

## JOURNAL OF SCIENTIFIC & INNOVATIVE RESEARCH

### An overview on Flavonoids: chemistry and metabolism

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**Abstract:** Flavonoids are a class of phenolic compounds widely distributed in plants. They occur either as free molecules or as glycosides. Over 1000 individual types are known, and the list is constantly expanding. The term flavonoid is used to include all of the pigments that possess structure based upon C6-C3-C6 carbon skeleton found in flavones, chalcones, anthocyanins, etc. Flavonoids have shown potential health benefits arising from the Antioxidative effects of these phytochemicals whose properties are attributed to phenolic hydroxyl group attached to the flavonoid structure. Scavenging of free radical seems to play a considerable part in the antioxidant activity of flavonoid compounds. In very recent years flavonoids as potent radical scavengers have attracted a tremendous interest as possible therapeutic against free radical mediated disease.

**Keywords:** Flavonoids, chemical structure, metabolism, Antioxidant

**Introduction:** Flavonoids are a group of polyphenolic compounds diverse in chemical structure and characteristics. They occur naturally in fruit, vegetables, nuts, seeds, flowers, and bark and are an integral part of the human diet.<sup>1-3</sup> They have been reported to exhibit a wide range of

biological effects, including antibacterial, antiviral, 4 anti-inflammatory, antiallergic,<sup>1, 4, 5</sup> and vasodilatory<sup>6</sup> actions, in addition, flavonoids inhibit lipid peroxidation (LPO)<sup>2, 7</sup> platelet aggregation,<sup>8-12, 13</sup> capillary permeability, and fragility,<sup>14, 15</sup> and the activity of enzyme systems including cyclo-

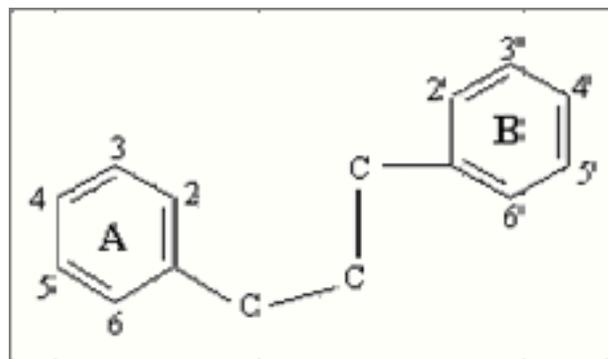
oxygenase and lipoxygenase.<sup>1, 5, 15, 16</sup> Flavonoids exert these effects as antioxidants, free radical scavengers,<sup>4, 17-19</sup> and chelators of divalent cations.<sup>20</sup>

Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. By virtue of their capacity to inhibit LDL oxidation, flavonoids have demonstrated unique cardioprotective effects.<sup>21, 22</sup> Flavonoid-rich diets have been shown to reduce myocardial post-ischemic damage in rats.<sup>23</sup> A protective role in the diet of humans has also been indicated in some large, prospective studies. For example, high flavonoid intake predicted lower mortality from coronary heart disease and lower incidence of myocardial infarction in older men<sup>24</sup> and reduced the risk of coronary heart disease by 38% in postmenopausal women.<sup>25</sup> The Zutphen Elderly Study demonstrated an inverse relationship between consumption of catechin, a predominant flavonoid in tea, and ischemic heart disease mortality in a cohort of 806 men.<sup>26</sup>

### CHEMISTRY OF FLAVONOIDS<sup>27-31</sup>

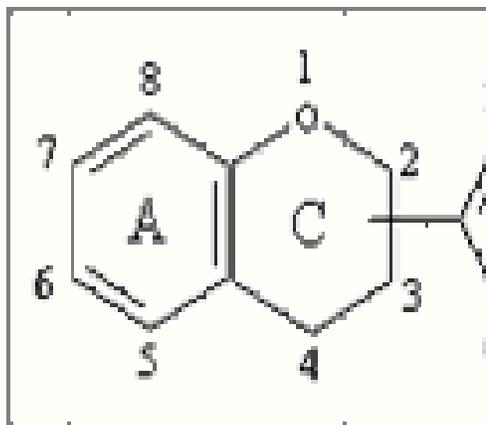
The flavonoids are polyphenolic compounds possessing 15 carbon atoms; two benzene

rings joined by a linear three carbon chain.<sup>32-34</sup>



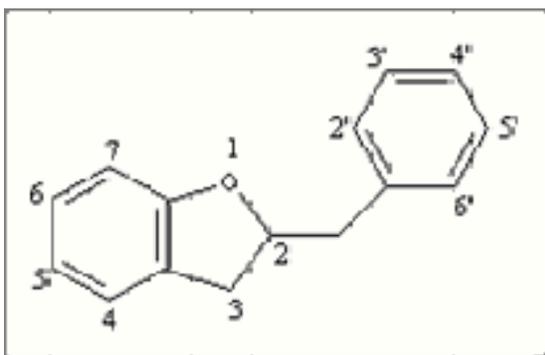
The skeleton above, can be represented as the C6 - C3 - C6 system.

Flavonoids constitute one of the most characteristic classes of compounds in higher plants. Many flavonoids are easily recognized as flower pigments in most angiosperm families (flowering plants). However, their occurrence is not restricted to flowers but include all parts of the plant. The chemical structure of flavonoids are based on a C15 skeleton with a CHROMANE ring bearing a second aromatic ring B in position 2, 3 or 4.



Various subgroups of flavonoids are classified according to the substitution patterns of ring C. Both the oxidation state of the heterocyclic ring and the position of ring B are important in the classification. Examples of the 6 major subgroups are:

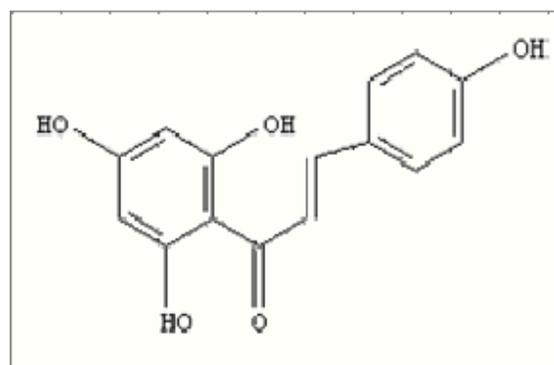
In a few cases, the six-membered heterocyclic ring C occurs in an isomeric open form or is replaced by a five-membered ring.



#### AURONES (2-benzylidene-coumarone)

The oxygen bridge involving the central carbon atom (C2) of the 3C - chain occurs in a rather limited number of cases, where the resulting heterocyclic is of the FURAN type.

#### 1. Chalcones



Chalcone do not have a central heterocyclic nucleus and are characterized by the presence of a three carbon chain with a ketone function and an  $\alpha$ ,  $\beta$  unsaturation, substitutions on the A ring are most often identical to those of other flavonoids, where as the B ring is fairly often unsubstituted. Isoprenyl and pyranochalcones seem rather common, especially in the Fabaceae. Aurones are characterized by a 2 benzylidenecoumarone structure.

#### Classification of Flavonoids:<sup>32-34</sup>

## 2. Flavone

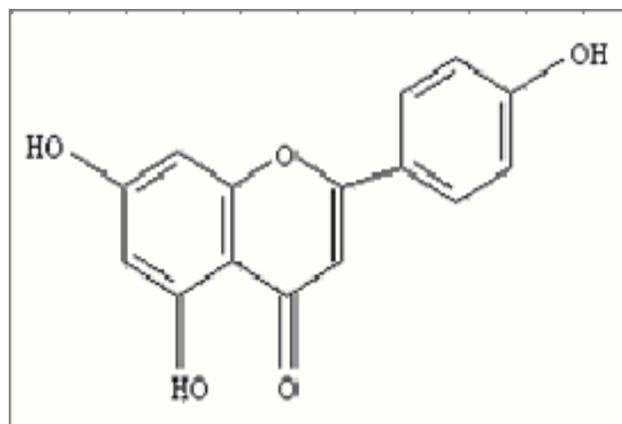
(Generally in herbaceous families, e.g. Labiatae, Umbelliferae, Compositae).

Apigenin (*Apium graveolens*, *Petroselinum crispum*).

Luteolin (*Equisetum arvense*)

In this ring A in over 90% of the cases is substituted by two phenolic hydroxyl groups at C-5 and C-7. These hydroxyl groups are either free or etherified, and one of them may be engaged in a glycosidic linkage. Other substitutions are possible, free or etherified hydroxyl groups at C-6 or C-8 or both in a carbon – carbon bond with a saccharide.

The ring B, substituted in the 4'-position in 80% of cases, may be 3',4'-di-substituted or, less frequently, 3',4',5'-trisubstituted; the substituents are OH or –OCH<sub>3</sub> groups. The other positions (2' and 6') are substituted only exceptionally.



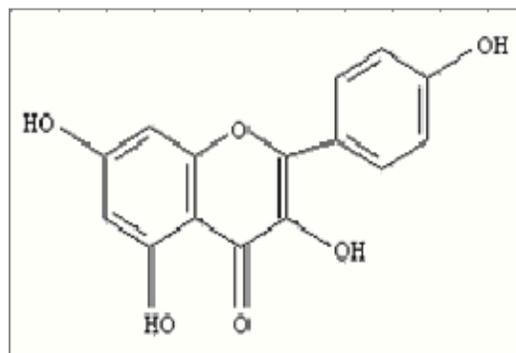
## 3. Flavonol

(generally in woody angiosperms)

Quercitol (*Ruta graveolens*, *Fagopyrum esculentum*, *Sambucus nigra*)

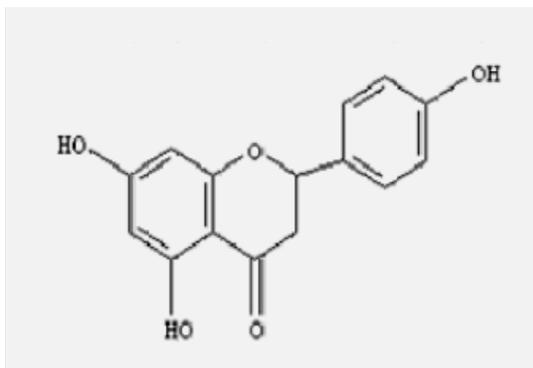
Kaempferol (*Sambucus nigra*, *Cassia senna*, *Equisetum arvense*, *Lamium album*, *Polygonum bistorta*).

These flavonols and their glycosides are universally distributed, but some of the substitution patterns are restricted to some families. Lamiaceae, rutaceae, and asteraceae.



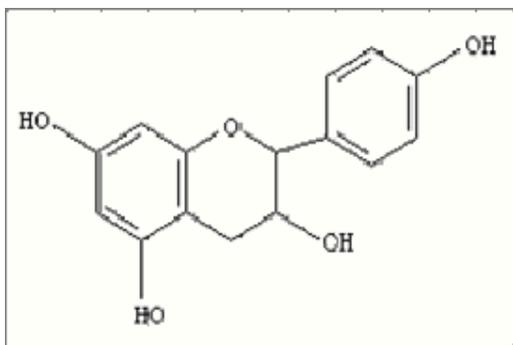
#### 4. Flavanone

These molecules are characterized by the absence of a 2,3-double bond, and by the presence of at least one asymmetric center. In natural flavanones C-2 is normally in the 2S configuration. These flavonoids are somewhat less common than their unsaturated homologs, and it is noteworthy that some families tend to accumulate their C-alkylated derivatives (Asteraceae, Fabaceae).



#### 5. Anthocyanins

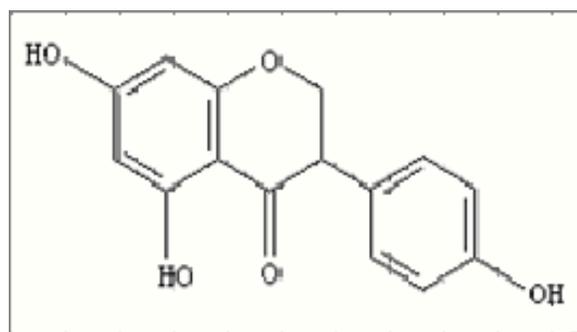
The term anthocyanins initially coined to designate the substance responsible for the color of the corn flower, applies to a group of water soluble pigments responsible for the red, pink, mauve, purple, blue, or violet color of most flowers and fruits. These pigments occurs as glycosides (the anthocyanins), and their aglycones (the anthocyanidins) are derived from 2-phenylbenzopyrylium cation. Anthocyanins are present in all of the angiosperms, although they are generally characteristics of flower petals and of the fruits, anthocyanins can also be found in the bracts (bromeliaceae). Anthocyanins whose vivid color attracts insect and birds play a major role in pollination and seed dispersal. A high coloring power and the absence of toxicity lend to these natural coloring glycosides the potential to replace synthetic color in food technology. Therapeutical applications of anthocyanins are limited to treatment of vascular disorders; the drug containing them are used for the extraction of anthocyanins and the preparation of galenicals designed to treat the symptoms linked to capillary and venous fragility.



in coumaranochromones. Other isoflavonoids have a coumarinic structure induced by the oxidation of an isoflavene. Some polycyclic compounds have an additional carbon atom, for example rotenoids arising from the oxidative cyclization products of a 2'-methoxyisoflavone.

## 6. Isoflavonoids

All molecules in this group can be related to skeleton of 3-phenylchromane. They are present in Dicotyledons, they are in fact almost specific to Fabaceae only. Nearly 700 isoflavonoids are known they are classified into dozens of types, in all types we can note the high frequency of isoprenylated derivatives, and consequently of furan, – dihydrofuran, and pyran type structures. The most common compounds are isoflavone, which occur in the free state, or more rarely as glycosides. Isoflavonoids have an additional ring such as in case of pterocarpan and their derivatives and also



Most of these (flavanones, flavones, flavonols, and anthocyanins) bear ring B in position 2 of the heterocyclic ring. In isoflavonoids, ring B occupies position 3. The Isoflavonoids and the Neoflavonoids can be regarded as ABNORMAL FLAVONOIDS.

### Flavonoids Types and Examples<sup>35</sup>

TYPE	COMPOUND
Flavone	Chrysin, Butin, Apigenin, Luteolin, Fistin
Flavonol	Quercetin, Kaempferol

Flavonone	Eriodictyol, liquiritigenin
Chalcones	Unstable isomes of flavonones
Xanthone	Gentisin
Isoflavone	Formononetin, Genistein
Biflavone	Amentoflavone

### **Solubilities and extraction of flavonoids:**

Although as a general rule, glycosides are water soluble and soluble in alcohol a fair number are sparingly soluble. (rutin, hesperidin), aglycones are soluble in a polar organic solvents :when they have at least one free phenolic group, they dissolve in alkaline hydroxide solutions. Lipophilic flavonoids of leaf, tissues are directly extracted by solvents of medium polarity. Glycosides can be extracted, at high temperature, by acetone or alcohol (ethanol, methanol) mixed with water (20 to 50% depending on whether the drug is fresh or dried ). Solvent evaporation under vacuum can be next followed, when only the aq. phase is left, by a series of liquid liquid extraction by non miscible solvents :petroleum ether which eliminates chlorophyll and lipids; diethylether which

extracts free aglycones and ethyl acetate which dissolves the majority of glycosides. The free saccharides remains in the aqueous phase with the most polar glycosides when these are present.

The separation and purification of the different flavonoids is based on the usual chromatographic techniques (on polyamide, cellulose or sephadex gel ) as in case of the most of the secondary metabolites, in the last few years HPLC has taken a place of choice in the battery of isolation techniques for glycosylflavonoids.

### **Characterization of Flavonoids:**

Although several color reactions allow the characterization of aglycone and glycosides in crude extracts, preliminary work on these extracts is classically dominated by TLC analysis. (but paper chromatography has not been abandoned) The chromatogram can be studied:

Directly, since chalcones and aurones are usually visible, and turn orange and red, respectively in the presence of ammonia vapors. By examination under UV light before and after spraying with alluminium trichloride, and before and after exposure to ammonia vapors.

After spraying with a 1% solution of the ester of 2-amino ethanol and diphenylboric acid, in other words the "Naturstoff ReagenzA", by examination under UV light then under visible light. After spraying with ferric chloride, anisaldehyde diazotized, sulfanilic acid and other general reagents for phenols.

By utilizing more or less specific reactions or properties such as: reaction with magnesium powder—for flavanones and dihydroflavanones, or with zinc for flavonoids, both in presence of hydrochloric acid, reaction of dihydrochalcones, first with sodium borohydrides, then with 2,3-dichloro-5,6-dicyano-1,4- benzoquinone.

Structural elucidation, Mass spectrometry and NMR techniques are generally emphasized, UV also can provide very useful information. The usefulness of UV

data extends to the use, in routine HPLC analysis, of diode array detectors.

### **Quantification of Flavonoids:**

The classic quantification methods are, colorimetric, or spectrophotometric. HPLC now makes possible, a rapid and precise estimate of all flavonoids present in a drug, therefore it is widely used.

### **Antioxidant Flavonoids:**<sup>13, 20, 36, 37</sup>

Flavonoids or bioflavonoids, are biquitous group of polyphenolic substances which are present in most plants, concentrating in seeds, fruits, skin or peels, bark and flowers.

A great number of plant medicine contain flavonoids, which have been reported by many authors as having antibacterial antiinflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, antithrombotic and vasodialator actions. The structural componants common to these molecule includes two benzne rings on either side of a 3- carbon ring, multiple combinations of hydroxyl groups, sugars, oxygens, and methyl group attached to

these structures create the various classes of flavonoids : flavonols, flavanones, flavones, flavan-3-ols(catechins ), anthocyanins, and isoflavones, flavonoids have been shown in no. of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals .Free radicals are responsible for many diseases .These radical oxygen species (ROS) are produced as a normal consequence of biochemical processes in the body and as a result of increases exposure to environmental and /or dietary xenobiotics. ROS are also beneficial component of the immune response, hepatic cytochrome P450-mediated detoxification processes (oxidative stress) that is thought to cause the subsequent cellular damage which leads to the disease processes. The body's antioxidant system including superoxide dismutase, catalase and glutathione, should keep the oxidative process in check, however deficiencies of nutritional antioxidants (flavonoids, vitamins A,C,E the minerals selenium and zinc coenzyme Q10, lipoic acid, and L-cysteine), and / or an overwhelming oxidant stress can overload this system.<sup>38</sup>

In one study 41 flavonoids of the flavone and flavonol types were investigated for their antioxidative property using a lipid peroxidation generation system, the results showed that both types specifically and markedly reduced the proportion of peroxidants induced by H<sub>2</sub>O<sub>2</sub>, Fe<sup>2+</sup>, or a fenton type reaction.<sup>39</sup>

### **Epidemiological Studies:**

Two recent epidemiological studies reveal an inverse correlation between dietary flavonoid intake and coronary heart disease mortality. Study found that those with the highest intake of flavonoids (mostly from onions and apples) had a reduced risk for coronary disease. The mechanism of free radical damage includes ROS induced peroxidation of polyunsaturated fatty acids in the cells membrane, lipid bilayer, which cause a chain reaction of lipid peroxidation, thus damaging the cellular membrane and causing further oxidation of membrane lipids and proteins. Subsequently, cell contents, including DNA, are damaged. It is this free radical induced damage which is thought to precede these overt disease processes.<sup>40</sup>

**Mechanism of action of Quercetin<sup>41-43</sup>**

Flavonoids as a rule are antioxidant and a number of Quercetin's effects appear to be due to its antioxidant activity. Quercetin scavengers oxygen radicals, inhibit xanthine oxidase and inhibits lipid peroxidation in vitro, As another indicator of its antioxidant effects, quercetin inhibits oxidation of LDL cholesterol in in vitro, probably by inhibiting LDL oxidation itself, by protecting vit. E in LDL from being oxidized or by regenerating oxidized vit E, by itself and pair with ascorbic acid, quercetin reduce the incidence of oxidative damage to neurovascular structures in skin, and inhibited damage to neurons caused by experimental glutathione depletion. Quercetin's anti-inflammatory activity appears to be due to its antioxidant and inhibitory effects of inflammation producing enzymes cyclooxygenase, lipoxygenase and the subsequent inhibition of inflammatory mediators Quercetin exerts antiviral activity against reverse transcriptase of HIV and other retroviruses.

**Conclusion:**

The existing knowledge of absorption and metabolism is confined to a select group of

dietary flavonoids, sufficient evidence supports that most of these compounds are absorbed sufficiently to exact a marked decrease in various parameters of plasma oxidant status. Most of the beneficial health effects of flavonoids are attributed to their antioxidant and chelating abilities. The protective effects of flavonoids in biological systems are ascribed to their capacity to transfer electrons free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals, and inhibit oxidases.

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