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Biosynthesis of Diverse Class Flavonoids *via* Shikimate and Phenylpropanoid Pathway

Mohd Rehan

Abstract

Flavonoids are natural products, which are useful in the protection of various types of human diseases. Several bioactive flavonoids as chalcones, flavonols, flavanol, flavones, flavanone, flavan, isoflavonoids, and proanthocyanidin, are found in parts as leaves, root, bark, stem, flowers, weed, fruits of plant species. Flavonoids are synthesized in higher plant species via the shikimate pathway, phenylpropanoid and polyketide pathway. The chalcones and flavanones are central intermediates of the pathway, which give several diverse classes of flavonoids. Central intermediates pathway (chalcones and flavanones pathway) depends on plants species and group of enzymes such as hydroxylases, reductases and isomerases to give different classes of flavonoids skeleton. The anthocyanins, isoflavonoids and condensed tannin (proanthocyanidins) are an important class of flavonoids, which synthesized by flavanones. Mostly, biosynthesis of flavonoids start from phenylpropanoid pathway. The phenylpropanoid pathway starts from shikimate pathway. The shikimate pathway starts from phosphoenol pyruvate and erythrose 4-phosphate.

Keywords: flavonoids, biosynthesis, shikimate pathway, phenylpropanoid pathway, tannins

1. Introduction

Flavonoids, are the largest class of secondary metabolites, having polyphenolic structure, which widely distributed in several parts as leaves, root, stem, bark, fruit, flower, weed, of diverse plant species [1]. The flavonoids play a key role to provide pigments in plant as dark blue and red color of berries, yellow and orange color of citrus fruits. These flavonoids play similar role as vitamins in the human body [2]. The flavonoids are constituted by 15 carbon atoms, which are arranged in C₆-C₃-C₆ backbone skeleton rings, in which ring A and ring B are linked by three carbon ring C [3]. The skeleton of ring represented in **Figure 1**.

On the basis of substitution pattern, flavonoids can be classified into major subgroups as chalcone, flavanone, dihydroflavonol, flavanol, flavones, isoflavone, flavonol, leucoanthocyanidin, proanthocyanidin (condensed tannins), anthocyanin [4]. The nature of these flavonoids depends on the basis of degree of hydroxylation, structural class, conjugations, substitutions and degree of polymerization [5]. Approximately, 9000 diverse type flavonoids have been reported and sure this number will be increased [6]. The diverse type flavonoids show diverse biological function as protection from UV radiation, apoptosis,

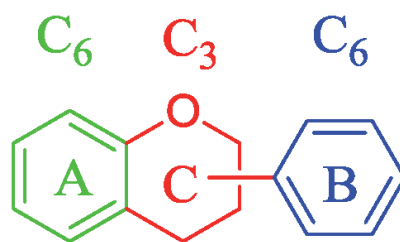


Figure 1.

Basic skeleton $C_6-C_3-C_6$ of ring A, B, and C in flavonoids.

treatment of psoriasis [7, 8]. The diverse class of flavonoids have been isolated from several plant species as quercetin and apigenin from *Cymbopogon citratus* [9], pinostrobin and cardamonin chalcone from rhizomes of *Boesenbergia rotunda* [10], 6-aldehydo-isoophiopogonanone A, 6-aldehydo-isoophiopogonanone B, methylophiopogonanone A and methylophiopogonanone B from fibrous roots of *Ophiopogon japonicus* [11]. The diverse type flavonoids were synthesized in plant species via shikimate and phenylpropanoid pathway. Several types enzyme as DAHP synthase, DHQ synthase, SA kinase, PAT, ADT, 4CL, CHS, CHI, F3H, DFR play key role in the biosynthesis of flavonoids [12, 13].

2. Shikimate pathway

Shikimate pathway plays high potential role in the biosynthesis of flavonoids. Several key enzymes are involved in this pathway for biosynthesis of shikimic acid. This pathway starts with the aldol condensation reaction of phosphoenol pyruvate (PEP) and D-erythrose 4-phosphate (E4P) to generate seven carbon keto acid, 3-deoxy-D-arabino-heptulosonate –7-phosphate (DAHP). This reaction catalyzes by 3-deoxy-D-arabino-heptulosonate –7-phosphate synthase (DAHPS) enzyme. The DAHPS is a highly potential enzyme of the shikimate pathway. Two DAHPS genes as DHS1 and DHS2 are found in *Arabidopsis thaliana* plants [14]. From literature, it is identified that DHS1 is more produced by infiltration or by physical wounding with pathogen in both tomato and *Arabidopsis* [15]. The DAHP is transformed to 3-dehydroquinic acid (DHQ) by intramolecular cyclization reaction in presence of DHQ synthase enzymes.

In most bacteria, DHQS is monofunctional and in some organism, it behaves multifunctional enzyme, which catalyze 2, 3, 4, and 5 steps of the shikimate pathway. The DHQS is a small part of larger AROM protein, which is pentafunctional peptide containing enzyme [16, 17]. The *Neurospora crassa* and *Aspergillus nidulans* DHQS enzyme found in nature as part of the AROM protein [18]. The DHQ converts into 3-dehydroshikimic acid (DHS) by losing a water molecule.

In the fourth step, DHS is transformed into shikimic acid by removing water molecule. The phosphorylation of shikimic acid is done by activating of shikimate kinase enzyme in the fifth step reaction. The shikimic acid with ATP is phosphorylated at the 5-OH group of shikimic acid converts into shikimic acid 3-phosphate (S3P). The shikimate kinase enzyme is not found in the human cell, but is an essential enzyme of many bacterial pathogens [19, 20]. The shikimic acid 3-phosphate converts into 3-enolpyruvyl shikimate –5-P (EPSP) by EPSP synthase enzymes.

The EPSPS is activating of shikimic acid 3-phosphate in the sixth step reaction of the shikimate pathway. According to intrinsic glyphosate sensitivity, it enzyme has been classified as a class I EPSP synthases and class II EPSP synthases [21, 22].

The class I EPSP synthases are found in plant and some bacteria as *Escherichia coli* and *Salmonella typhimurium*. The class II EPSP synthases is found several bacteria species as *Streptococcus pneumonia*, *Streptococcus aureus*. The EPSP converts into chorismic acid (CHA) by eliminating of the pi group at C-3.

The end product of shikimate pathway is chorismic acid, which found in plants, fungi, bacteria and some parasites [23]. The chorismate synthases (CS) is divided

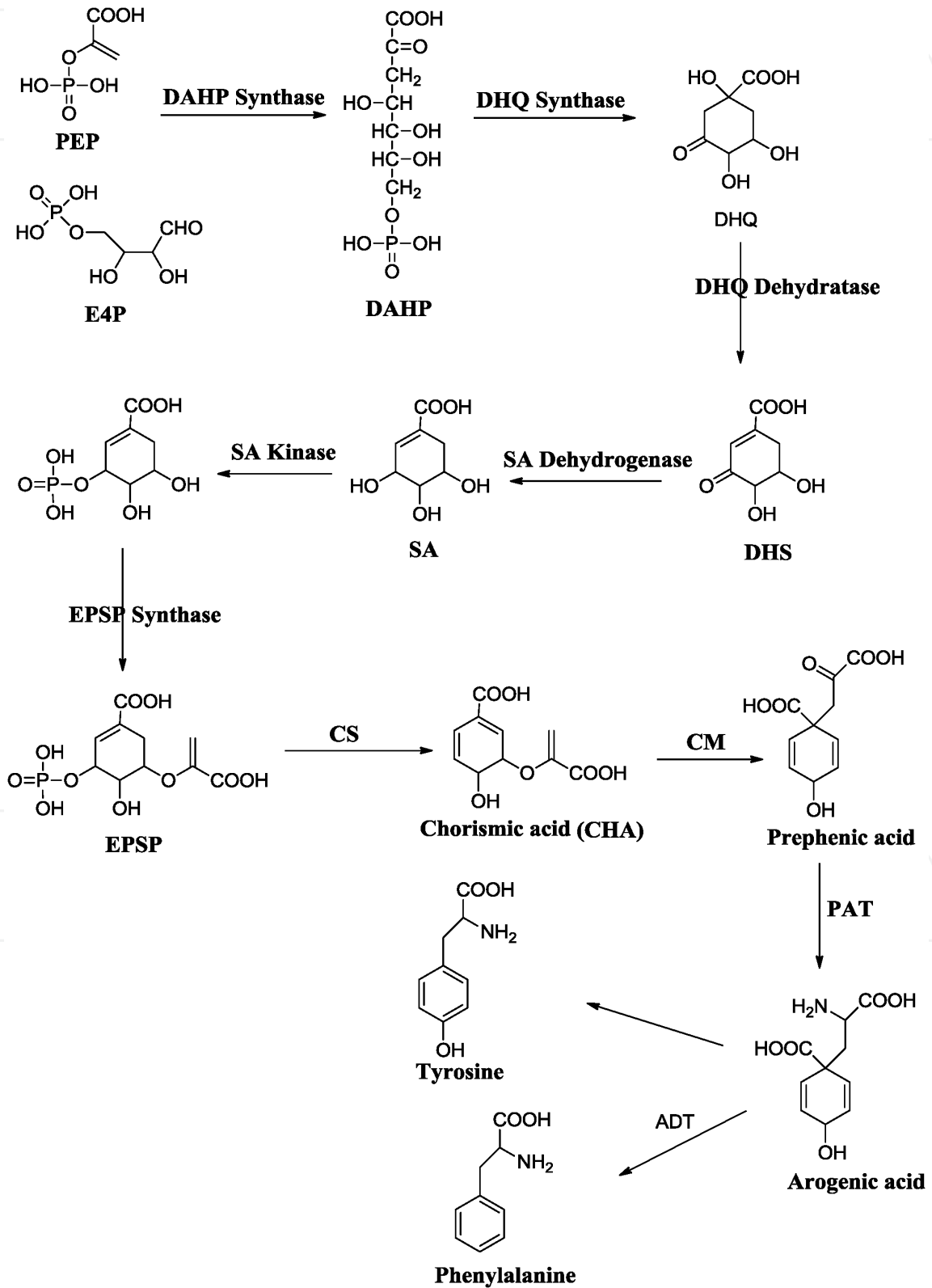


Figure 2.
Shikimate pathway in biosynthesis of flavonoids.

within one of two functional groups as fungal type bifunctional CS and plant, bacterial type monofunctional CS [24, 25].

The chorismate mutase (CM) is a first step enzyme of the tyrosine and phenylalanine biosynthesis. It activates of chorismic acid, which converts into prephenic acid by claisen rearrangement [26]. On the basis functional and structural, multiple form of this enzyme exists. Some monofunctional example from *Serratia rubidaea*, *Bacillus subtilis* [27], *Aspergillus nidulans* [28]. In presence of this enzyme, chorismic acid change into prephenic acid.

The prephenate aminotransferase (PAT) play a key role in phenylalanine biosynthesis. It catalyzes first step product (prephenic acid) into arogenic acid [29]. The arogenate dehydratase (ADT) is a last step enzyme of phenylalanine biosynthesis, which catalyzes of arogenic acid into amino acid phenylalanine [30]. In the arabidopsis genome, six ADT genes as ADT1-ADT6 are found, whereas ADT4 and ADT5 were dominant in roots and stems [31]. The shikimate pathway with enzyme activity is summarized in **Figure 2**.

3. Phenylpropanoid pathway

The shikimate pathway plays the main role in the biosynthesis of flavonoids, which provides amino acid phenylalanine. The phenylalanine ammonia lyase (PAL) is an enzyme of first step reaction in phenylpropanoid pathway. The presence of this

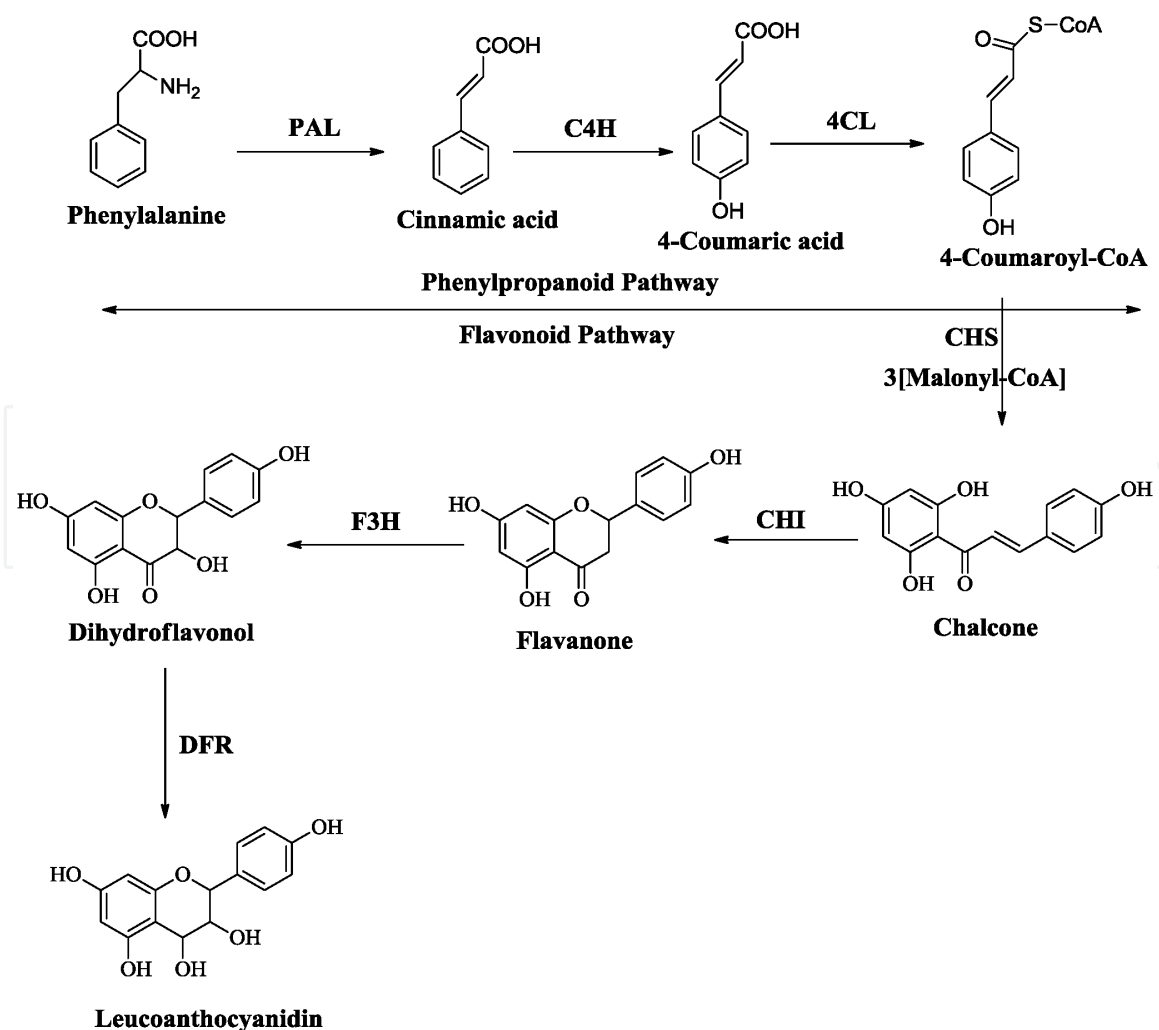


Figure 3.
Phenylpropanoid pathway in biosynthesis of flavonoids.

enzyme has been reported in different types of plant species [32] as certain fungi [33], few prokaryotic organisms, including *Streptomyces* [34, 35], algae, including *Dunaliella marina* [36] and detected in several species representing gymnosperms, ferns, lycopods, monocots, and dicots [37]. This enzyme converts phenylalanine into cinnamic acid and remove the ammonium ion.

The cinnamate –4-hydroxylase (C4H) plays a crucial role in conversion of trans-cinnamic acid in 4-coumaric acid. This acid, yielding 4-coumaroyl-CoA by catalyzing of 4-coumaroyl-CoA-ligase (4CL). The 4-coumaroyl-CoA-ligase (4CL) plays a pivotal role in phenylpropanoid biosynthesis pathway and produced coumarin skeleton. Mostly, a multiple isoform of 4CL are found in higher plants. These isoforms have distinct catalytic properties and expression profiles in plant tissue [38].

The initial step of flavonoids biosynthesis is the condensation reaction of one molecule 4-coumaroyl-CoA with three molecules of malonyl-CoA to yielding chalcone (2',4',6',4-tetrahydroxy chalcone) by catalyzing the chalcone synthase (CHS) enzyme [39]. chalcone synthase (CHS) enzyme plays key role in the biosynthesis of flavonoids and isoflavonoids. The plant polyketide synthase is a big family called superfamily, CHS is a member of this family [40]. The chalcone isomerized into flavanone by activating of chalcone flavanone isomerase (CHI) enzyme. The flavanone is the intermediate pathway of flavonoids, which divided into many different flavonoids classes [41, 42]. The modification of flavanone into the basic skeleton of flavonoids, depends on the species and a group of enzymes as hydroxylases, reductases, isomerases [43]. The phenylpropanoid pathway in the biosynthesis of flavonoids summarized in **Figure 3**.

4. Flavonoids pathway

The shikimate and phenylpropanoid pathway play important role in biosynthesis of flavonoids. After this pathway flavonoids pathway starts, which produce various diverse type flavonoids in presence of several enzymes. The isoflavonoid synthase (IFS) is a main enzyme, which converts a flavanone into isoflavone. In soybean, two isoform of IFS genes as IFS-1 and IFS-2 are found, which play a crucial role in the isoflavones biosynthesis [44, 45]. The role of this enzyme summarized in **Figure 4**.

The flavonol synthase (F3H) is a key enzyme of the biosynthesis in the central flavonoid pathway. It plays a pivotal role in the conversion of flavanone into dihydroflavonol. It has been isolated from various plant species (more than 50 plants) [46, 47]. The flavonol synthase (FLS) is a highly activating enzyme, which converts of dihydroflavonol into flavonol. The first FLS gene was known from *P. hybrida* [48] and other FLS gene were known from various plant species as *A. thaliana* [49], *E. grandiflorum* [50] etc.

The dihydroflavonol reductase (DFR) is a essential enzyme, which catalyzes dihydroflavonol into leucoanthocyanidin and are precursors of anthocyanidins and proanthocyanidins [51]. The DFR genes have been cloned in several plant species as *Lotus japonicas* [52], *Ginkgo biloba* [53], *Brassica rapa* [54]. *The DFR can overexpression in apple and tobacco, which increase anthocyanin production* [55, 56].

The proanthocyanidins is known condensed tannins (polymers), which produced by condensation of flavan-3-ol monomeric units as epicatechin and catechin. It catalyzes in the presence of two enzymes as leucoanthocyanidin reductase (LAR) and anthocyanidin reductase (ANR). The LAR is the main enzyme of anthocyanin biosynthesis pathway, which converts leucoanthocyanidin into catechin, while ANR converts anthocyanidin into epicatechin [57–59]. *The CsLAR gene is found in tobacco, which*

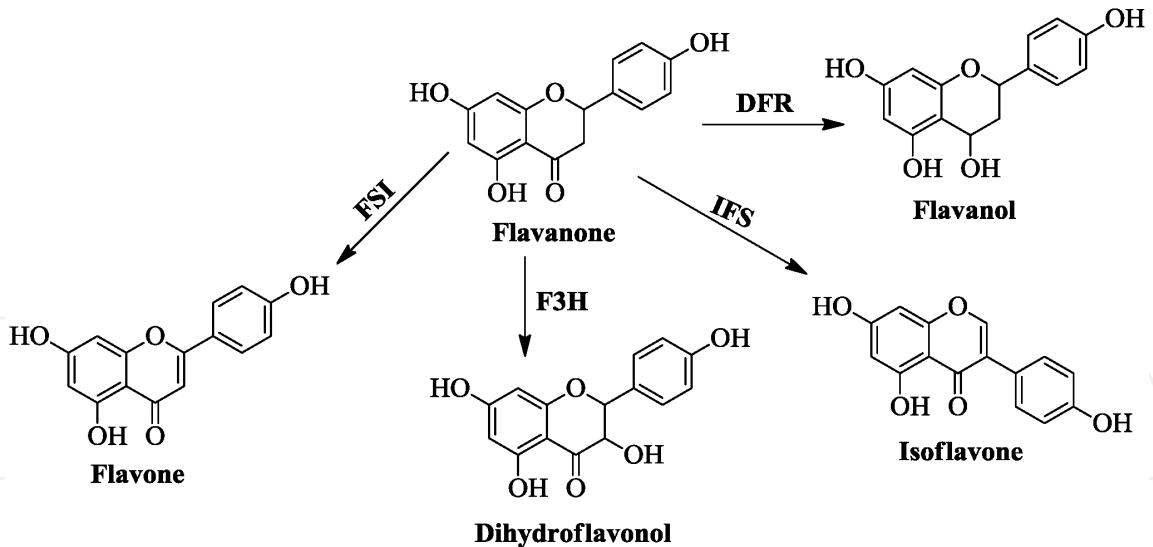


Figure 4.
The essential role of enzyme in flavonoids pathway.

accumulation of higher level of epicatechin than catechin while ANR in tea and grapevine is involved in biosynthesis of mixture of catechin and epicatechin from anthocyanidin [60, 61]. The proanthocyanidins have been reported from various plant species [62, 63]. The catalyzing properties of these enzymes are showed in **Figure 5**.

4.1 Chalcones

Chalcone synthase plays potential role in the biosynthesis of flavonoids/ isoflavonoids pathway. The CHS is a member of the polyketide synthase family, which play a key role flowering plant as providing floral pigment, insect repellents, UV Protectants and antibiotics [64]. The chalcones are called open chain

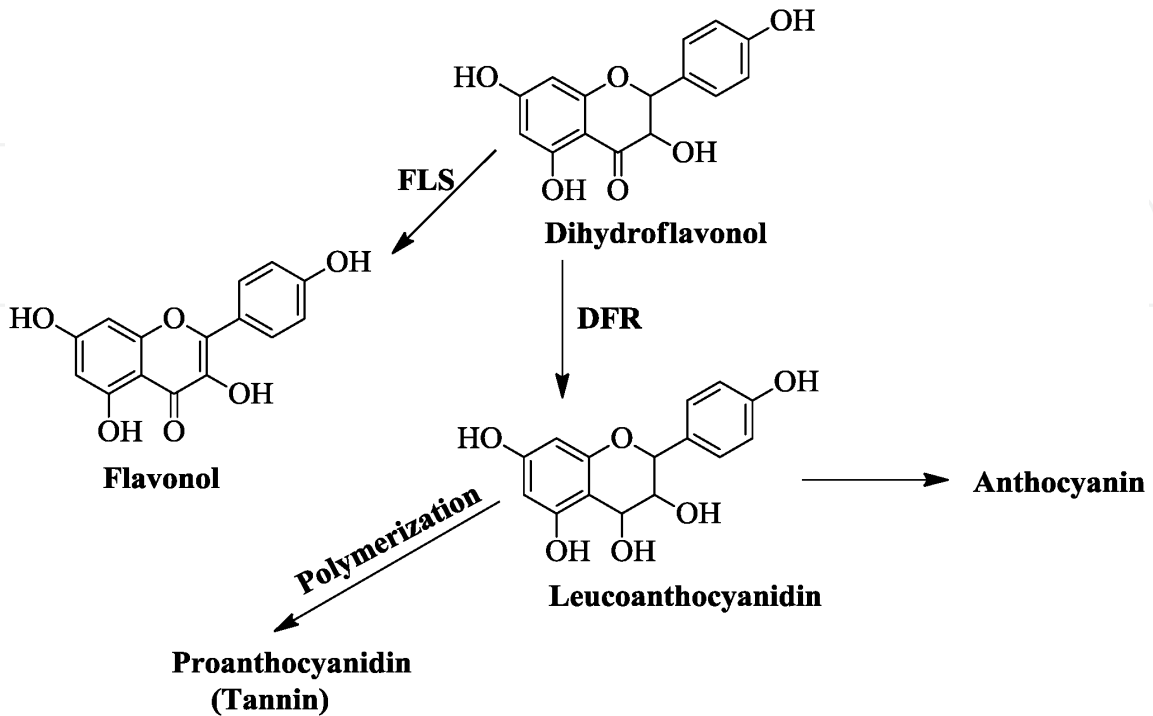


Figure 5.
Biosynthesis of tannins and anthocyanin in flavonoids pathway.

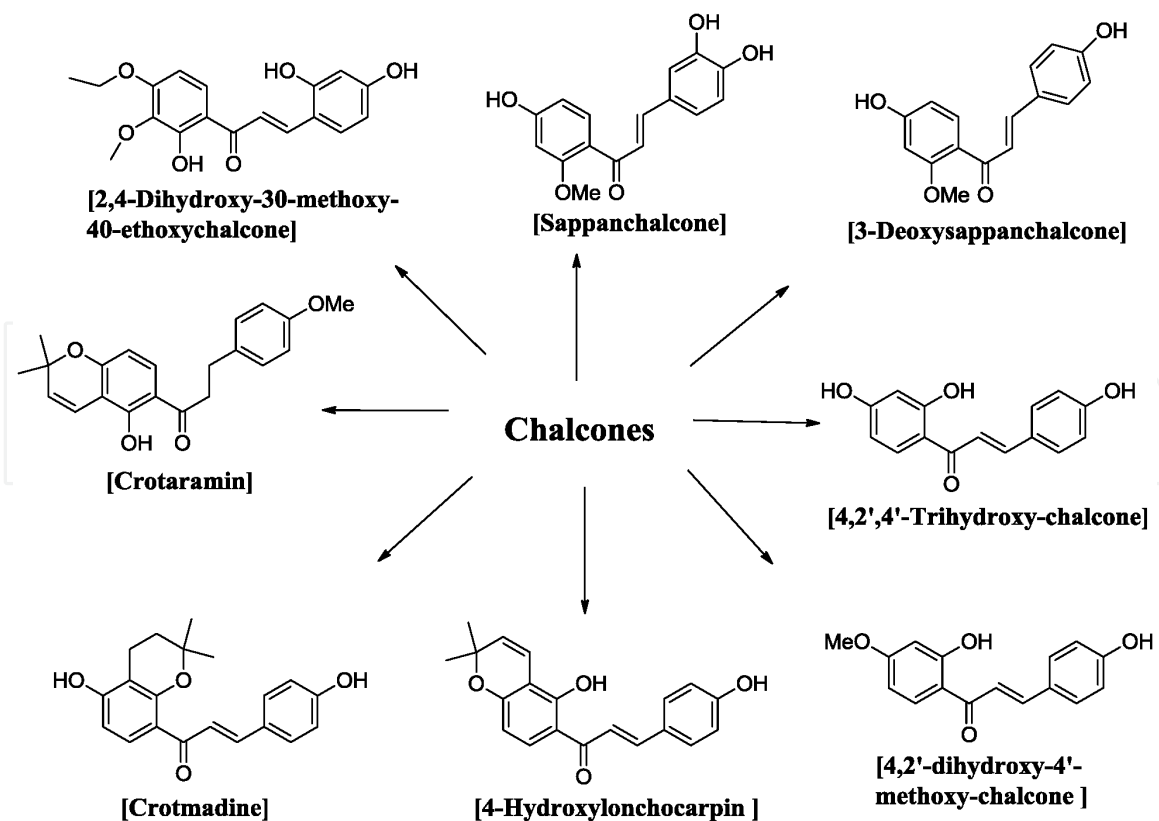


Figure 6.
Various types chalcones isolated from several plants.

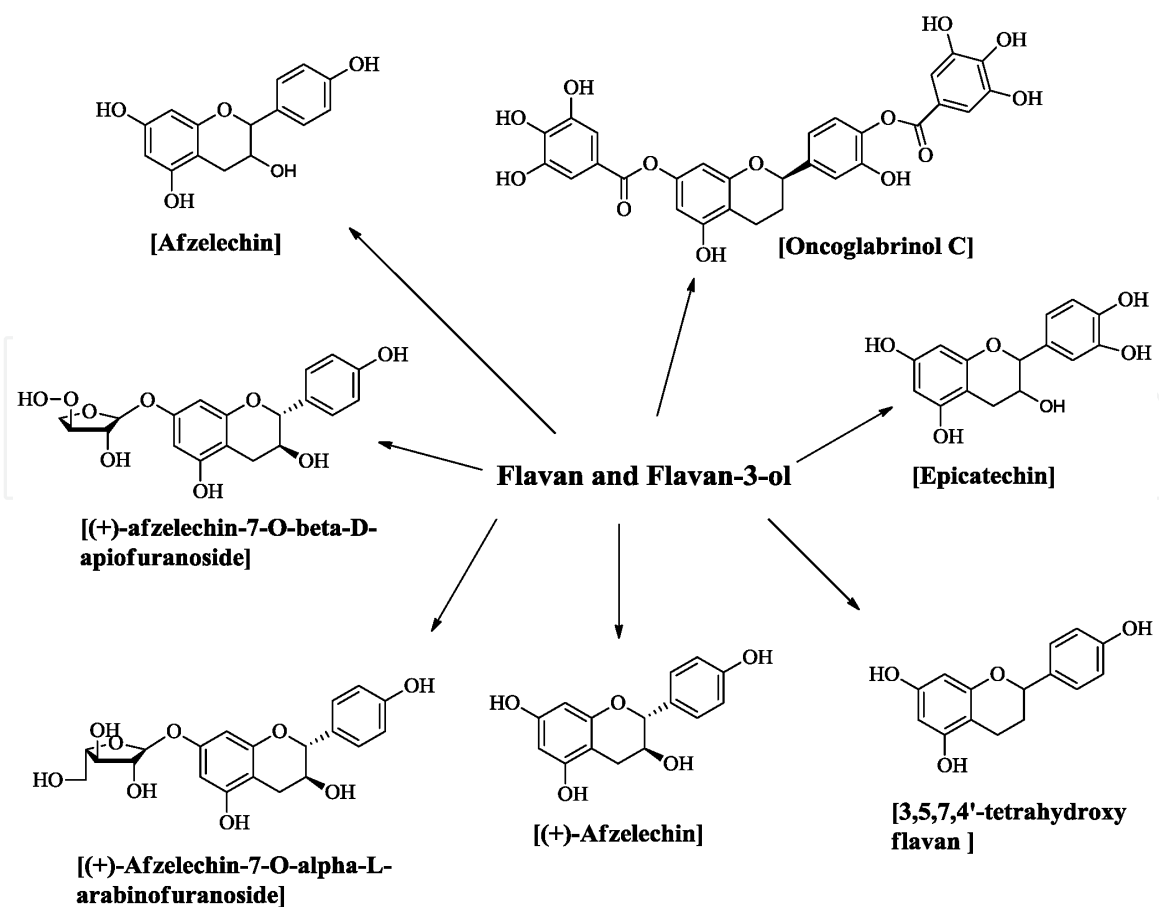


Figure 7.
Diverse type of flavan and flavan-3-ol reported from parts of plants.

flavonoids, which have 15 carbon structure and arranged in C₆-C₃-C₆ skeleton. The modification of chalcones can be done by methylation, condensation, and hydroxylation. These chalcones can be distributed in many parts of plants as fruits, seed, bark, stem, flowers [65].

Various diverse type chalcones have been reported from many plant species such as 2,4-dihydroxy-30-methoxy-40-ethoxychalcone from *Caragana pruinosa* [66], two chalcones, sappanchalcone and 3-deoxysappanchalcone from *Haematoxylum campechianum* [67], 4,2',4'-trihydroxy-chalcone 4,2'-dihydroxy-4'-methoxy-chalcone, 4-hydroxylonchocarpin, crotmadine chalcones *Codonopsis cordifolioidea* root [68], and crotrammin chalcone from *Crotalaria ramosissima* plant [69]. These chalcones are showed in **Figure 6**.

4.2 Flavan and Flavan-3-ol

Many different flavan and flavan-3-ol are summarized in **Figure 7**, which have been isolated from many plants as afzelechin from steam bark of *Pinus halepensis* [70], oncoglabinol C from *Oncocalyx glabratus* [71], epicatechin, and 3,5,7,4'-tetrahydroxy flavan from stem bark of *Embelia schimperi* [72], three flavan-3-ol derivatives as (+)-afzelechin, (+)-afzelechin-7-O- α -L-arabinofuranoside and (+)-afzelechin-7-O- β -D-apiofuranoside from *Polypodium vulgare* L. rhizomes [73].

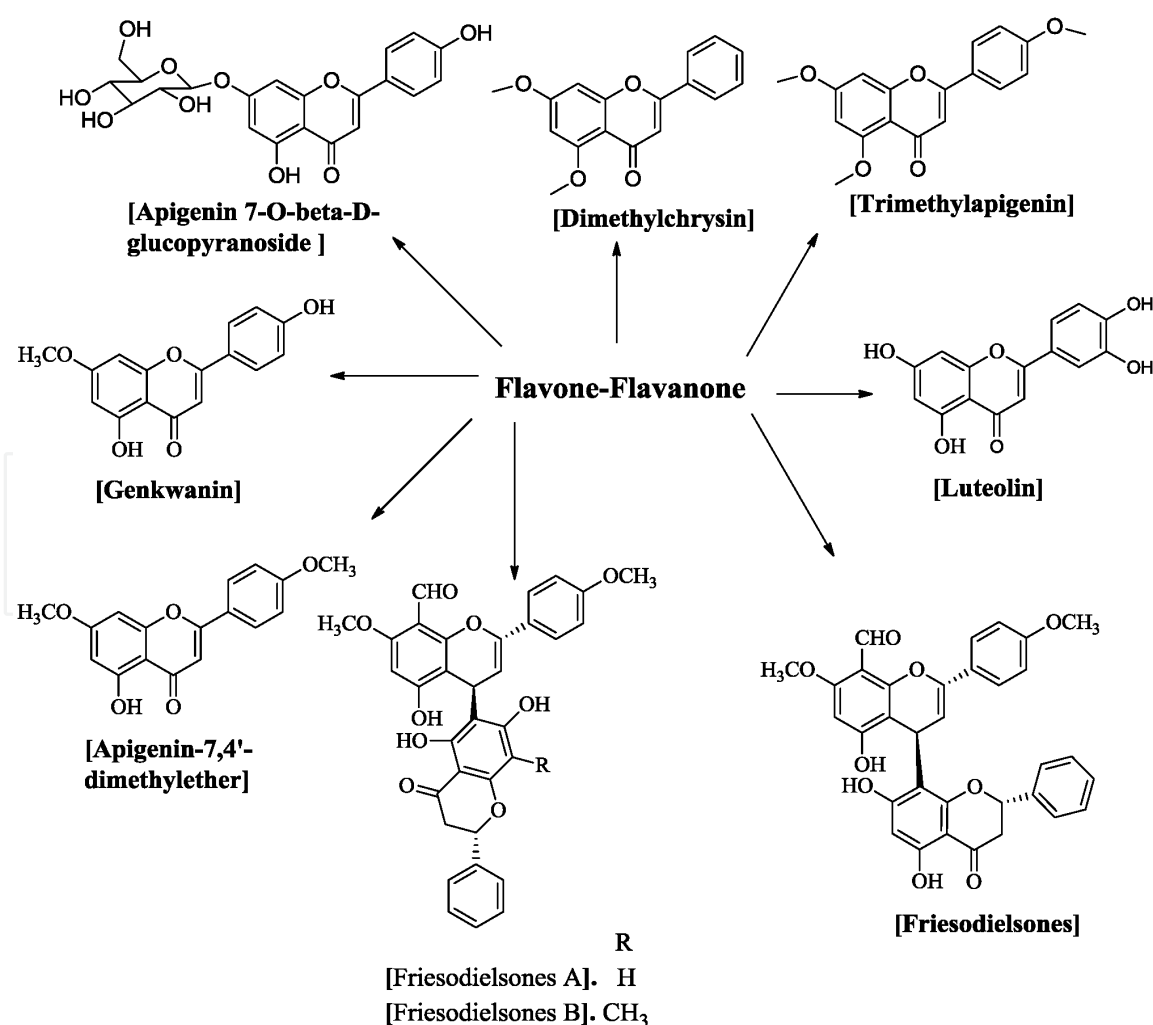


Figure 8.
Structural diversity of flavones and flavanone.

4.3 Flavone-flavanone

Many different structures of flavones and flavanone are synthesized via shikimate and flavonoids pathway. These structures of these are showed in **Figure 8**. Several type of flavones and flavanone were isolated such as apigenin 7-O- β -D-glucopyranoside, dimethylchrysin, trimethylapigenin, 5,7,3',4'-tetrahydroxyflavone (Luteolin) from *Sterculia foetida* leaves [74], three new flavan-flavanones as friesodielsones A, friesodielsones B, friesodielsones, from *Friesodielsia desmoides* leaves [75], and flavonoids (flavones) as apigenin-7,4'-dimethylether, genkwanin from *Aquilaria sinensis* leaves [76].

4.4 Isoflavonoids

The diverse type structure of isoflavonoids was synthesized from flavanone, which have been reported several plants as corylifol A, neobavaisoflavone, and iris-florentin from *Cytisus striatus* [77], formoninetin and biochanin A from *Hylastinus obscurus* [78]. One new leptoisoflavone A (a rare 5-membered dihydrofuran ring) from *Limonium leptophyllum* [79], 2,2'-trimethoxy-6,8-dihydroxy-isoflavone from the ethanol extract of *Thespesia populnea* bark [80] and isoflavones, genistein and daidzein from *Hericium erinaceum* (**Figure 9**) [81].

4.5 Flavonol

Several type of flavonol were reported from parts of plants as myricetin 3-O-(2'',4''-di-O-acetyl)- α -L-rhamnopyranoside from *Myrsine Africana* [82], flavonoid glycoside named as 3'-O-methyl quercetin-3-glucose-6-gallic acid from *Cordia oblique* leaves [83], 2-(3, 4-dihydroxyphenyl)-3, 5, 7-trihydroxy-4H-chromen-4-one

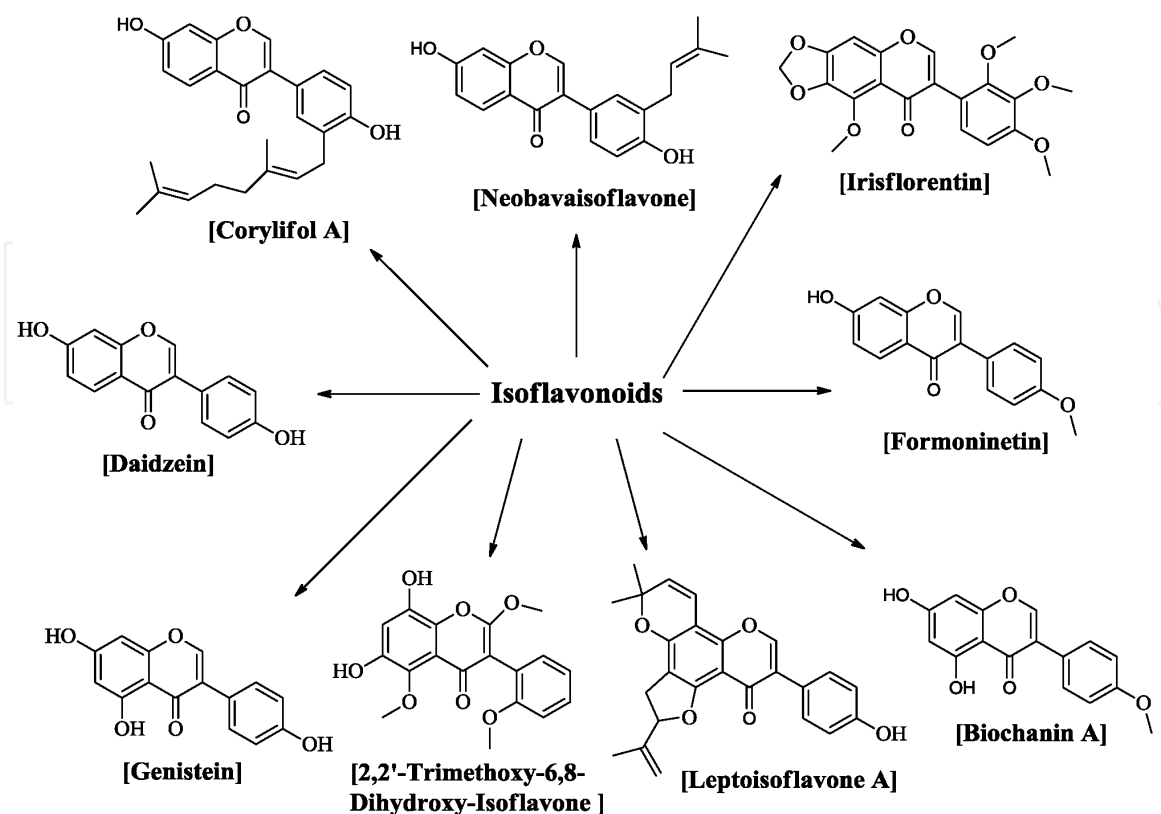


Figure 9.
Diverse structure of isoflavonoids from plants species.

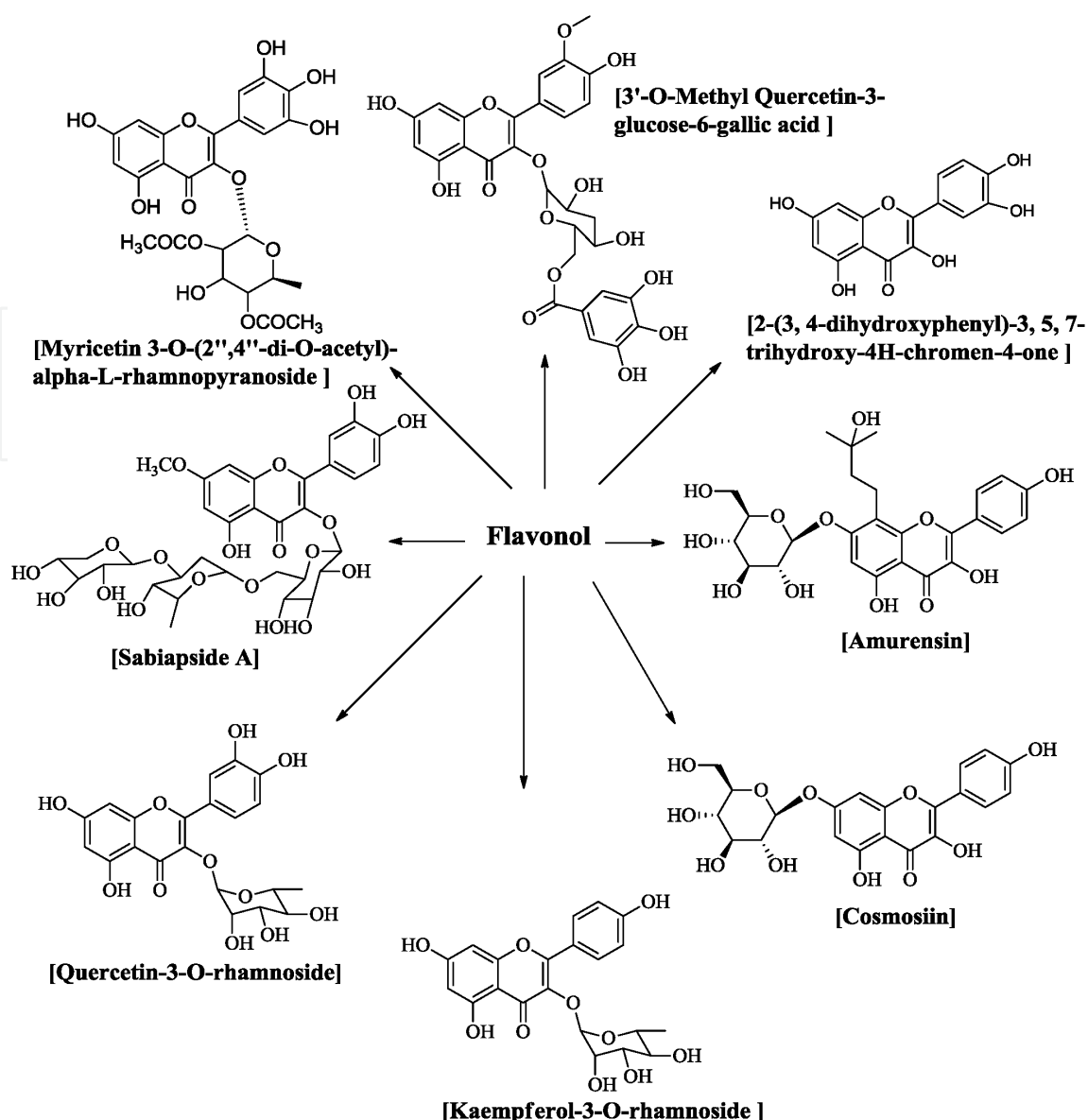


Figure 10.
Several different structures of flavonol isolated from parts of plant species.

from aerial parts of *Chenopodium album* [84], amurensin and cosmosiin from *Trigonella foenum graecum* [85], rhamnosides flavonol, kaempferol-3-O-rhamnoside and quercetin-3-O-rhamnoside from leaves of *Pometia pinnata* [86] and a new flavonol glycoside, sabiapside A from *Sabia parviflora* [87] (**Figure 10**).

5. Conclusions

Flavonoids are a large class of natural compounds, which isolated from various of plants as seed, root, bark, flower, leaves, fruit etc. and prevent from various diseases. The biosynthesis of flavonoids is highly complicated because a group of enzyme (DHAP synthase, SA kinase, EPSP synthase, PAL, 4CL, CHS, CHI, F3H, DFR) plays a key role in the pathway of flavonoids biosynthesis. These enzymes play a potential role in modification of flavonoids via isomerases, hydroxylases, reductases, and polymerises reaction. The proanthocynidins are interested natural compounds, which formed via polymerization reaction of flavonoids. The flavonoids are synthesized in various plant species via shikimate and phenylpropanoid pathway.

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Conflict of interest

The author declares no conflicts of interest.

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