting their vital bioactivities. This can be addressed by the study of absorption, distribution, metabolism and excretion (ADME), which is crucial in defining the pharmacokinetics and pharmacodynamics of polyphenols, most importantly addressing the fate of the glycosides and aglycones. Reviews by Walle et al., Zhang et al. and Prasain et al. provide detailed explanations on the possible transport mechanisms and metabolisms of polyphenols in the body. The absorption patterns between glycosides and the aglycones are distinctively different mainly due to the variation in size and polarity. Polyphenol aglycones are more permeable across human intestinal, therefore more readily absorbed while the glycosides are hydrolyzed into their aglycones prior to absorption. Hydrolysis of these glycosides could be accomplished by β-glucosidase in the small intestinal epithelium of human or lactase phlorizin hydrolase (LPH) bound to the intestinal lumen[28]. LPH was initially known only to hydrolyze lactose. Besides being metabolized, the glycosides can also remain intact and directly be absorbed using the sodium-dependent glucose transporter 1 (SGLT-1)[28, 29], with absorption efficiency greatly determined by the sugar moiety of individual molecules[28]. These absorbed glycosides could possibly be transported back into lumen by the multidrug resistance protein 2 (MRP2). Although the majority of the flavonoids absorbed are aglycones followed by a minute amount of glycosides, both groups are found in very low concentrations in the plasma. Compounds that predominately the blood circulation are primarily phase II metabolites such as glucuronides, sulfo and methyl conjugates as a result of glucuronidation, sulfonation and methylation[30]. Glucuronidation reaction is mediated by UDP-glucuronosyltransferases (UGTs), sulfonation is mediated by sulfortransferases (SULTs) while methylation is regulated by catechol-O-methyl transferases (COMTs)[29, 30]. Walle et al. also suggested a few additional metabolic pathways including bacteria metabolism that happened mostly in the colon and metabolizes oxidation by reactive oxygen species (ROS)[29]. Flavonoids and their metabolites that reach the colon may be further metabolized and degraded by bacterial enzymes into phenolics, carboxylic acids and carbon dioxide. On the other hand, oxidized flavonoids were reported to be able to covalently bind to DNA and protein in human intestinal and hepatic cells[31].

This review focuses on investigating the bioavailability of individual flavonoids/stilbene, the different approaches attempted to improve polyphenols’ bioavailability as well as their current advances in biological activities. This review includes in vitro studies, in vivo studies in both animals and humans as well as a few clinical trials. This study seeks to offer a greater understanding of the bioavailability of different polyphenols and their metabolites, their role in preventing and curing diseases, and ultimately help improve human well-being.

**BIOAVAILABILITY AND BIOEFFICACY FLAVANONES**

Flavanones are among the most prominent phenolics due to their role as the direct precursors of all other flavonoids. The most representative flavanones are naringenin, hesperetin as well as their glycosides. Flavanones are most abundant in citrus fruit and juice compared to other food sources[32], with hesperidin and narirutin, the 7-O-rutinoside version of the aglycone hesperetin and naringenin respectively, as the major flavanonone glucosides in oranges. Several studies have looked into the in vivo bioavailability of flavanone after
consumption of citrus fruit or juice and cooked tomato paste[33-35]. In the plasma and urine, flavanones and their metabolites were determined by measuring their concentration throughout the course of the studies.

Overall, flavanones are poorly bioavailable and their reported peak plasma concentrations are in the low μM range[32, 33, 36]. Manach et al. conducted a human study in which 0.5 or 1 L of commercial orange juice (444mg/L of hesperidin and 96.4 mg/L narinrutin) was ingested. The peak plasma concentration of aglycones hesperetin and naringenin were 0.46μM and 1.28μM, respectively[33]. In another human study, 150 subjects whose age ranged from 18 to 80-year-old were given either orange fruit (150g) or juice (300g). The reported mean peak plasma concentration of flavanone was in the range of 0.05-0.1μM. This particular study reported a few important observations: first, there were no significant bioavailability variation in flavanones ingested from fruit or juice and second, there was a huge inter-individual flavanone excretion variation but it was independent of age, sex, body mass index, or use of contraceptive pill[32]. The major reason behind the low bioavailability and absorption of flavanone glycosides in human is the individual sugar moieties[37]. Hesperidin with a rutinoside is one of the glycosides with low bioavailability. By treating the consumed orange juice with hesperidinase enzyme to remove the rhamnose group yielding hesperetin-7-glucoside, Nielsen et al. were able to improve the bioavailability of hesperetin by 4-fold compared to volunteers who consumed orange juice with natural hesperidin alone, and by 1.5-fold compared to those who consumed modified orange juice with 3 times more hesperidin. The researcher proposed that the improved bioavailability was caused by the change in the absorption site from the colon to the small intestine[38].

In order to eliminate doubts that other active compounds present in orange that decrease the overall flavanone bioavailability, some studies used pure flavanones to scrutinize flavanone bioavailability. Six healthy volunteers were orally administered with 135 mg of hesperetin or naringenin under fasting conditions. Both the flavanone aglycones were rapidly absorbed and detected in the blood samples 20 minutes after dosing. Their mean peak plasma concentrations were about 2.3μM and 7.3μM respectively; however, their urinary excretions were merely 3.26 percent and 5.81 percent the administered dose, respectively[39]. These results confirmed the low bioavailability of flavanone aglycones.

The flavanone metabolites found in the circulating plasma, urinary excretion were mainly glucuronides and sulfoglucuronides[40]. Manach et al. reported four major metabolites: 4'- and 7-O-monoglucuronides of naringenin and the 3'- and 7-O-monoglucuronides of hesperetin in addition to two hesperetin diglucuronides and a hesperetin sulfo-glucuronide in plasma and urine[33]. Phenolic acids were also reported as flavanone metabolites resulted from intestinal microflora degradation[33, 41]. Kanaze et al. attributed the low cumulative flavanone urinary recovery to possible cleavage of flavanone aglycones on the C ring[39].

Even though flavanone aglycone and glycosides possess very low bioavailability, numerous in vivo animal and human as well as in vitro studies have shown that flavanones exhibit an array of health benefits including antioxidant, anti-inflammatory, anti-tumor and anti-carcinogenic activities. There have been reports in the past two decades suggesting the antioxidant activities of flavanone in vitro[42, 43]. On the contrary, Andrade et al. reported no antioxidant or pro-oxidant effects in the rat model when the rats were fed with 3 increasing concentrations of naringenin (30, 60 and 120mg/kg or diet)[44].

Several in vivo animal studies looked into the function of flavanones as anti-carcinogenic and anti-cancer agents. Oral administration of hesperidin on azoxymethane (AOM)-induced colon carcinogenesis in rats, significantly decreased the incidence and multiplicity of neoplasms in the rats’ large intestines, reduced the 5'-bromodeoxyuridine-labeling index and argyrophilic nuclear organizer region’s number in crypt cells, colonic mucosal ornithine decarboxylase activity, and polyamine levels in the blood, suggesting hesperidin possible chemoprevention effect against colon carcinogenesis[45]. They later reported a similar chemopreventive effect of a commercial Satsuma mandarin (Citrus unshiu Marc.) juice, which is rich in hesperidin (3.58 percent) and β-cryptoxanthin (0.67 percent)[46]. Later studies were conducted to investigate whether citrus juices could modify carcinogenesis in other organs. A modified Satsuma mandarin juice with higher hesperidin (100mg) and β-cryptoxanthin (3.9mg) was reported to possess a chemopreventive ability against 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone-induced mouse lung tumorigenesis[47]. Apart from the glycosides, flavanone aglycones themselves were also reported similar anti-carcinogenic effect. In 1,2-dimethylhydrazine-induced colon carcinogenesis rats, hesperetin reduced the formation of preneoplastic lesions and modulated the xenobiotic-metabolizing enzymes in rats[48], whereas in N-methyl-N'-nitro-N'-nitrosoguanidine-induced gastric carcinogenesis rats, naringenin significantly up-regulated the redox state in rats to decrease the risk of cancer[49]. Liquiritigenin, a naringenin analog lacking the 5'-hydroxyl group also showed potent cytotoxic effect against human cancer cells in vitro via reactive oxygen species production and subsequently lead to cell apoptosis[50, 51].

Besides the evidence on the anticarcinogenic and anti-tumor activities in animals, flavanones have been shown to protect against various inflammatory injuries. Vafeiadou et al. studied the role of flavanones as neuroprotective agents. In vitro assays performed to assess flavonoids’ role in glial cells suggested that naringenin had one of the strongest activity among flavonoids tested against attenuating LPS/IFN-γ-induced TNF-α production and inhibiting LPS/IFN-γ-induced iNOS expression, p38 mitogen-activated protein kinase phosphorylation and nitric oxide production. Moreover, it also protected inflammatory-induced neuronal death in a primary neuronal-glial co-culture system[52]. In periodontitis, an inflammatory disease of tooth supporting tissues, naringenin could act as a potent inhibitor of the pro-inflammatory cytokine response induced by lipopolysaccharide in both macrophages and in human whole blood model[53]. Both naringin and the aglycones were evaluated for their anti-inflammatory effect on acute model of induced colitis in mice, a disease affecting the tissue that lines the gastrointestinal system. Results revealed both the flavanones were capable of reducing edema of the gut and the levels of nitrate/nitrites through inhibition of inducible nitric oxide synthetase, and minimized lipid peroxidation by oxygen radicals scavenging; hence suggesting anti-inflammatory potentials[54]. Another flavanone derivative, isopedin also showed potent anti-inflammatory function in inhibiting superoxide anion production in formyl-l-methionyl-l-leucyl-l-phenylalanine (FMLP)-activated human neutrophils[55].

Hypcholesterolemic and cardioprotective activities of flavanones also attracted a few animal studies and human clinical trials. Supplementation of naringenin in 1 percent high-cholesterol diet-fed rats effectively improved the plasma or hepatic lipid profiles and antioxidant capacities compared to the control group[56]. Naringenin also showed cardioprotective role in ISO-induced myocardial infarction in rats that were pretreated with 10 to 40mg/kg for a period of 56 days[57]. In contrast, one study contradicted the benefits declared. 50mg/kg of naringenin ingested in both control and ethanol-drinking rats for 2 weeks only exerted minor influence on lipid metabolism. Likewise, isolated adipocytes incubated with naringenin revealed similar lack of effect[58]. In a human clinical trial, orange juice fed three times daily to thirteen healthy subjects for three weeks successfully decreased the low density lipoprotein to high density lipoprotein cholesterol (LDL/HDL) ratios but cholesterol concentrations in the plasma did not vary significantly[59].

In anti-diabetic related area, Ghanim et al. employed a different approach using orange juice as possible glucose substitution. Glucose intake could induce oxidative stress and inflammation but these effects were absent following fructose, orange juice and water ingestion. In vitro proved that reactive oxygen species in mononuclear cells was suppressed by naringenin and hesperetin in
orange juice but not by fructose or ascorbic acid[60]. Naringenin was also shown to facilitate extra-pancreatic action and to inhibit carbohydrate absorption from intestine, thereby promoting anti-diabetic effect on diabetic rat models[61].

**ISOFLAVONES**

Leguminous plants such as soya are major source of isoflavone. Isoflavones glycosides predominate in soy compared to the aglycons, but once it is fermented, the soya product primarily contains the aglycons.

When it comes to the bioavailability of isoflavones (aglycones and glycosides), the results are highly contradictory. There are reports suggested that isoflavone aglycones are more rapidly absorbed than their glycosides[62-64]; whereas Setchell et al. reported higher bioavailability of glucosides form of isoflavones[65]. More interestingly, Richelle et al. and Zubik et al. did not observe any absorption difference when either aglycons or glucosides were administered[66, 67]. Discrepancy among the reports could be attributed to the used of soybean extract (mixture of isoflavones) compared to pure compounds in some other studies. Setchell credited the inconsistent results to the importance of multiple time points sampling during the elimination phase and after attaining steady-state rather than sampling merely 2 time points in the elimination phase[65].

In the plasma, concentration of genistein is consistently higher than daidzein[64, 65]. Similar to most other flavonoids, isoflavones glycosides were not found in human plasma after ingesting either 50 mg of isoflavone glycosides (daidzin or genistin) or 250mL of soymilk[68]. Isoflavone aglycones could be detected when pure daidzein and genistein were administered[65]. In addition, data of individual conjugates of daidzein and genistein have also been reported. Glucuronidates predominates in human plasma after metabolism. Clarke et al. determined the intact isoflavone conjugates in human urine and the average pattern was found to be 54 percent 7-glucuronide, 25 percent 4-glucuronide, 13 percent monosulfates, 7 percent free daidzein, 0.9 percent sulfoglucuronides, 0.4 percent diglucuronide, and less than 0.1 percent of disulfate[69].Cassidy et al. investigated the effect of age, gender, and the food matrix on the bioavailability of isoflavones. Firstly, they showed gender differences in peak concentrations, in which women attained higher levels of daidzein in the plasma. Consumption of predominantly isoflavone aglycons (tempoh) attained a higher serum peak and area than the administration of corresponding isoflavones glucosides (textured vegetable protein). At last, consuming isoflavone through liquid matrix reached a higher absorption rate and earlier peak than solid soy food[64]. Another study looked into the role of ethnic difference in isoflavones pharmacokinetic and bioavailability. On acute intake of soya cheese, Asians absorbed daidzein and genistein better than Caucasians, while on chronic ingestions, the concentration of isoflavones in the plasma increased in Caucasians but not in Asians[70].

Isoflavone family attracted a lot of in vivo studies and they remain one of the most investigated polyphenols mainly due to their estrogen-like structures[71]. They belong to the phytoestrogen family and have been demonstrated to have a plethora of pharmacological benefits in illnesses such as cardiovascular disease, breast and prostate cancer, post-menopausal syndrome and osteoporosis. In cardiovascular-related area, genistein was shown to improve endothelium function in postmenopausal women after one year of therapy with dose of 54 mg/day, and the result was comparable to a similar extent as the estrogen/progesterin treatment[72]. A review by Siow et al. concluded that isoflavones are able to activate intracellular signaling pathways, leading to increased NO bioavailability and improving antioxidant gene expression via key transcription factors NFκB and Nrf2, and hence protecting ones from cardiovascular-related diseases[73].

Breast cancer is the most common and second leading cause of cancer deaths among women in the United States. One possible reason Asian women are less prone to breast cancer compared to American women could be that traditional Asian diet is rich in soy products such as miso, tofu and soymilk. This is supported by the fact that Asian population has a higher mean daily soy intake of 10 to 50g compared to the intake of 1 to 3g in the USA[74]. In a rodent study, Lamartiniere et al. looked into the mammary glands of immature rats after genistein treatment. The expression of epidermal growth factor was up-regulated shortly after treatment, suggesting that early genistein action promotes cell differentiation that might result in a less active epidermal growth factor signaling pathway in adulthood, and eventually lead to the suppression of the development of mammary or breast cancer[75]. In a clinically-relevant breast tumor animal model, bioactive genistein was shown to enhance Tamoxifen (an estrogen antagonist that interferes with the activity of estrogens in most estrogen sensitive tissues) activity in a synergistic manner via induction of tumor cell apoptosis and inhibition of tumor cell proliferation[76]. In addition, isoflavones have also shown positive effects in decreasing the likelihood of prostate cancer. Dietary genistein dose of up to 250mg/kg was shown to decrease human prostate cancer metastasis in a dose-dependent manner through mediated inhibition of prostate cancer cell detachment[77]. Another cancer related case-control study was conducted in Japan on colorectal adenoma or colon cancer. The researchers observed a ceiling effect associated with higher isoflavone intake on colon cancer and showed an inverse association between isoflavone intake and the risk of colorectal adenoma[78].

In obese Zucker rat (OZR), high isoflavones soy protein consumption resulted in an improved lipid metabolism and activated peroxisome-proliferator activated receptors (PPARs), which subsequently led to an anti-diabetic effect[79]. Besides the potent effects of various illnesses, isoflavones could also decrease the risk of osteoporosis[80, 81], improve cognitive performance[82, 83] and reduce LDL cholesterol and LDL/HDL ratio[84, 85].

Despite all the aforementioned positive reports, there are also studies that presented contradictory results. Taku et al. reported no improvement of LDL cholesterol levels in normocholesterolic menopausal women ingested with 70 mg of extracted soy isoflavones/day for 1–3 months[86]. Krejikamp-Kaspers et al. found no apparent correlation between dietary isoflavone intake and cognition functions such as memory, processing capacity and speed, and executive function[87]. Furthermore, no beneficial effects of isoflavones were detected on vascular function in older postmenopausal women[88]. In a review, Cassidy et al. concluded that the evidence of isoflavone potentials on cancer, diabetes and cognitive function are inconclusive due to the lack of appropriately designed studies and inconsistent methodological approaches[89].

**FLAVONES**

Flavones present in diet primarily as 7-O-glycosides and C-glycosides[13]. Chrysir, apigenin and luteolin are the most popular flavones with 0, 1 and 2 hydroxyl substitutions on the B ring respectively. To date, only a handful of studies have reported the absorption and bioavailability of flavone family. Shimoi et al. looked into the bioavailability of luteolin in both rats and humans[90]. Plasma of rats orally administered with luteolin contained free luteolin, glucuronide, sulfate and methyl conjugates. For the rats administered with luteolin 7-O-β-glucoside, the glucoside was not detected in the plasma but luteolin glucuronides were identified. The author suggested that flavone glucoside was hardly absorbed by itself; it was hydrolyzed to luteolin by the microbacteria presented on the surface of the intestinal mucosa and later glucuronidated. Pharmacokinetic studies revealed the rats’ luteolin plasma concentration was about 15μM, 30 minutes after 50μmol/kg was administered. In the same study, two volunteers were involved in which they ingested 50mg of luteolin. In the serum of both volunteers, free luteolin and its monoglucuronide were detected[90]. Similar study with human subjects was conducted, in which two different artichoke leaf extracts with varied luteolin glucoside concentrations (equivalent to 14.4mg and 35.2mg of luteolin respectively) were administered to 14 healthy volunteers. The
reported peak luteolin plasma concentration was 206nM (extract 1) and 546nM (extract 2) after 0.36 and 0.46 hours respectively[91]. In another human study, 11 healthy subjects were administered a single oral bolus of 2g/kg blanched parsley (65.8μmol equivalent of apigenin) to scrutinize the bioavailability of apigenin. The peak apigenin plasma concentration in urine was reported to be 127 nM after about 7.2 hours, and the average apigenin content for the first 24hour was 144nmol or 0.22 percent of the ingested dose[92]. Walle et al. looked into the bioavailability of chrysin, after 7 subjects ingested two 200mg capsules of chrysin. The highest chrysin peak plasma concentration among the volunteers was 16mg/mL or 64nM and surprisingly, the chrysin sulphate concentrations were found to be 30-fold higher than the aglycone concentration; whereas in urine, free chrysin and its glucuronide accounted for 0.2–3.1mg and 2–26mg, respectively and only trace amounts of chrysin sulphate were found[93]. In their earlier in vitro study in human intestinal Caco-2 and hepatic Hep G2 cells, the only detected metabolites were flavone conjugates[94, 95].

In order to overcome the low bioavailability bottleneck that flavonoids possess, Walle et al. proposed methyl-capping all free hydroxyl groups of flavonoids. In rats, the oral bioavailability of methylated flavone was tested by comparing chrysin and its methylated version 5,7-dimethoxyflavones. When both compound were co-administered, only the methylated flavone was detected in the plasma with a peak concentration of 2.3 μM at 1 hour, and it was found to be accumulated highly in tissue, especially in the liver[96]. In conclusion, methylated flavone could resulted in higher bioavailability and improved metabolic stability compared to the aglycone[97-99]. This approach might be a way to overcome the bottleneck of flavonoids being such low bioavailable polyphenols.

Similar to the bioavailability studies, the most investigated flavones are the aglycones luteolin, chrysin, apigenin, and their derivatives. Based on numerous in vitro studies and animal experiments, flavones exhibit a wide range of biological and pharmacological benefits. Michels et al. used H4IIE rat hepatoma cells as a model system for studying luteolin capability on inducing oxidative stress and apoptosis. It was reported that luteolin is relatively toxic and it triggered cell apoptosis through the mitochondria pathway. At a concentration of 250μM, luteolin was capable of activating and at the same time increasing the activity of different caspases(used in cell apoptosis)[100]. Besides the capability of inducing oxidative stress, surprisingly flavone apigenin was found to have a protective role against oxidative stress and DNA damage in N-nitrosodihydimethane (NDEA)-induced hepatocellular carcinogenesis in Wistar albino rats[101]. Apigenin was also demonstrated to successfully manage the evasiveness of tumor cells by preventing them from penetrating healthy tissues[102]. In human pancreatic cancer cells MiaPaCa2. 20μM of luteolin successfully blocked the epidermal growth factor receptor (EGFR) tyrosine kinase activity, inhibited the growth of tumor cells and induced apoptosis[103]. In human prostate cancer DU145 and PC-3 cells, apigenin was reported to significantly inhibit insulin like growth factor (IGF)-1-stimulated cell proliferation, dephosphorylate Akt and induced cell death, both in vitro and in vivo[104, 105]. Flavones also showed potent effects on many other cancer diseases, for example leukemia[106], breast cancer[107] and etc.

In cardiovascular-related diseases, Jin et al. investigated apigenin potential on endothelium-dependent vasorelaxation in isolated rat aortic rings exposed to superoxide anion. 0.5 to 72.0 μM of apigenin were used and resulted in a concentration-dependent relaxation in aortas and also provided protection against the dysfunction caused by the superoxide anion[108]. Similarly, Ma et al. verified superoxide anion capability to impair acetylcholine-induced relaxation and hyperpolarization of smooth muscle cells in resistance arteries, and both luteolin and apigenin protected resistance arteries from injury, which further verified flavonols’ vasoprotective activity[109]. Another article looked into the effect of flavones as angiotensin converting enzyme (ACE) inhibitors, which are commonly used to treat cardiovascular diseases. Among the flavones tested, apigenin, luteolin and luteolin-7-O-β-glucopyranoside appeared to be potential inhibitor candidates with IC50 values of 280, 290 and 280μM respectively, as compared to the therapeutic drug captopril (IC50 0.02 μM). Yamagata revealed apigenin function as a arteriosclerotic vascular disorder protectant by regulating the activation of NF-κB[110]. Some studies also underlined the anti-inflammatory effect of flavones. Chrysin concentrations of 1, 5 and 10mg/kg were administered orally to dextran sodium sulfate (DSS)-induced colitis in mice and flavone was able to attenuate symptoms and colitis DAI scores of DSS-induced colitis, and inhibited NF-κB activation in TNF-α-stimulated intestinal epithelial cells[111]. These results suggested chrysin as a potential therapeutic agent for intestinal inflammation. Also in mouse model of LPS-induced acute pulmonary inflammation, two less common flavones, fisetin (3',4',7-tetrahydroxyflavone) and tricetin (3',4,5,5',7-pentahydroxyflavone) were compared to an anti-inflammatory glucocorticoid dexamethasone as potential therapeutic agents against pulmonary inflammation. Fisetin was shown to have the highest potential and it significantly reduced lung myeloperoxidase-levels and gene-expression of different inflammatory mediators[112]. Other studies also examined flavone therapeutic effects on anti-aging, antibacterial, neuprotective, etc.

Lack of medicinal effects of flavones in some studies was also reported. Human leukemia cells HL60 and their MDR-1 resistant subline HL60/VCR were treated with both luteolin and apigenin, but neither of them regulated the cell apoptotic program[113]. Janssen et al. reported that at a relatively high concentration of 2500 μmol/L in vitro, apigenin inhibited collagen- and ADP-induced platelet aggregation; whereas at lower concentration, it showed no effect. The hypothesis was again tested in vivo and no significant effects were found on platelet aggregation, thromboxane B2 production, factor VII, or other hemostatic variables[114].

In more recent years, methylated flavones have attracted voluminous studies mainly due to their improved bioavailability compared to the flavone aglycones. The downside of using the methylated compounds is associated with their antioxidant potentials. The major determinants for radical-scavenging ability are based on the presence of catechol group in the B ring, 2,3-double bond conjugated with the 4-oxo group in the C ring[115]. O-methylation of the hydroxyl groups particularly on the B-ring greatly reduces the overall antioxidant capacity of the molecules. Michels et al. demonstrated that the anti-oxidative activity of luteolin was lowered by methylolation (5,3’-dimethylflavone and 5,7,3’,4’-tetramethylflavone) in H4IIE rat hepatoma cells, and hence led to the decline of radical-scavenging activity and to the reduction of luteolin aglycone’s inducing-apoptotic potential[116]. Walle et al. who suggested the methyl-capping approach confused the fact that even though the methylated molecules lack antioxidant properties, their effects as modulators of protein and lipid kinase signaling remain and in some cases even more potent[97, 117]. For instance, 30μM of nobiletin (3’,4’,5,6,7,8-hexamethoxyflavone) was capable of attenuating the apoptosis induced by H2O2 exposure in human neuroblastoma SH-SY5Y cells[118]. Methylated flavone also showed potent inhibitory activity on carcinogen bioactivating enzymes CYP1A1 and CYP1B1 at low concentration[119, 120]. On cancer chemoprevention in vitro, methylated flavones were ten times more potent inhibitors of cell proliferation than the unmethylated flavones on humanoral SCC-9 cancer cells[96]. Likewise, Cai et al. revealed the effectiveness of the methoxy group in promoting gastrointestinal cancer chemopreventive efficacy both in vitro and in vivo[121]. The issue of using methyl-capping to significantly improve the bioavailability of not just flavones but also flavonoids and stilbenes requires further investigation as its possible consequences: lower antioxidant activity might jeopardize a handful of polyphenols vital bioactivities.

**FLAVONOLS**

Flavonols occur as glycosides in diet. Onion, kale and apple are a few major sources of flavonols and they are best represented by
Quercetin, kaempferol and myricetin. It has long been known that flavonol aglycones' bioavailability is low. A number of studies have looked into the bioavailability of quercetin, mostly by using onion as the source. Quercetin glycosides were generally more efficiently absorbed than quercetin aglycones, except rutin (quercetin-3-O-rutinoside), which was reported to be less rapidly absorbed mainly due to its sugar moeity[122], and these results were confirmed by Graefe et al.'s study. A four way crossover study was conducted, in which 12 subjects were administered with two isolated quercetin glucosides (quercetin-4′-O-glucoside and quercetin-3-O-rutinoside) and two plant extracts (onion and buckwheat tea). Quercetin glucuronides were found in human plasma but not the aglycones and the peak plasma concentrations were 7.0, 1.1, 7.6 and 2.1µM respectively. It was concluded that sugar moiety, glycosylation position and the dietary sources contribute to the overall flavonols' bioavailability[123]. Flavonol bioavailability studies up to date concluded that flavonols are less bioavailable than their glucosides; however, this conclusion have defied the typical assumption of aglycone should be more bioavailable than its glucosides due to the requirement of either preliminary hydrolysis to aglycones or active transport for absorption. Wiczkowski et al. attributed the low bioavailability of the aglycones to their low solubility in the digestive tract, and they suggested dispersing flavonol quercetin from shallot into the food matrix. As a result, a higher quercetin bioavailability than its glucosides was reported[124].

Similar to most flavonoids, flavonol glucuronides and sulfates occurred predominantly in the circulating plasma. Major metabolites of quercetin detected in human plasma 1.5 hours after consumption of onions were quercetin-3-glucuronide, 3'-methylquercetin-3-glucuronide and quercetin-3-sulfate[125]. Isorhamnetin (3'-methylquercetin) contributed to 20 percent of the absorbed quercetin, one third was sulfated and the remaining were quercetin glucuronides or mixed conjugates[40]. Walle et al. also found oxidized quercetin as a result of ROS oxidation and these quercetin were reported to covalent bound to DNA and cellular protein in hepatic cells and human intestinal[31].

There are also some studies that looked into amending the bioavailability of flavonols, intending to maintain a higher flavonol plasma concentration for greater distribution and subsequently greater bioactivity. In a review, Manach et al. reported the baseline quercetin plasma concentration after overnight fasting and promising improvement on the baseline after prolonged supplement of flavonols was reported[126]. A rat study was conducted to further elucidate the phenomenon. Male CD rats were fed with two distinct administration procedures: intragastric administration (single 50mg/kg dose) and free access administration (diet with 1 percent quercetin for 4 weeks). The total quercetin concentration was higher in the free access group (7µM) compared to intragastric group (2.5µM), which supported the improved flavonol baseline hypothesis. The methylated to non-methylated metabolites ratio was also accounted in which the free access group had a much higher ratio of 3.79 compared to 0.39 for the intragastric group[127]. On the other hand, experiments by Bieger et al. in pig reported no greater flavonol accumulation in any tissue or plasma between orally administered a single dose quercetin aglycone (25mg/kg) and twice a day orally administered at the same dosage for 4 weeks[128]. These results indicated possible bioavailability variation among organisms of interest and this issue requires further elucidation. Dietary quercetin combined with pectin (a heteropolysaccharide used as a thickening agent) could significantly enhance the intestinal absorption of quercetin. Male mice fed with pectin-rutin (PR) diet or cellulose-rutin (CR) diet for 14 days resulted in varied plasma concentration of quercetin (PR 2.5µM versus CR 0.89 µM) and of isorhamnetin (PR 6.66 µM versus CR 0.64 µM)[129]. Similarly, the effect of apple pectin (AP) on quercetin and rutin bioavailability was investigated. Suprisingly, for rats with quercetin administration, the maximum plasma concentration of quercetin metabolites was significantly higher in AP-fed rats. In contrast, this phenomena was not seen in AP-fed rats administered with rutin. In short, the apple pectin did not affect rutin bioavailability[130]. The researchers attributed the disagreement between the 2 studies to the manner in which rutin was fed.

Flavonols have prominent antioxidant properties which are among the best among flavonoids, particularly with quercetin. This compound fulfilled all requirements as a potent free radical scavenger, with 3′ and 4′ hydroxyls on the B ring (catechol group), 2,3-double bond conjugated with the 4-oxo group and 3-hydroxyl on the heterocyclic C ring[115]. In a recent study, rats with nicotine-induced prooxidant and antioxidant imbalance in circulation were given quercetin by intragastric intubations for 22 weeks. Quercetin modulated the lipid peroxidation and antioxidant status of nicotine-induced rat, decreased DNA damage and showed protective effect against nicotine toxicity to the extent of N-acetylcyesteine (NAC), a popular antioxidant[131]. In plant, it has long been demonstrated that adaptation of plants to high light stress involves accumulation of protective pigments namely, flavonols which absorb solar radiation in broad spectral ranges extending from UV to the green and, in some cases, to the red regions of the spectrum. The build-up of such substances in specific cell and tissue structures reduces the fraction of radiation absorbed by potent photosensitizers, and thereby diminishes light-induced damage. As an important defence mechanism against the deleterious effects of solar radiation, long-term adaptation of higher plants involves synthesis of relatively stable flavonols capable of serving as light screens and/or internal traps. Similar photoprotection effect was also demonstrated on mice skin by Campos et al., in which flavonols was applied on dorsal skin of hairless mice irradiated by UVA/B for 15 minutes[132]. The association of flavonols to the vitamin was shown to further improve the antioxidant activity in vitro and protect the mice skin from UV damage by reducing the number of sunburn cells. Similar outcome was seen when topical application of 0.2mg/cm² standardized black tea extract in SKH-1 hairless mice prior to UVB exposure resulted in 40 percent reduced incidence. 64 percent reduced severity of erythema and 50 percent reduction in skinfold thickness by day 6 when compared to nontreated UVB-exposed mice[133].

In addition to antioxidant and photoprotective potentials, flavonols also possess anti-inflammatory effect. In a very recent study, kaempferol, quercetin and other flavonoids were found to block tumor necrosis factor (TNF)-α induced interleukin-8 promoter activation and gene expression in HEK 293 cells. Interestingly, kaempferol was the only flavonoid compound that did not affect the cell viability during pretreatment[134]. Similarly, quercetin effect as an anti-inflammatory compound on neuroinflammation was investigated and it showed potent effect on lipopolysaccharide (LPS)-induced mRNA levels of two proinflammatory genes, interleukin 1-α and tumor necrosis factor-α, in which both were greatly reduced. Moreover, the inflammation-mediated apoptotic death of neuronal cells was also inhibited[135]. During inflammation, nitric oxide (NO) formation is enhanced and Hamalainen et al. revealed that both the flavonols: kaempferol and quercetin repressed inducible nitric oxide synthase (iNOS) protein and mRNA level as well as NO production by first inhibiting the activation of nuclear factor-kappaB (NF-κB) as well as signal transducer and activator of transcription 1 (STAT-1), both transcription factors of iNOS[136].

Quercetin is a potent molecule for treating or preventing cancer due to its anti-mutagenic, anti-proliferative, antioxidative activities and its role in cellular receptor interactions and modification of signal transduction[137]. Ginkgo biloba extract was proven to induce apoptosis by activation of caspase-3 in oral cavity cancer cells[138] and the individual molecules responsible for the anti cancer potential were the flavonols, kaempferol and quercetin. Both at concentration of 40µM were able to induce apoptosis on oral cancer cell lines SCC-1483, SCC-25 and SCC-QLL1, and showed cleavage of poly (ADP-ribose) polymerase[139]. Quercetin also inhibited cell proliferation and induced apoptosis in HepG2 cells in dose- and time-dependent manner through activation of caspases and down-regulation of two anti-apoptosis gene, survivin and Bcl-2[140]. Shan et al. demonstrated quercetin’s antitumor potential in SW480 colon cancer cells by inhibiting expression of cycline D1 and survivin through
Reports on quercetin anti-cancer potentials in vivo have been published. Administration of a diet containing 4.5g/kg of quercetin was capable of suppressing the formation of early preneoplastic lesions in azoxymethane (AOM)-induced rat colon carcinogenesis, together with inhibition of cell proliferation and induction of apoptosis[142]. Similar studies were done by comparing the anti-cancer properties of rutin to quercetin but results are strongly contradictory. Compared to the control (no test reagent) in AOM-induced rat colon cancer model, quercetin and rutin decreased the number of aberrant crypt foci, a histological tumor marker by 4 and 1.2 fold respectively[143]. In contrast, with dextran sulfate sodium (DSS)-induced colitis in mice, diet containing 0.1 percent of rutin but not quercetin attenuated DSS-induced body weight loss and shortening of the coloectum, improved colitis histological scores and significantly blunted colonic mucosal IL-1β levels[144]. The author attributed the discrepancy again to the model of study and pointed out that there are differences among mouse, rat and human gut with respect to the mechanisms each organism utilize or exclude luminal flavonoids. Quercetin also showed promising effect on breast cancer[107, 145] and prostate cancer[146, 147].

Apart from inducing apoptosis in flavonols' anticancer activity, they can also reverse apoptosis when cells are under oxidative stress[137]. Chow et al. showed that quercetin prevented H2O2-induced apoptosis in macrophages through its antioxidant potential and heme oxygenase 1 expression; but similar effects were not seen in both rutin and quercitin (quercetin 3-O-rhamnose)[148]. Quercetin also facilitated anti-apoptosis in its anti-neurodegenerative potential, in which it regulated the mRNA levels and protein expression of pro-apoptotic (Bax) and anti-apoptotic Bcl-2 genes and as a result, the apoptotic neuronal cell death was diminished[149]. Similar effect of quercetin was observed in neural cultures treated with amyloid beta (Aβ), an Alzheimer disease protein[150, 151]. Apart from inhibiting apoptotic cell death, quercetin also attenuated the peptide-induced cytotoxicity, protein oxidation and lipid peroxidation. Interestingly, this promising Alzheimer disease treating approach was only attained at lower quercetin doses (5µM); higher doses (20 µM or 40 µM) were not only non-protective but toxic effects are observed[152].

In cardiovascular-related diseases, flavonol has also been shown to be a powerful cardioprotective agent. Daunorubicin (DNR) was commonly used for cancer treatment, but its use was limited because of its association with the development of cardiac toxicity. Mozijosva et al. revealed quercetin at concentrations between 10µM and 100 µM were able to protect H9c2 cardiomyocyte cells against DNR-induced damage[153]. As mentioned earlier, flavonol metabolites predominate in plasma while the aglycone exists in a tiny amount. Lodi et al. assessed quercetin and its glucuronidated, methylated and sulfated metabolites vasorelaxant effects, and their roles on NO bioavailability and endothelial function in rat aorta. It was found that the free quercetin and its metabolites offered protection against endothelial dysfunction. The only downside was these conjugates lack direct vasorelaxant effects, unlike the quercetin aglycone[154].

Flavonol's other reported health benefits involved life-prolonging property in Caenorhabditis elegans[155], anti-leishmaniacal activity[156], anti-viral activity[157] etc. A recent review by Bischoff et al. provides a comprehensive assessment of the biological effects of quercetin[158].

Despite promising results related to the effects of flavonol on human health, possible adverse outcomes or lack of effects are also matters of debate. Back in 1997, Hertog et al. investigated the hypothesis that high flavonols intake resulted in low rate of ischemic heart disease (IHD). This was done in 1900 Welsh man aged range from 45 to 59 years old but surprisingly, no correlation was found[159]. Similarly, effects of the dietary antioxidant supplementation (quercetin and vitamin C) at 900mg/day on the blood inflammatory biomarkers and on the severity of rheumatoid arthritis in patients were assessed after treatment. Again, no significant differences of pro-inflammatory cytokines such as C-reactive protein (CRP) in the plasma after 4 weeks of supplementation were observed. Moreover, the scores of disease severity measurement were not significantly decreased[160]. Kääriäinen et al. investigated quercetin antioxidant and cytotoxicity in vitro and neurotoxicity in vivo. Quercetin showed significant antioxidant effect against 6-hydroxydopamine(6-OHDA)-induced oxygen radical formation in catecholaminergic SH-SYSY neuroblastoma cells. At low concentration in the range of 10 to 100µM, quercetin protective effect was seen by a reduction of caspase-3 like activity, but at the highest concentration tested (100 µM), enhanced toxicity occurred reversing the protective effect. In vivo results showed no consistent neuroprotective effect of quercetin concentration of up to 200mg/kg in 6-OHDA rat lesion models of Parkinson’s disease[161]. In a later study, it was revealed that quercetin’s protective in vitro was time-dependent, in which initially, quercetin protected against 6-OHDA-induced cell death, but after prolonged treatment, the protective effect began to diminish[162]. The author attributed the discrepancy to the fact that articles reporting quercetin anti-neuroprotective property only analyzed results in the early protective stage, missing the toxicity effect that might have followed. In a phase I clinical trial, cancer patients who were given intravenous quercetin at escalating doses reported renal toxicity, chest pain, nephrotoxicity, nausea[163].

FLAVANOLS

One unique characteristic of flavanols that distinguish them among other flavonoids is instead of being present in glycosides, they can occur in diet as aglycones. Flavanols are abundant in tea, chocolate, fruits and red wine[13, 126], and the most representative molecules of flavanols are (-)-epicatechin and (+)-catechin epimers, gallatechins, and catechin gallates, the gallic acid esters of catechins. Bioavailability and pharmacokinetic studies of flavanols have been investigated mainly after the administration of tea, cocoa or red wine[126]. In some studies, pure flavanol compounds were used. In tea, the most abundant flavanols are (-)-epicatechin gallate (ECg), (-)-epigallocatechin (EGC) and (-)-epigallocatechin gallate (EGCG)[13, 164, 165]. In red wine, relatively high levels of (-)-epicatechin and (+)- catechin epimers were found[13], while in cocoa (-)-epicatechin and (-)-catechin, instead of the more common (+)-enantiomer, were reported[166]. The peak plasma concentrations (Cmax) of EGCg, EGC, and EC were found to be 0.17, 0.73 and 0.43µM, respectively at Tmax 1.3 to 1.6 hours after a single dose of 20mg green tea solids/kg was administered[165]. The results elucidated the relatively low bioavailability of all three catechin found in green tea. However, Nakagawa et al. report a much higher EGC Cmax of 4.3 and 4.4µM, 1.5 hours after ingesting 375mg and 525mg EGCG equivalent of green tea capsules respectively[167]. Flavonol bioavailability was also tested in cocoa beverage and red wine. After 5 healthy adults were given 0.375 g cocoa/kg as a beverage, the Cmax for (-)-epicatechin, and (+)-catechin were 5.92 µM and 0.16 µM, respectively after 2 hours. Interestingly, epicatechin was the predominant plasma flavanol even though the measured ratio during cocoa preparation of epicatechin to catechin was 1:1[168]. Possible explanation for this observation can be that the cleavage of dimers or other procyanidin oligomers that form epicatechin monomers were then absorbed[168, 169]. In another study that involved the consumption of black chocolate, much lower Cmax of 0.383 and 0.7µM were reported after the administration on epicatechin equivalent dose to 82 and 164mg in chocolate. In wine, the bioavailability of catechin is extremely low compared to other flavanol food sources. An intake of 120mL of red wine (35mg catechin) resulted in a catechin Cmax of 91nM[170].

Investigations on flavanols using pure individual tea catechins also revealed low bioavailability of each flavanol. It was reported that no EGCG or EC in the plasma after administration of pure EGCg and the reported EGCG Cmax was 0.96µM[164]. Van Amelsvoort et al. reported similar Cmax of 1.3µM, but traces of EGC (5.1 percent of AUC) was detected in the plasma, while after ingesting 663mg of EGc, its Cmax was found to be 3.1µM with traces of the
epicatechin (3.3 percent of AUC). Interestingly, after the consumption of 459 mg of EGC (the only tea polyphenol without gallic acid substituent), a separate peak was found in addition to EGC, which was confirmed as the O-methylated EGC. EGC Cmax was 5.0 μM whereas the Cmax for the methylated EGC was reported to be 1.9 μM. In urine, no traces of EGCg or ECG were found but 13.6 percent of EGC was excreted (9.8 percent aglycone and 3.8 percent methylated) [171].

In plasma, flavanols can appear as aglycones or as glucurononated, sulfated or methylated conjugates [13]. For (+)-catechin, Donovan et al. reported the formation of 3′-O-methylcatechin and their sulfate, glucuronide, sulfo-glucuronide conjugates after consuming 35 mg of catechin in 120 mL of red wine [172]. On the other hand, for (+)-epicatechin, three metabolites namely (+)-epicatechin-3′-O-glucuronide, 4′-O-methyl- (+)-epicatechin-3′-O-glucuronide, and 4′-O-methyl- (+)-epicatechin-5′ or 7-O-glucuronide were purified from human urine [173]. Lu et al. investigated extensively the enzymology of the human cytosol catechol-O-methyltransferase (COMT)-catalyzed methylation of EGCg and EGC. This enzyme catalyzed the methylation of EGC to form 4′-O-methylEGC and at a lower concentration, 3′-O-methyl-EGC. For EGCg, it was first metabolized to 4′-O-methyl-EGCG, then to 4′,5′-di-O-methyl-EGCG. The possible glucuronidation sites of EGCG were reported at 7 position on the A ring, 3′ position on the B ring and 3′,4′ position on the gallate ester D ring. Besides, the structure-inhibition activity was also proposed. It was found that glucuronidated EGCG on the B- or the D-ring greatly inhibited methylation on the same ring but glucuronidation on the A ring of both EGC and EGCG did not affect methylation [174]. Apart from the flavan-3-ol conjugates, phenolic acids were also reported after the consumption of flavanol-rich food. Colon microflora has been reported to degrade flavanols to simple phenolic acids or ring-fission metabolites, valerolactones. 6 phenolic acids were found in the urine after chocolate intake: m-hydroxyphenylpropionic acid, furfural acid, 3,4-dihydroxyphenylacetic acid, m-hydroxyphenylactic acid, vanillic acid, and m-hydroxybenzoic acid [175] while 2 ring-fission metabolites (-)-5-(3′,4′,5′-trihydroxyphenyl)-γ-valerolactone and (-)-5-(3′,4′-dihydroxyphenyl)-valerolactone were also identified [165].

Higher and repeated dosing were shown to improve flavanol’s bioavailability [164, 176]. Compared to a single grape seed polyphenolic extract (GSPE) dose, repeated daily exposure to GSPE in mouse models was found to significantly improve the bioavailability of catechin and epicatechin by 253 and 282 percent respectively [176]. The effects of alcohol and milk on the bioavailability of flavanols were also scrutinized. No difference was seen in the proportion of individual flavanol metabolites after the consumption of red wine and de-alcoholized red wine, but ethanol was able to reduce the elimination half-life of catechin in plasma by facilitating elimination in urine [172]. Results on the effect of milk on plasma flavanol levels are mixed. Serafini et al. reported the absorption of (-)epicatechin into the bloodstream after ingestion of chocolate was significantly less when chocolate was accompanied by milk (-46.4 percent) or chocolate itself containing milk (-69.1 percent). The author hypothesized a formation of secondary bonds between the flavanols and milk proteins that reduced the absorption of flavanols [177]. Other studies clearly oppose the reported effects. Roura et al. demonstrated that milk did not affect the bioavailability of flavanols [178]. Likewise, Mullen et al. revealed negligible effects on the plasma pharmacokinetics of catechin metabolites when cocoa beverage was made with water or milk. However, milk had a major effect on the flavanol metabolite concentrations in urine, with significant reductions in the excreted 4 flavanol metabolites [179]. The author credited such discrepancies to the amount of flavanols available in cocaos. With high flavanol concentrations, milk that reduces absorption have minimal effect compared to typical commercial cocaos with lower flavanol content in which milk is capable to affect the absorption.

Besides flavanol, flavanols also possess prominent antioxidant properties, with both ECG and ECG being the best of breed, comparable to or even greater than that of quercetin. The presence of pyrogallol group in the B ring greatly increases the radical scavenging activity and this pyrogallol ring is known to outperform the typical catechol ring. In addition, galloylation of the 3 position further enhances the radical scavenging capacity. Catechin, which lacks all these features, had a 50 percent lower Trolox Equivalent Antioxidant Capacity (TEAC) than both ECG and ECG [115]. Tea flourishing with flavonoids, especially flavanols, is not surprising to possess potent beneficial antioxidant effects. In a recent study on experimentally induced cerebral hypoperfusion rat models, 400 mg/kg of green tea polyphenols were found to scavenge oxygen free radicals, enhance antioxidant potential, decrease lipid peroxide production and reduce oxidative DNA damage in rats. In addition, the green tea treated rats had better spatial learning and memory compared to the control rats [180]. Similarly, on cholesterol-fed rats, green tea polyphenol reduced the susceptibility of LDL to oxidation, decreased malondialdehyde (oxidative stress marker), improved antioxidative activity of serum and increased HDL cholesterol level [181]. However, an in vivo human study showed that single or double dosage of tea polyphenol extract did not improve the antioxidant activity measured by ferric reducing antioxidant power (FRAP) assay. No antioxidant activity increment was detected even after 7 days of continuous consumption of tea catechins but significant decrease in the activity was observed 7 days after the withdrawal from the intake [182]. Cocoa, another food source that contains abundant flavanols was also shown to exert strong antioxidant effects. After heat exposure, flavanols in rats fed with cocoa polyphenolic extract inhibited the generation of free radicals by activated leukocytes and protected the rat from subsequent cognitive impairments [183]. Consumption of cocoa also contributed to the inhibition of lipid peroxidation in human [184] and attenuated in vitro LDL oxidation [185].

Besides boosting individuals’ antioxidant defense system, flavanol-rich green tea and cocoa can affect cardiovascular health and function, by modulating inflammation, platelet aggregation, nitric oxide availability and lipid profile that directly affects blood pressure [186, 187]. In a human study, one week of dark chocolate consumption elucidated flavanols’ cardioprotective properties, in which they improved lipid profile (lowered LDL by 6 percent, increased HDL by 9 percent) and decreased platelet reactivity [188]. Similarly, a 5-week treatment with green tea extract, or equivalent of 270 mg of ECG on 14 healthy women resulted in 37.4 percent reduction in the oxidized LDL concentration [189]. The role of platelet in the development and manifestation of atherosclerosis, myocardial infarction and stroke is well documented [186]. Compared to the placebo group, Murphy et al. reported significantly decreased platelet function, P-selectin expression, and ADP- as well as collagen-induced aggregation after healthy volunteers were administered with 234 mg cocoa flavanols and procydamins for 28 days [190]. When human umbilical-vein endothelial cells were treated with pure EC, ECG, EGC and ECG, only ECG showed inhibition properties on endothelial exocytosis in a dose-dependent manner. Besides, ECG increased Akt phosphorylation, eNOS phosphorylation, and nitric oxide (NO) production, which help attenuated vascular inflammation [191].

Many experimental animal studies and in vitro cell culture studies have also tested the antimutagenic, anti-carcinogenic and anticancer potential of flavanols. Kavanagh et al. revealed the effect of green tea on breast cancer both in vivo and in vitro. Green tea ingested by 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary cancer in rats significantly improved the mean latency of first tumor, reduced the tumor burden and decreased the number of invasive tumors. In vitro experiment with the treatment on EGC on breast cancer cell lines further confirmed that it inhibited cell proliferation [192]. Similar in vitro studies on the effect of flavanols on mammary cancer elucidated two diverse mechanisms of EGC and EGC independently inhibited heregulin-beta1 (HRG)-induced migration/invasion of MCF-7 human breast carcinoma cells and prevented the metastasis of the cancer cells. EGC inhibited the
migration/invasion through downregulation of ErbB2/ErbB3/P3K/Akt signaling, while EGC functioned through the disruption of the HRG-stimulated activation of ErbB2/ErbB3 but not Akt[193]. Aqueous black tea extract reversed testosterone-induced oxidative stress in Wistar rats that if untreated might result in development of prostate cancer[194]. In vitro studies also showed that at 0.2 percent, cocoa polyphenols extracts had a growth inhibition effect on two prostate cancer cell lines (nonmetastatic 22Rv1 cells and metastatic DU145 cells) but not on normal prostate cells. The anti-proliferative activity was significantly greater than β-sitosterol, the most common phytosterol used which only showed minor growth inhibition. Interestingly, there was no synergistic effect when both cocoa extract and β-sitosterol were combined for treatment[195]. Lee et al. presented a detailed review article on the possible effects of tea and coffee on cancer, particularly prostate cancer[196]. Green tea, cocoa and pure flavanols have also been reported to treat other cancer diseases, for instance oral and gastrointestinal cancer[197-199], lung cancer[200], pancreatic cancer[201] and liver cancer[202, 203].

Flavanol consumption has also been associated with life prolonging benefits. Saul et al. found that catechin treatment caused a marked reduction in Caenorhabditis elegans' body length, but an increase in lifespan, which supported the “Disposable Soma” theory. Further investigation revealed the importance of catechin in assisting modulation of stress response and repair system that resulted in a prolonged lifespan[204]. This result was replicated by Abbas et al. with the use of main tea polyphenols, EGCg[205]. Flavanols are also considered to be beneficial for providing ultraviolet protection, especially with ECGG[206]. Some studies also suggest that flavanols’ neuroprotective and anti-neurodegenerative activities[207, 208]. In addition, flavanols administration led to improved body composition and possible anti-obesity potential[209, 210]. Finally, flavanols are also associated with anti-diabetic effect, such as improved glucose tolerance and insulin sensitivity[211, 212].

There are also studies that reported unfavorable or lack of beneficial effects associated with flavanols. Ried et al. reported no blood pressure lowering effect of flavonol-rich dark chocolate, after 50g or 750 mg polyphenols equivalent of consumption for 8 weeks[213]. Moreover, neuroprotective effect of catechin was not observed in nigrostriatal dopaminergic neurons in Parkinson’s disease rat model, unlike other flavonoids, such as flavones, procyanidins and isoflavones that could attenuate the 6-hydroxydopamine-induced dopaminergic loss[214]. A cohort study conducted by Kikuchi et al. revealed no association of green tea consumption and prostate cancer incidences among 19561 Japanese men, who on average consumed more tea than the Westerners[215]. Some adverse effects after ingestion of flavanols were reported. Bonkovsly et al. and Stevens et al. both demonstrated hepatotoxicity following the consumption of supplements containing tea extracts: green tea extract from Camellia sinensis and Hydroxyxcut, a concoction of plant extracts respectively[216, 217].

ANTHOCYANINS

Studies investigating the bioavailability of anthocyanins that looked into the pharmacokinetics, absorption, metabolic fate and excretion have increased exponentially over the past decades in both human and animal models. In plants, both cyanidin aglycone and its glycosides, with hydroxyl on the 3-position on the C ring of the aglycone being occupied by different sugar groups, exist in leaves, fruits, vegetables and flowers. Anthocyanins pH dependent colorations from red, purple to blue have attracted interest from food industries due to their promising properties as natural food colorants. They can be used to replaced artificial food color that was reported to increase hyperactivity in children[218]. In order to study the bioavailability of anthocyanins, animal or volunteers are generally administered with anthocyanin-abundant berries (elderberry, blackcurrant, blackberries, chokeberry, etc), juices, wines, extracts or pulps. After a certain period, plasma and urinary excretion are collected for analysis. In general, the peak plasma concentrations (C_max) of anthocyanins were extremely low, varying from the mid to low nM range[219]. The highest reported anthocyanin C_max at 96nM was attained 2.8 hours after volunteers were administered with 7.1g of encapsulated chokeberry extract containing 721.4mg of cyanidin-3-O-glycosides[220]. A merely 5.17nM and 2.53nM C_max were reported after consumption of acai pulp and clarified acai juice, respectively[221]. Similarly, the anthocyanin urinary excretions were relatively low. Elimination of plasma total anthocyanins followed first-order kinetics, with rate of urinary excretion reaching maximum 3-4 hours after post-administration and then decreased exponentially[222, 223]. Kay et al. reported the total urinary excretion of metabolites and parent compounds of merely 0.15 percent of the initial dose over the course of 24 hours[220]. An even lower total anthocyanin urinary excretion of 0.05 percent of ingested dose was reported after the consumption of 12g of vitis vinifera grape peel extract (equivalent anthocyanin dose of 183.9mg)[224].

Unlike flavonoids, in which glucuronidated, sulfated or methylated conjugates are generally uncovered in plasma and urine with little or no native forms found, both intact anthocyanin glycosides and the metabolized derivatives have been identified in both plasma and urine[126, 219]. Various glycosides (glucoside, galactoside, rutinoside, sambubioside) glycosylated at the 3-position and anthocyanin derivatives on the B ring (delphinidin, malvidin, etc) could be absorbed directly into the plasma. Miyazawa et al. showed that in both humans and rats, cyanidin-3-O-glycoside and cyanidin-3,5-diglycoside were absorbed from the digestive tract into the blood circulation system in structurally intact forms[225]. In addition, cyanidin 3-galactoside, cyanidin 3-rutinoside, cyanidin 3-sambubioside, cyanidin-3-sambubioside-5-glucoside have also been detected unaltered in plasma, urine or both[226-228]. In a recent human study, following 12g of grape extract oral ingestion, 3-monoglucosides of delphinidin, cyanidin, petunidin, peonidin and malvidin, were detected in their intact forms in both plasma and urine, except for cyanidin 3-glucosides, which could not be identified in the plasma alone[224]. No further explanation was given on the undetected glucosides.

In addition to the intact anthocyanin glycosides, their metabolites have also been profoundly investigated. Tian et al. showed that the methylation patterns of anthocyanin glycosides were primarily affected by the structures of the glycoside moieties (monoglycosides, diglycosides or triglycosides). Methylation occurred on the 3'-hydroxy on the B-ring of anthocyanin monoglycoside and diglycoside, whereas for anthocyaniglycosides triglycosides like cyanidin 3-xylosylrutinoside, methylation could occur at either 3'- or 4'-hydroxyl[219, 229]. Both glucuronide and sulfate conjugates of cyanidin have been studied. In most of the studies, mono-glucuronidated anthocyanin like peonidin, isopeonidin, cyanidin and cyanidin-3-glucoside monogluconurides were reported[220, 224, 230-233]. The urinary excretion of cyanidin 3-glucoside diglucuronide was also detected after consumption of steamed red cabbage[234]. In the case of sulfoconjugates, pelargonidin- and cyanidin-sulfates have been identified in the urine[235, 236]. Anthocyanin aglycones or anthocyanidins have been identified only in a appreciable amount in a few studies due to their relative instability. Hassimotto et al. attributed the trace amounts of the aglycones in small and large intestines to the action of endogenous and microbial β-glycosidases, which hydrolyze or deglycosylate flavonoid glycosides for subsequent glucuronidation and sulfoconjugation[230].

The main metabolites resulted from the degradation by the intestinal micro flora are phenolic acids and aldehydes. Woodward et al. elucidated rapid degradation of anthocyanins to phenolic acids (4-hydroxybenzoic acid, protocatechuic acid, gallic acid) and aldehydes (phloroglucinol aldehyde) under simulated (in vitro) physiological conditions. In addition, the author also revealed the reversed correlation between the number of hydroxyl groups on the B-ring and the stability of anthocyanin molecules[237]. In an earlier study conducted by Vitaglione and colleagues, protocatechuic acid was found to be the major metabolite of cyanidin-glycosides in humans,
accounting for 44 percent of the ingested cyanidin-3-glucosides in the plasma (6 hours) and 28.1 percent in the feces (24 hours) after administration of blood orange juice[238]. However, no protocatechuic acid was detected by Hassimotto et al. after Wistar rats were orally administered with anthocyanin-rich extract from wild mulberry[230].

The diverse result among studies in the area of bioavailability, including plasma and urine concentrations as well as the availability of certain compound can be attributed to a few factors including source of anthocyanins, food matrix, acylation, dosage, individual variation, analytical methodology and the sensitivity of detection method[219, 234]. A very recent study revealed the effect of dosage, acylation and plant matrix on the bioavailability of anthocyanin in purple carrot juice. Highest peak anthocyanin plasma concentration was obtained with 250mL of carrot juice containing 380.2µM of anthocyanin compared to 2 other lower doses of 50mL (76.1µM) and 150mL (228.1µM). Two major findings in this report were nonacylated anthocyanins were more bioavailable than acylated anthocyanins and secondly, increased administration dose resulted in a increased absorption but decreased absorption efficiency[234]. On an even greater administration dosage of 714µM equivalent anthocyanin published in later publications, no peak plasma concentration was observed suggesting saturation of anthocyanin occurred at dosage range from 250 to 350µM[234, 239]. These results highlighted the fact that increased dosage resulted in improved peak plasma concentration up until a saturation point. In addition, sample preparation techniques and the molecule structure can also contribute to the overall recovery of anthocyanins. Woodward et al. demonstrated that solid-phase extraction (SPE) adopted by different studies was seriously affected by hydroxylation on the B-ring and resulted in compound degradation and lost of anthocyanins[237].

Similar to other polyphenols, anthocyanins affect a huge array of biological activities and many of the potentials are related to their inherent antioxidant capacities[71]. Anthocyanins and the aglycones antioxidant activities are equipotent to quercetin and catechin gallates (EGCg), especially with the aglycone cyanidin. Similar to other antioxidants, catechol structure of the B-ring like cyanidin drastically affects the overall radical-scavenging capacity of the molecules; for example pelargonidin with the missing 3'-hydroxy group had a lower overall antioxidant capacity by more than 3-fold compared to cyanidin[115]. These results were further confirmed by Fukumoto et al. who also reported improved antioxidant activity with increased number of hydroxy groups on the same ring whereas decreased activity with the glycosylation of anthocyanidins[240]. However, in vitro and in vivo studies have presented anthocyanins compelling potentials on modulating nitric oxide(NO) production[241, 242], protecting cells against oxidative stress[243] and oxidative damage[244], inhibiting lipid peroxidation[245], reducing reactive oxygen species (ROS)[246].

Anthocyanins anti-carcinogenic, anti cancer and chemopreventive activities were extensively assessed in in vitro, animal cancer models and also controlled human dietary intervention studies. In vitro tests have been performed on different human cancer cell lines under different experimental conditions to show anthocyanins anti-proliferative, pro-apoptotic and pro-oxidant potentials by regulating gene and protein expression[71, 219]. Hafeez et al. showed that delphinidin was able to induce apoptosis on human prostate cancer cells both in vitro and in vivo. Delphinidin treatment of human prostate cancer LNCaP, C4-2, 22Rnu1, and PC3 cells gave rise to cell growth inhibition and induced apoptosis in a dose-dependent manner by activation of caspases. Similar results were also presented in vivo in athymic nude mice implanted with PC3 cells, in which delphinidin significantly inhibited tumor growth[247]. In rats, black raspberries and anthocyanin-rich fractions were shown to prevent esophageal tumorigenesis by inhibiting cell proliferation, inflammation, and angiogenesis as well as inducing cell death in both preneoplastic and papillomatous esophageal tissues[248]. Mirtoselect, an anthocyanin-rich standardized bilberry extract as well as isolated cyanidin-3-glucoside were tested on ApcMin mouse models of intestinal carcinogenesis for 12 weeks at a concentration of up to 0.3 percent in the diet. As a result, the number of intestinal adenomas was decreased by 45 and 30 percent, respectively compared to the untreated mouse[249]. Similarly, the chemopreventive role of Mirtocyanin (previously Mirtoselect) was later elucidated on 25 colorectal cancer patients. The consumption of up to 2g of anthocyanins daily for 7 days before surgery inhibited the tumor tissue proliferation by 7 percent compared to pre-intervention values[250].

Several studies looking into anthocyanins cardioprotective effects were intended to investigate the underlying mechanisms in the vascular system. Human umbilical vein endothelial cells (HUVECs) were pretreated with Aronox, an anthocyanin-rich extract from Aronia melanocarpa E before 7β-hydroxycholesterol-induced apoptosis treatment. Aronox significantly decreased apoptosis by inhibiting cytochrome c release, reversing both the down-regulation of Bcl-2 and up-regulation of caspase-3[251]. In another study, pure cyanidin- and peonidin-3-glucosides were treated on CD40-mediated endothelial activation and apoptosis in HUVECs. Similarly, both anthocyanins inhibited CD40-induced apoptosis, JNK and p38 activation whereas endothelial activation was also prevented by limiting the production of pro-inflammatory cytokines and matrix metalloproteinases[252]. Anthocyanins cardioprotective effects were also confirmed in vivo. Wistar rats fed with anthocyanin-rich diet for 8 weeks successfully protected the myocardium from ischemia-reperfusion injury ex vivo as well as in vivo by possibly improving the heart endogenous antioxidant defenses[253]. Atherosclerosis-induced mice fed with anthocyanin-rich extracts from black rice for 20 weeks had significantly improved lipid profile by decreasing serum triglyceride, total cholesterol and non-HDL cholesterol, and hence, resulted in a smaller atherosclerotic plaque area[254]. In dyslipidemic patients, the intake of berry-derived anthocyanin supplement was capable of increasing HDL-cholesterol concentrations, decreasing LDL-cholesterol concentrations and improved the cellular cholesterol efflux to serum[255].

Significant life-prolonging effect of anthocyanin was also demonstrated by Butelli et al. The group engineered tomato intended to boost the suboptimal anthocyanin level. By expressing two transcription factors of snapdragon in tomato, they successfully induced the high accumulation of anthocyanin in tomato to the level found in berries, which enhanced the overall antioxidant capacity of tomato fruit by 3-fold and resulted in fruit with intense purple coloration in both peel and flesh. The life span of cancer-susceptible Trp53(-/-) mice fed with 10 percent of the engineered tomato powder was significantly enhanced with average lifespan of 182.2 days compared to the control diet (142 days) and red tomato diet (145.9 days)[256]. Anti-inflammatory effects exhibited by dietary anthocyanins have also been evaluated. Recently, a study by DeFuria et al. utilizing mice on high-fat diet showed that blueberry powder and anthocyanin displayed protective potential against adipose tissue inflammation by attenuated the up-regulation of inflammatory genes including tumor necrosis factor-α, interleukin-6, monocyte chemoattractant protein 1 and inducible nitric oxide synthase[257]. Evidences suggesting anthocyanin vision improvement potential have also been demonstrated. Matsumoto et al. verified the effects of purified cyanidin 3-glucoside and cyanidin 3-rutinoside on accelerating the regeneration of rhodopsin, most probably through improved formation of a regeneration intermediate[258]. In a human study, administration of high doses of anthocyanoside oligomer daily for 4 weeks, improved morphoscopic objective contrast sensitivity in myopia subjects[259]. In addition to the plethora of bioactivities discussed, anthocyanins also possess anti-obesity and anti-diabetic[257, 260], neuroprotective[246, 261], and gastroprotective properties[262].

Despite all the health benefits, Galvano et al. doubted the relevance of in vitro culture experiments performed exclusively with anthocyanin aglycones and the significance of in vitro studies evaluating the bioactivity anthocyanins possess. This is mainly due to the nature of anthocyanins at physiological pH that can be easily
...degraded to protocatechuic acid and other benzoic acids, resulting in the low amount of actual anthocyanins to act on cells as well as tissues, and promote their health benefits[263]. Similarly, there are also reports declaring absolutely no effect on certain reported bioactivities. Moller et al. showed no effect of blackcurrant juice or an anthocyanin drink prepared from blackcurrant anthocyanin concentrate on DNA damage markers in 57 healthy volunteers[264]. Possible explanation to this observation was that the antioxidant activity might only show protective effect on oxidatively stressed subjects.

STILBENES

Trans-resveratrol is by far the most extensively investigated and reported stilbene, as compared to other analogs, like its cis counterpart, pinosylvin (trans-3,5-dihydroxystilbene) and piceatannol (trans-3,5,3',4'-tetrahydroxystilbene). Pharmacokinetic studies indicate that circulating resveratrol in the plasma is extensively metabolized in human body and the oral bioavailability of resveratrol is close to zero[265], being restricted by limited absorption, limited chemical stability, and degradation by intestinal microflora and intestinal enzymes[94, 95, 266]. In Walle et al.’s bioavailability study, when 14C-resveratrol doses of 25 mg were orally administered to six healthy volunteers, the peak resveratrol and metabolite plasma concentration was 491 ng/mL or equivalent to 2µM after an hour, followed by a second peak of 1.3 µM and plasma concentrations declined exponentially thereafter[267]. Similarly, Yu et al. reported that virtually no unconjugated form of resveratrol was found in the plasma or urine samples[268]. Resveratrol is mainly distributed to various tissues in its conjugated forms in humans, and resveratrol glucuronides as well as sulfates predominate the plasma[267, 269].

The fact that resveratrol remains intact after incubation with human liver microsomes shows that phase I enzymes are inactive in resveratrol metabolism[268, 270]. Phase II enzymes, which are active in conjugation reaction, are the ones that actively metabolizing the intact resveratrol and resulting in the formation of the conjugates.

Resveratrol received little interest until 1992, when it was postulated to explain the “French paradox”, the phenomenon that describes the inverse correlation between the highly saturated fat consumption and the number of cardiovascular diseases in France compared to the USA. This led to the hypothesis that consumption of relatively high amount of red wine might lead to cardioprotective effects. Since then, dozens of publications have revealed different human related health benefits associated with resveratrol, including antioxidant, anticancer, anti-inflammatory, antivirus, anti-aging and life-prolonging activities[14, 271-273].

Out of all the health associated benefits resveratrol possesses, the most intriguing properties are its anti-aging and life-prolonging potentials. A discovery in the 1930s revealed the inverse relationship between calorie intake and lifespan, and recently, sirtuin, a highly conserved class of NAD+-dependent deacetylase, has been shown to play a significant role in calorie restriction[274, 275]. Up to 20000 compounds were screened for sirtuin activator compounds (STAC) using fluorescent deacetylation assay and resveratrol was found to be the most potent sirtuin activator, up to 14-fold improvement[271]. Howitz et al. also demonstrated resveratrol potential in yeast on mimicking calorie restriction, increasing DNA stability and eventually extending their lifespan by up to 70 percent[271]. Besides, resveratrol has been demonstrated to extend the lifespan of evolutionary distant species such as C. elegans, D. melanogaster and vertebrate fish[276-278]. In a more recent publication by Baur et al., mice models were used to study the life prolonging properties of resveratrol. They were divided into 3 main groups: standard diet mice (SD), high calorie diet mice (HC) and high calorie diet mice with resveratrol (HCR). After 114 weeks, resveratrol successfully reduced the risk of death from HC by 31 percent to a point similar to the SD or in other words, resveratrol successfully shifted the physiology of HC towards that of mice on a SD without the need of calorie restriction. Furthermore, resveratrol opposed the effects of high calorie diet in 144 out of 153 significantly altered pathways and was found to improve insulin sensitivity, improve liver histology, increase mitochondrial number, etc[14]. It was later found that resveratrol did not actually extending the lifespan of mice, but it delayed age-related deterioration, such as reduction of osteoporosis, cataracts, vascular dysfunction and declined in motor coordination[279].

There are a few limitations involved in the anti-aging effect of resveratrol. First, these results are yet to be replicated in human and a human clinical trial that is specifically for anti-aging study would take a considerable long period of time. An alternative to the difficulties is instead of anti-aging, focuses could be directed to the potentials on age-related diseases, such as diabetes, heart attack and cancer. Second, to achieve the equivalent dose of resveratrol fed to the high calorie diet mice that resulted in a longer lifespan[271, 276-278], a equivalent human dose of about 1000 bottles/day of red wine or 60L/day of red wine with high resveratrol concentration is required, which is unfeasible. Therefore, more efforts are needed to be laid on elucidating the “French Paradox” because the apparent cardioprotective potentials are probably unachievable through daily consumption of wine. The only way to achieve the anti-aging and cardioprotective dose has to be done through pharmacology approach, for instance with resveratrol concentrated capsules or supplements. However, clinical studies using high concentrated polyphenols have showed potential toxicity effects in treated patients. Williams showed that up to 700mg/kg of Resvida (high purity trans-resveratrol) fed in rats daily for 90 days caused no adverse effects, such as toxicity and it was also found that high concentration of resveratrol was well-tolerated both in vivo and in vitro (rat hepatocytes and Caco-2 cells)[280]. Human clinical studies also showed no serious adverse effect after ingesting a single dose of up to 5g of resveratrol[281].

Besides strong evidence of its anti-aging and life-prolonging properties, resveratrol also showed potential benefits in the treatment of diabetes, neurodegenerative disorder and cancer. In an animal study, streptozotocin-induced rats pretreated with resveratrol showed an enhancement in catalase activities, nitric oxide and zinc levels, and a decrease in lipid peroxidation product malondialdehyde (an oxidative stress marker) and copper concentrations[282]. In addition, resveratrol helped maintain insulin sensitivity in diet-induced obese mice[283]. Regarding neurological disorders, Yousuf et al. underlined the significance of resveratrol in the preservation of ischemic neurovascular units and its ability in the treatment of ischemic stroke in cerebral ischemia/reperfusion induction (IR)-induced mitochondrial dysfunctions in rats[284]. Also in Alzheimer’s disease, resveratrol reduced the plaque formation in a region specific manner, including media cortex (~48 percent), striatum (~89 percent) and hypothalamus (~90 percent) after 45 days administration of resveratrol in Alzheimer’s disease transgenic mice[285].

Resveratrol also thrives in its anticancer activity at the initiation, and progression stages[270]. This has been shown in both animal and in vitro studies. Resveratrol suppressed prostate cancer in transgenic adenocarcinoma mouse prostate when 625 mg resveratrol was administered per kg diet for 28 weeks[286]. Similarly, resveratrol reduced both the number and weight of the lung metastases in rat models[287]. In human in vitro studies, a 48 hour treatment with 100µM resveratrol activated the “death pathway” in human colorectal cancer DLD1 and HT29 cells[288]. Resveratrol also inhibited tumor growth of in mouse xenograft models of human neuroblastoma. It was reported that the loss of mitochondrial membrane potential was an early response to resveratrol. When the isolated mitochondria were treated with resveratrol, depolarization occurred, and mitochondria released cytochrome c and Smac/Diablo and subsequently activated the caspases[289]. Zhou et al. utilized human hepatocellular carcinoma-derived HepG2 cells as a model to study resveratrol effect on cell growth, cell cycle progression and apoptosis. At low concentration (6.25 to 25µM), resveratrol did not cause cytotoxicity or cell apoptosis but it slowed down cell cycle progression by prolonging the synthesis phase (S phase). The slowing down of the cell cycle at this stage allocated more time to repair damaged DNA, hence reduced the chance of tumorigenesis and mutagenesis.
Figure 3. Summary of flavonoid and stilbene bioefficacy and protective potentials against various diseases in humans.

However, S-phase arrest was not observed at high concentration (50 to 100 μM); indeed resveratrol induced apoptosis and growth inhibition mediated by mitochondria pathway[290]. Resveratrol has also been reported to show cardioprotective effects. Recently, Yang et al. underlined the protective effect of resveratrol in monocrotaline (MCT)-induced right ventricular hypertrophy in rats by improving various detriment caused by MCT, such as increased right ventricular wall thickness, systolic pressure and hypertrophy, mitochondria swollen and cardiomyocyte apoptosis[291]. In doxorubicin-induced cardiomyocyte death, resveratrol also reversed the oxidative stress and cell death induced by doxorubicin through manipulation of mitochondrial function[292]. Other possible therapeutic potentials of resveratrol are anti-bacterial[293], anti-viral[294], anti-asthmatic[295], etc.

There were also articles reporting the lack of bioactivity on several area of interest. 10ppm of resveratrol showed no activity on pancreatic carcinogenesis in either the initiation or post-initiation stages of pancreatic carcinogenesis models in hamsters, which possibly due to the insufficient dosage used for pancreatic protection[296]. In two eight-weeks long rat feeding experiments, rats fed with either high resveratrol diet (300mg/kg body weight) or low resveratrol diet (50mg/kg) revealed no effect on different chemopreventive parameters, except for the total antioxidant activity[297]. Researchers attributed the lack of effect to the formation of resveratrol conjugates, which subsequently lowered the resveratrol bioavailability.

CONCLUSION AND FUTURE PERSPECTIVES

Polyphenols are common in our daily diet and ample analytical studies have provided good indications of individual flavonoids and stilbene distribution in nature with flavanones found predominantly in citrus fruits, flavones in parsley, isoflavones in leguminous plant, flavonols in onion, flavanols in green tea and cocoa, anthocyanins in berries, and stilbenes in red wine. Voluminous studies have identified the tremendous health potentials of polyphenols varying from cardioprotective, anti-carcinogenic, vasoprotective to lifespan prolonging and anti-aging shown both in vivo and in vitro (Fig. [3]).

In order to benefit from these health potentials that polyphenols offer, bioavailability studies are essential. Absorption, distribution, metabolism and excretion (ADME) studies allow precise investigation of the actual metabolites presented in the plasma and urine, their peak plasma concentration (Cmax), the duration to reach the respective peak (tmax), the duration the compound remains in the body before excretion and the percentage of excretion compared to the ingested amount. As presented in this review, most of the flavonoids and stilbene showed low bioavailability mainly due to efflux transporter and more importantly extensive metabolism through glucuronidation, sulfation and methylation[98]. The free hydroxyls exist in polyphenols are major targets for these UDP-glucuronosyltransferases (UGTs), sulforotransferases (SULTs) and catechol-O-methyl transferases (COMTs). In addition to flavonoids metabolites, phenolics, carboxylic acids and carbon dioxide are detected as a result human gut microbial degradation. Simons et al. studied in vitro flavonoids degradation using cultivated gut microflora[298]. The degradation rates for 5,7,4′-trihydroxy flavonoids, such as naringenin, genistein, apigenin, and kaempferol were significantly higher than other structural motifs. The structure-activity relationship studies were generated to examine the effect of A, B, C rings and substitution patterns on the degradation kinetics. No rate differences were observed with A-ring variations; on the B-ring, 4′-hydroxy was crucial for rapid degradation but only with the present of 5- and 7-hydroxyls; on the C-ring, existence of the double bond at the 2-3 position and 3-hydroxy did not affect the rate of microbial degradation while substitution of B-ring at position-3 (genistein) instead of position-2 (apigenin), did not affect the degradation rate. Interestingly, daidzin (genistein glycosylated at position-7) was rapidly hydrolyzed to daidzein while puerarin (genistein glycosylated at position-8) was not. Puerarin was the only tested compound that was resistant to degradation[298]. In conclusion, polyphenol aglycones and glucosides that predominate in food sources are not the primary compounds circulating the plasma and subsequently acting on target tissues. Instead, the glucuronidated, sulfoconjugated and methylated derivatives as well as the phenolic acids, resulting from intestinal, hepatic conjugation and microflora degradation, are the ones that possibly give rise to the reported health potentials. The low bioavailability of flavonoids and stilbene can be mainly attributed to the hydroxyl substitutions on the A and B rings. Furthermore, sugar moieties, number of hydroxyls and position of glycoside substitution can greatly affect the overall bioavailability of polyphenols.
In vitro studies looking into health benefits are useful in identifying potential target molecules and understanding individual mechanism of action. However, to identify therapeutic potential, animal and human studies are more appropriate because most concentrations used in vitro are significantly higher and unrealistic compared to the actual concentration distributed to the target tissues in vivo. This factor can explain the results of numerous human and animal studies that cannot be extrapolated from in vitro observations.

This review provides a comprehensive discussion on the bioavailability as well as the bioefficacy of stilbene and 6 subclasses of flavonoids. In terms of bioavailability, they are generally low but uncertainties still exist particularly in some contradictory results in absorption, metabolism and tissue distribution that are yet to be clarified. Also, given the contradictory in vivo results reported previously, more efforts need to be laid on elucidating the issues specifically on the bioactivity, toxicity and the adverse effects of individual compounds.

Future in vitro studies exploring the benefits of polyphenols should include main metabolites, and the concentration treated has to be close to the concentration detected in plasma or at least physiological viable. In vivo experiments should investigate the use of well-defined mixtures of pure compounds in addition to whole food or food extracts. This could possibly assist in identifying not only the true effects of individual compounds, but also the possible synergistic effects of different analogs that might exist. In terms of bioavailability studies, future experiments can focus on more strategies to improve bioavailability of different compounds, in addition to the ones mentioned in this review: dispensing in food matrix, methyl-capping exposed hydroxyls, combining with thickening agent (pectin), prolong supplement, etc. Methyl-capping is one promising approach that involves modifying the tested compound. It has been shown to improve bioavailability but at the same time decrease antioxidant potential. Improved polyphenols' bioavailability could help maintain a higher plasma concentration for the same time duration. One promising approach that involves modifying the tested compound.

In vitro studies in addressing each of them independently. Pharmacokinetics-pharmacodynamics relationships are crucial and have to be clarified to avoid question on whether the compounds themselves or their derivatives that are offering the plethora of pharmacological benefits.

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