Flavonoids as nutraceuticals. A review


D. J. College of Pharmacy
Modinagar, India

*corresponding author: phone: 09811767325,
e mail: pushpendra_utd@rediffmail.com, pushpendra.utd@gmail.com

Summary

Phenolic compounds form one of the main classes of secondary metabolites. They display a large range of structures and are responsible for the major organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties. They also contribute to the nutritional qualities of fruits and vegetables. Among these compounds, flavonoids constitute one of the most prevailing groups among plant phenolics. Owing to their importance in food organoleptic properties and human health, a better understanding of their structures and biological activities indicates their potentials as therapeutic agents and also for predicting and controlling food quality. Due to the variety of pharmacological activities in the mammalian body, flavonoids are more correctly referred as “nutraceuticals”.

Key words: bioflavonoids, structure-classification, nutraceuticals, antimicrobial activities, antioxidant activity, metabolic effects

INTRODUCTION

Phenolic compounds constitute one of the main classes of secondary metabolites. They display a large range of structures and they are responsible for the major organoleptic characteristics of plant-derived foods and beverages, particularly color and taste properties and they also contribute to the nutritional qualities of fruits and vegetables. The most important natural pigments are carotenoids which are tetrpyrrole derivatives of naturally occurring phenolic compounds ubiquitously distributed in plant kingdom. Among these compounds, flavonoids constitute one of the most ubiquitous groups of all plant phenolics. Up to now,
over 8,000 varieties of flavonoids have been identified [1]. Even 50 years ago, information on the working mechanisms of flavonoids was scarce. But it has been widely known for centuries that compounds of plant origin possess a broad spectrum of biological activity [2]. In 1930, Szent-Gyorgyi isolated a new substance from oranges and classified it as vitamin P, but later it became clear that this substance was actually a flavonoid [3]. Flavonoids drew greater attention of researchers with the discovery of the French Paradox, i.e., the decrease in cardiovascular incidents observed in the Mediterranean population which was associated to red wine consumption, and a greater amount of saturated fat in the average diet than in other countries [3].

STRUCTURE AND CLASSIFICATION OF FLAVONOIDS

Flavonoids occur as aglycones, glycosides and methylated derivatives. (4) In plants, flavonoids aglycones (i.e., flavonoids with no sugar attached) occur in a variety of structural forms. All of them contain fifteen carbon atoms in their basic nucleus: two six-membered rings linked with a three carbon unit which may or may not be a part of a third ring [5]. For convenience, the rings are labeled A, B, and C (fig. 1). The individual carbon atoms are based on a numbering system which uses ordinary numerals for the A and C and “primed” numerals for B-ring [1]. Primed modified numbering system is not used for chalcones [2] and the isoflavones derivatives [6]: the pterocarps and the rotenoids [6]. The different ways to close this ring associated with the different oxidation degrees of ring A provide the various classes of flavonoids. The six-membered ring condensed with the benzene ring is either a pyrone flavones [1] flavonols [3] or its dihydroderivative flavanones (4) and flavan-3-ols (5). The position of the benzenoid substituent divides the flavonoids into two classes: flavonoids [1] (2nd position) and isoflavonoids [6] (3rd position). Most of flavonoids occur naturally associated with sugar in conjugated form and, within any one class, may be characterized as monoglycosidic, diglycosidic, etc. The glycosidic linkage is normally located at position 3 or 7 and the carbohydrate unit can be L-rhamnose, D-glucose, glucorhamnose, galactose or arabinose [8].

FLAVONOIDS AS NUTRACEUTICALS

“Nutraceutical” is a term coined in 1979 by Stephen DeFelice. (9) It is defined “as a food or parts of food that provide medical or health benefits, including the prevention and treatment of disease.” Nutraceuticals may range from isolated nutrients, dietary supplements, and diets to genetically engineered “designer” food, herbal products and processed products such as cereals, soups, and beverages. A nutraceutical is any nontoxic food extract supplement that has scientifically proven health benefits for both the treatment and prevention of disease [10]. The increasing interest in nutraceuticals reflects the fact that consumers hear about
epidemiological studies indicating that a specific diet or component of the diet is associated with a lower risk for a certain disease. The major active nutraceutical ingredients in plants are flavonoids. As is typical for phenolic compounds, they can act as potent antioxidants and metal chelators. They also have long been recognized to possess anti-inflammatory, antiallergic, hepatoprotective, antithrombotic, antiviral, and anticarcinogenic activities, as discussed in the subsections that follow (fig. 1, fig. 2, tab. 1):

Figure 1. Chemical structures of some representative flavonoids

Figure 2. General structures for various classes of flavonoids
Substitution patterns of series of flavonoids

<table>
<thead>
<tr>
<th>Group</th>
<th>3</th>
<th>5</th>
<th>6</th>
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<th>3'</th>
<th>4'</th>
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<td>OH</td>
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<td>H</td>
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<td>Tectorigenin</td>
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<td>OH</td>
<td>OCH3</td>
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<td>H</td>
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<td>Daidzein</td>
<td>-</td>
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<td>Formononetin</td>
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<td><strong>Flavan-3-ols</strong></td>
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<td>(+) Catechin</td>
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<td><strong>Flavylum Salts</strong></td>
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<td>Cyanidin</td>
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</tbody>
</table>

Antioxidant activity (tab. 2, tab. 3)

The best-described property of almost every group of flavonoids is their capacity to act as antioxidants. The flavones and catechins seem to be the most powerful flavonoids protecting the body against reactive oxygen species (ROS). Body cells and tissues are continuously threatened by the damage caused by free radicals and ROS which are produced during normal oxygen metabolism or are induced by exogenous damage [11, 12]. Free radicals and ROS have been implicated in a large number of human diseases [13, 14]. Quercetin, kaempferol, morin, myricetin and rutin, by acting as antioxidants, exhibited beneficial effects such as anti-inflammatory, antiallergic, antiviral as well as anticancer activity. They have also been suggested to play a protective role in liver diseases, cataracts and cardiovascular diseases. Quercetin and silybin, acting as free radical scavengers, were
shown to exert a protective effect in liver reperfusion ischemic tissue damage [15, 16]. The scavenging activity of flavonoids has been reported to be in the order: myrcetin > quercetin > rhamnetin > morin > diosmetin > naringenin > apigenin > catechin > 5,7-dihydroxy-3’,4’,5’-trimethoxyflavone > robinin > kaempferol > flavone [17].

Table 2.
Reactive oxygen species that can be scavenged or whose formation can be inhibited by flavonoids [18, 19]

<table>
<thead>
<tr>
<th>Species</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>O_2 (Superoxide anion)</td>
<td>One-electron reduction product of O_2. Produced by phagocytes, formed in autoxidation reactions (flavoproteins, redox cycling), and generated by oxidases (heme proteins).</td>
</tr>
<tr>
<td>HO_2 \text{H}^+</td>
<td>Potonated form of O_2. \text{H}^+</td>
</tr>
<tr>
<td>H_2O_2 (Hydrogen Peroxide)</td>
<td>Two-electron reduction product of O_2 formed from O_2 by \text{H}^+ dismutation or directly from O_2. Reactivity of O_2 and H_2O_2 is amplified in the presence of heme proteins.</td>
</tr>
<tr>
<td>OH (Hydroxy radical)</td>
<td>Three-electrons reduction product of O_2 generated by Fenton reaction, transition metal (iron, copper)-catalysed Haber-Weiss reaction; also formed by decomposition of peroxynitrite produced by the reaction of O_2 with NO (Nitric oxide radical). Example: Lipid radical (LO). \text{H}^+</td>
</tr>
<tr>
<td>RO (Alkoxyl radical)</td>
<td>Example: Lipid peroxyl radical (LOO) produced from organic hydroperoxide (e.g. lipid hydroperoxide, LOOH), ROOH by hydrogen abstraction.</td>
</tr>
<tr>
<td>ROO (Peroxyl radical)</td>
<td></td>
</tr>
<tr>
<td>'O_2</td>
<td>Singlet oxygen</td>
</tr>
</tbody>
</table>

Table 3:
Characteristics of flavonoid structure for most effective radical-scavenging activity [20-22]

1. The catechol (O-dihydroxy) group in the ring confers great scavenging ability.
2. A pyrogallol (trihydroxy) group in ring B of a catechol, as in myricetin, produces even higher activity. The C2-C3 double bond of the C ring appears to increase scavenger activity because it confers stability to the phenoxy radical produced.
3. The 4-oxo (keto double bond at position 4 of the C ring), especially in association with the C2-C3 double bond, increases scavenger activity by delocalizing electrons from Bring.
4. The 3-OH group on the C ring generates an extremely active scavenger; in fact, the combination of C2-C3 double bond and 4-oxo group appears to be the best combination on the top of the catechol group.
5. The 5-OH and 7-OH groups may also add scavenging potential in certain cases.

Antimicrobial activity

Flavonoids and esters of phenolic acids have also been investigated for their antibacterial, antifungal and antiviral activities.
Antibacterial activity

Antibacterial activity has been displayed by a number of flavonoids. Quercetin has been reported to completely inhibit the growth of *Staphylococcus aureus*. Most of the flavanones having no sugar moiety showed antimicrobial activities whereas none of the flavonols and flavonolignans tested showed inhibitory activity on microorganisms [23].

Antifungal activity

A number of flavonoids isolated from the peelings of tangerine orange, when tested for fungistatic activity towards *Deuterophoma tracheiphila* were found to be active; nobiletin and langeritin exhibited strong and weak activities, respectively, while hesperidin could slightly stimulate fungal growth. Chlorflavonin was the first chlorine-containing flavonoid-type antifungal antibiotic produced by strains of *Aspergillus candidus* [24].

Antiviral activity

Naturally occurring flavonoids with antiviral activity have been recognized since the 1940s but only recently attempts have been made to make synthetic modifications of natural compounds to improve antiviral activity. Quercetin, morin, rutin, dihydroquercetin (taxifolin), apigenin, catechin and hesperidine have been reported to possess antiviral activity against some of 11 types of viruses [25]. The antiviral activity appears to be associated with the nonglycosidic compounds and hydroxylation at the 3-position is apparently a prerequisite for antiviral activity. It has been found that flavonols are more active than flavones against *Herpes simplex* virus type 1. The order of importance was galangin > kaempferol > quercetin [26]. Recently, a natural plant flavonoid polymer of molecular weight 2,100 daltons was found to have antiviral activity against two strains of type 1 Herpes simplex virus and type 2 Herpes simplex viruses [27]. Because of the worldwide spread of HIV since the 1980s, the investigation of the antiviral activity of flavonoids has mainly focused on HIV [28]. There have appeared several recent reports on the anti-AIDS activity of flavonoids. Out of twenty eight flavonoids tested, the flavans were generally more effective than flavones and flavanones in the selective inhibition of HIV-1 and HIV-2 or similar immunodeficiency virus infections [29].

Effect on gastrointestinal system

Antiulcer activity exerts significant anti-inflammatory activity in the animal model of both acute and chronic inflammation when given orally or topically [34, 35]. Hesperidin, a citrus flavonoid, possesses significant anti-inflammatory and analge-
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sic effects [36]. Recently, apigenin, luteolin and quercetin have been reported to exhibit anti-inflammatory activity [37]. A number of reports have been published which demonstrate that flavonoids can modulate arachidonic acid metabolism via the inhibition of cyclo-oxygenase (COX) and lipooxygenase activity (LO). It has been also speculated that the anti-inflammatory and antiallergic properties of flavonoids are the consequence of their inhibitory actions on arachidonic acid metabolism [38]. Some recent studies have indicated that flavonoids possess antiulcerogenic activity. Flavonoid glycosides of Ocimum basilicum (Labiatae) decreased ulcer index and inhibited gastric acid and pepsin secretions in aspirin-induced ulcers in rats [30]. Quercetin, rutin, and kaempferol administered intraperitoneally (25–100 mg/kg) inhibited dose-dependent gastric damage produced by acidified ethanol in rats [31].

Hepatoprotective activity

The liver is subject to acute and potentially lethal injury by several substances including phallolidin (the toxic constituent of the mushroom, Amanita phalloides), CCl₄, galactosamine, ethanol and other compounds. Flavonoids have also been found to possess hepatoprotective activity. In the study carried out in order to investigate the flavonoid derivatives: silymarin, apigenin, quercetin, and naringenin, as putative therapeutic agents against microcrystin Lr-induced hepatotoxicity, silymarin was found to be the most effective [32]. The flavonoid, rutin and venoruton showed regenerative and hepatoprotective effects in experimental cirrhosis [33].

Antiinflammatory activity

The antiinflammatory activity of flavonoids has been reported in many animal models. Flavone/flavonol glycosides as well as flavonoid aglycones have been reported to flavones/flavonols kaempferol, quercetin, myricetin, fisetin were reported to possess LO and COX inhibitory activities [39, 40].

Antidiabetic effects

Flavonoids, especially quercetin, have been reported to possess antidiabetic activity. Vessal et al reported that quercetin brings about the regeneration of pancreatic islets and probably increases insulin release in streptozotocin induced diabetic rats [41]. Also in another study, Hif and Howell reported that quercetin stimulate insulin release and enhanced Ca²⁺ uptake from isolated islets cell which suggests a place for flavonoids in noninsulin-dependent diabetes [42, 43].
Effect of vasorelaxant agent on cardiovascular system

The consumption of flavonoids may prevent endothelial dysfunction by enhancing the vasorelaxant process leading to the reduction of arterial pressure [44, 45]. Endothelial dysfunction represents a critical event in the development of cardiovascular diseases and the major complication of atherosclerosis and arterial thrombus formation [46].

The consumption of flavonoids can prevent a number of cardiovascular diseases including hypertension and atherosclerosis [47, 48]. Recently, many experimental studies have shown that these polyphenolic compounds may reduce the arterial pressure in rats and enhance the vasorelaxant process. The endothelium-dependent relaxation induced by flavonoids has been well documented. Furthermore, investigators have demonstrated that Anthocyanin delphinidin exerts a significant endothelium dependent vasorelaxation [49, 50].

Antiatherosclerotic effects

Oxidative modification of low-density lipoproteins (LDL) by free radicals is an early event in the pathogenesis of atherosclerosis effect [52]. Furthermore, a Japanese study reported an inverse correlation between flavonoid intake and total plasma cholesterol concentrations [53].

Antithrombogenic effects

Platelet aggregation plays a pivotal role in the physiology of thrombotic diseases. Activated platelets adhering to vascular endothelium generate lipid peroxides and oxygen free radicals which inhibit the endothelial formation of prostacyclin and nitrous oxide. It was shown in the 1960s that tea pigment can reduce blood coagulability, increase fibrinolysis and prevent platelet adhesion and aggregation [54]. Selected flavonoids, such as quercetin, kaempferol and myricetin were shown to be effective inhibitors of platelet aggregation in dogs and monkeys [55]. Flavonols are particularly antithrombotic because they directly scavenge free radicals, thereby maintaining proper concentration of endothelial prostacyclin and nitric oxide [56]. One study showed that flavonoids are powerful antithrombotic agents in vitro and in vivo because of their inhibition of the activity of cyclooxygenase and lipoxigenase pathways [57].

Cardioprotective effects

Recently, the interest in flavonoids has been stimulated by the potential health benefits arising from the antioxidant activity of these polyphenolic compounds.
These are the results of their high propensity to transfer electrons, chelate ferrous ions and scavenge reactive oxygen species [58]. Due to these properties, flavonoids have been considered as potential protectors against chronic cardiotoxicity caused by the cytostatic drug – doxorubicin. Doxorubicin is a very effective antitumor agent, although, its clinical use is limited by the occurrence of a cumulative dose-related cardiotoxicity, resulting in, for example, congestive heart failure (negative inotropic effect). In the recent report, the cardiotoxicity of doxorubicin on the mouse left atrium has been inhibited by flavonoids, 7- monohydroxyethylrutoside and 7', 3', 4'- trihydroxyethylrutoside [34, 59-61].

**Antineoplastic activity**

A sufficient number of flavonoids have exhibited antineoplastic activity. Several recent reviews have highlighted this activity. Detailed studies [62-64] have revealed that quercetin exerted a dose-dependent inhibition of growth and colony formation. The flavonoids: kaempferol, catechin, toxifolin and fisetin, also suppressed cell growth [65, 66]. On screening the antileukaemic efficacy of 28 naturally occurring and synthetic flavonoids on human promyelocytic leukaemic HL-60 cells, genistein, an isoflavone was found to have strong effect [67, 68].

**Effect on central nervous system**

Synthetic flavonoids, such as 6-bromoflavone and 6-bromo-3'-nitroflavones, were shown to displace [3H] flumazenil binding to membranes from rat cerebellum but not from spinal cord, indicating selectivity for the BZOMa receptor subtype, but the latter was more potent than 6-bromoflavone. Results from two conflict tests in rats showed that these synthetic flavonoids possess anxiolytic like properties similar or superior to that of diazepam [69].

**Toxicity of flavonoids**

Flavonoids are ubiquitous in plant foods and drinks and, therefore, a significant quantity is consumed in our daily diet. The toxicity of flavonoids is very low in animals. For rats, the LD50 is 2–10 g per animal for most flavonoids. Similar doses in humans are quite unrealistic. As a precaution, doses less than 1mg per adult per day have been recommended for humans [70]. Dunnick and Hailey reported that high doses of quercetin over several years might result in the formation of tumors in mice [71]. However, in other long-term studies, no carcinogenicity was found [72]. Moreover, as described earlier, quercetin has been reported to be anti-mutagenic *in vivo*. 
CONCLUSION

Flavonoids comprise a vast array of biologically active compounds that are ubiquitous in plants, many of which have been used in traditional eastern medicine for thousands of years. They also constitute an unavoidable component of the diet. In the present review, we have reviewed detailed structural aspects and biological properties of flavonoids. The chemical and structural similarities of flavonoids with numerous biomolecules as well as their crucial role in plant-insect and plant-bacterial interactions make them an attractive class of phytoconstituents for biological activity. Their widespread occurrence, broad spectrum diversity and natural origin make them appropriate chemical scaffolds for novel therapeutic agents. Of the many actions of flavonoids, antioxidant and antiproliferative effects stand out. Given that certain substituents are known to be required or increase their actions, the therapeutic potential of selected flavonoids is fairly obvious. These natural compounds have several great advantages over other therapeutic agents for the following reasons:

1. Many diets are rich in these phenolics and are daily consumed.
2. They rarely have any side effects.
3. They have relatively long half-life.
4. They can be easily absorbed in the intestine after ingestion.

The study of flavonoids is complex because of the heterogeneity of different molecular structures and the scarcity of data on bioavailability. There is a need to improve analytic techniques to allow collection of more data on absorption and excretion. Data on the long-term consequences of chronic flavonoid ingestion are especially scarce. Finally, we think that natural, hemsynthetic and synthetic flavonoids alone or in combination with other preventive and/or therapeutic strategies will become effective future drugs against the most common degenerative diseases such as cancer, diabetes and cardiovascular complications.

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FLAWONOIDY JAKO NUTRACEUTYKI – PRZEGŁĄD


D. J. College of Pharmacy
Modinagar, India

*autor, do którego należy kierować korespondencję : tel.: 09811767325,
e mail: pushpendra_utd@rediffmail.com, pushpendra.utd@gmail.com

S t r e s z c z e n i e

Związki fenolowe są jedną z głównych grup metabolitów wtórnych. Mogą mieć bardzo różną strukturę i są odpowiedzialne za większość cech organoleptycznych żywności pochodzenia roślinnego i napojów, szczególnie za ich właściwości kolorystyczne i smakowe. Podwyższają także wartości odżywcze owoców i warzyw. Flavonoidy są jedną z największych grup fenoli roślinnych. Lepsze zrozumienie budowy i właściwości biologicznych tych związków może wykazać ich potencjał jako związków leczniczych, a także mających znaczenie w jakości żywności. Ze względu na różnorodność działań farmakologicznych w organizmach ssaków, flavonoidy powinno się nazywać „nutraceutykami”.

Słowa kluczowe: bioflawonoidy, klasyfikacja ze względu na budowę, nutraceuticaly, działanie przeciwbakteryjne, działanie przeciwtleniające, wpływ na metabolizm