Review Article

Antioxidant properties of flavonoids

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Abstrak

Flavonoids, metabolit sekunder terbanyak yang bersumber dari tanaman, telah lama dimanfaatkan sebagai obat tradisional dan secara ilmiah juga telah terbukti memiliki efek farmakologi. Senyawa ini juga memiliki beragam manfaat terhadap kesehatan sehingga sangat berpotensi sebagai bahan baku untuk pengembangan obat baru. Penelitian terbaru memaparkan pemanfaatan flavonoids sebagai antioksidan untuk penyakit akibat radikal bebas. Sari pustaka ini menyoroti peran flavonoid sebagai anti oksidan.

Abstract

Flavonoids represent a remarkable group of plant secondary metabolites and have long been used as traditional medicines with scientifically proven pharmacological benefits. They serve vast-ranging medicinal activities that may lead drug discovery with novel and potential therapeutic evidence. Latest research magnifies primarily functional activity of flavonoids as antioxidant against oxidative stress. This review enlightens the prospective role of flavonoids as antioxidant.

Keywords: antioxidant, flavonoids, oxidative stress

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Oxygen is an essential molecule for the metabolism of living cells. However, this beneficial molecule can also be detrimental for the cells. Disequilibrium between the cellular production and the oxidative stress clearing process may induce the production of various free radical molecules.

Free radical molecules particularly reactive oxygen species (ROS) are derived from biotransformation of molecular oxygen. There are several types of ROS, including superoxide anion radical (O_2-.), singlet oxygen (O_2), hydrogen peroxide (H_2O_2), and the highly reactive hydroxyl radical (OH). The detrimental effects of oxygen are associated with its metabolic reduction to these highly toxic species. ROS which naturally present in all living cells correspond with biochemical antioxidants. The dominant mills of endogenous ROS are hydrogen peroxide and superoxide anion, which are produced as natural by products of cellular metabolism such as mitochondrial respiratory chain. In addition, the prominent sources of extracellular ROS are UV light and other ionizing radiation, bugs, xenobiotic, and pollutants.[1]

The oxidative damage produced by free radicals is referred to as oxidative stress, and has been associated with several degenerative diseases, such as osteoarthritis, cancer, diabetes, cardiovascular diseases, etc. Oxidative stress occurs when critical balance between ROS production and endogenous antioxidant defense mechanism is altered. To counterbalance the oxidant effects and to repair redox equilibrium, cells must readapt important homeostatic indices. The reactive molecules can affect macromolecules, such as DNA, proteins, carbohydrates, and lipids through oxidation process. Consequently, free radical damage can cause proteins denaturation, DNA mutation, and binding to unsaturated lipid membrane leading to lose of fluidity. However, ROS are not always harmful. When firmly controlled, it can also act as intracellular messenger.[2]

Considering many hazardous impacts of free radicals on essential elements in human body, extensive research on new compound of antioxidant agents from natural product is necessary. One of the most thoroughly studied bioactive antioxidant compound from plants is flavonoid.

Flavonoids are found ubiquitously among the plant kingdom. They appear as basic portion of our daily diet such as vegetables, fruits, nuts, seeds, stem, flowers, tea, and wine. Table 1 illustrates various flavonoids and their typical dietary sources in our daily food.[3]

The earliest study on flavonoid started in 1936, when a Hungarian Nobel prize winner, Albert Szent-Gyorgi, revealed the interaction between pure vitamin C and an unknown molecule from the peels of lemons, which he first introduced as citrin, and latter known as "vitamin P".[4] Flavonoids are class of low molecular weight compounds that assembled by a polyphenols skeleton (Figure 1). A classification based on the oxidation and saturation expressed in the heterocyclic C-ring structure is commonly used to classify different flavonoids, and categorized mainly into flavanols, flavones, flavonones, isoflavone, flavonol, and flavanonol (Figure 2).[5]

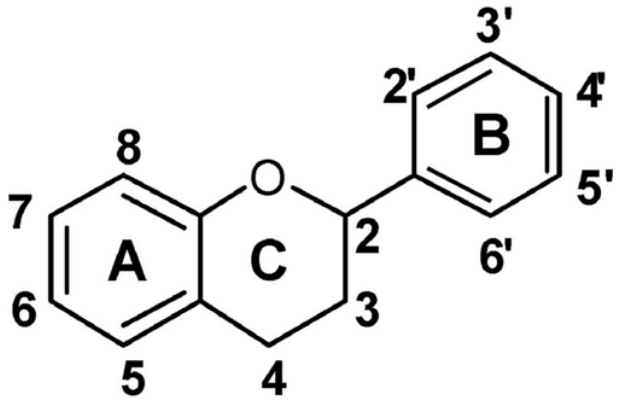

Figure 1. Basic structure of flavonoids. Reprinted, with permission, from Kumar, et al[5]

In this article, the mechanisms through which flavonoids act as an antioxidant and its therapeutic applications for oxidative stress related diseases namely cancer, diabetes, cardiovascular diseases, and neurodegenerative diseases, are discussed.

Antioxidant activities

Primarily, antioxidants perform by delaying, preventing or removing oxidative harm to a target molecule. The comprehensive mode of action of flavonoids includes (1) quenching free radical elements, (2) chelating metal, (3) suppressing the enzymes associated with free radical generation, and (4) stimulation of internal antioxidant enzymes.[6]

The best-described antioxidant property of flavonoids derives from its ability to directly scavenge the reactive oxygen species. Flavonoids are able to chelate free radicals immediately by donating a hydrogen atom or by single-electron transfer.[6]

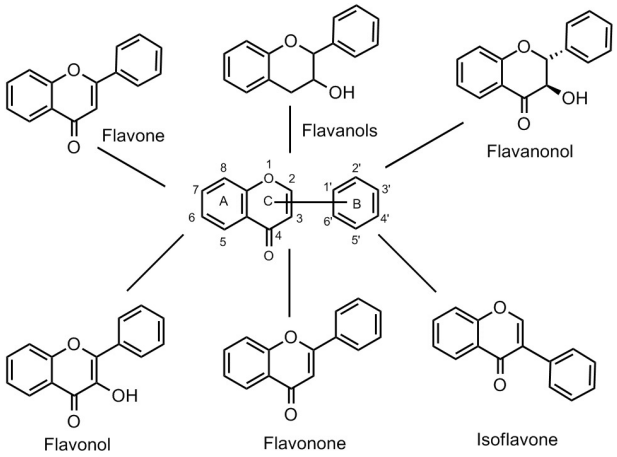

Figure 2. General chemical structure of flavonoids and their different classes. Reprinted, with permission, from Kumar, et al[5]

Table 1. Flavonoids subclasses and typical food sources

No.	Flavonoids subclass	Representative flavonoids	Food source
1	Flavonol	Kaempherol, myricetin, quercetin, rutin	Onion, kale, broccoli, apples, cherries, berries, black tea, red wine
2	Flavone	Apigenin, rutin, luteolin	Parsley, celery, thyme, red wine, tomato skin
3	Flavanone	Naringin, naringenin, hesperidin, taxifolin	Citrus, lemon, orange, grapefruit
4	Isoflavone	Genistin, genistein, daidzein	Soybean and products
5	Flavanol	Catechin, epicatechin	Tea
6	Anthocyanidin	Cyanidin, apigenidin	Cherry, raspberry, strawberry, colored fruits

Another possible mechanism of action of flavonoids is through the chelation of transition metal elements. Flavonoids have chelating property, which enabled them to chelate, or binds to metal ions in human body to prevent them being accessible for oxidation. Certain flavonoids have potential capacity to chelate trace metal ions such as Fe^{2+} and Cu^{+} that play a vital role in oxygen metabolism and free radical formation.[7]

Besides direct scavenging of free radicals and chelating of transition metal elements, flavonoids can also act as an intracellular antioxidant through inhibition of free radical generating enzymes such as xanthine oxidase, lipoxygenase, protein kinase C, cyclooxygenase, microsomal monooxygenase, mitochondrial succinoxidase, and NADPH oxidase.[6] Induction of internal antioxidant enzymes is other possible mechanism through which flavonoids act as an antioxidant. Phase II metabolizing enzymes (e.g. UDP-glucuronosyltransferases, sulfotransferases, N-acetyltransferases, glutathione S-transferases and methyltransferases) are the utmost defensive enzymes against intracellular toxicants and xenobiotic.[6]

Structure-activity relationships

Many of *in vitro* and *in vivo* investigations have been carried out using the natural flavonoids to verify the correlations between the flavonoid structure and their antioxidant activities. Distinctive chemical structures related with flavonoids antioxidant activities have been established including hydroxyl groups (C in figure 3), ortho-dihydroxy arrangement in the B ring (A in figure 3), C2-C3 unsaturated bond combined with C-4 carbonyl group in the C skeleton (B in figure 3), and O-methylation.[8]

The free hydroxyl group will donate its hydrogen atom to a radical molecule, thereby stabilizing it and generates a relatively stable flavonoid phenoxyl radical. Subsequently, this stable molecule may react with a second radical (Alkoxyl (RO^{*})), adopting a stable quinone structure.[9] Additionally, the position instead of total number of hydroxyl groups, considerably dominantly influence anti oxidative activity.[10]

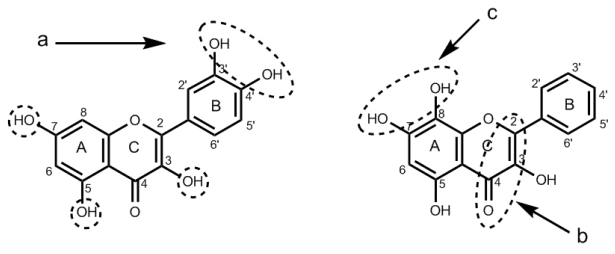

Figure 3. Summary of the antioxidant structure-activity relationships. Modified from Bubols, et al[8]

The B-ring hydroxyl structure is the utmost significant actor of scavenging of both oxygen and nitrogen-centered free radicals.[11] Hydroxyl groups on this nucleus donate hydrogen and an electron to hydroxyl, peroxyl, and peroxynitrite radicals, stabilizing them and giving rise to a relatively stable flavonoid radical. Moreover, the twisting angle of the B-ring aside from the rest of the molecule strongly influences free radical scavenging ability. Studies by Moalin, et al showed that higher antioxidant activity is in linear with the presence of hydroxyl groups in ring B and also total number of hydroxyl groups. Their studies described that among quercetin and its derivatives, quercetin harbors vigorous antioxidant activity compared to other compounds.[12] A study by Celik and Arinç[13] showed that quercetin also express higher antioxidant capacity than its flavonoids derivatives, like rutin and naringenin. Heijnen, et al[14] found that hydroxyl groups also act as vigorous scavengers not only for ROS but also for RNS. It appears that hydroxyl groups dominate the capability of flavonoids as antioxidant.

The influence of hydroxyl group on the capacity of flavonoids to induce antioxidant enzymes has also been studied. Wiegand and colleagues showed a significant increase in either the hepatic mRNA or NQO1 activity in rats fed with genistein.[15] It appears that genistein binds to the promoter-binding site following up regulation of mRNA transcription. Another study with Hepa-1c1c7 mouse hepatoma cells found that luciferase was substantially induced in groups given flavonoids contained 3-OH at the C-ring compared to no hydroxyl group. They assumed that activation of electrophile responsive element (EpRE), which is a regulatory sequence of a group of genes encoding for phase II enzymes, is highly associated with redox properties and antioxidant activity of flavonoids.[16]

In addition to this hydroxyl groups, several investigations have emphasized the importance of 2,3 unsaturation combined with a 4-carbonyl group. A study by Meyer, et al as cited by Wolfe and Liu[17] demonstrated that delocalization of electron from

the B-ring was due to the presence of the 2,3-double bond combined with the 4-keto groups, and the depletion of either one or both features significantly lowered cellular antioxidant activity. This is showed when the quercetin to taxifolin and catechin and that of kaempferol to naringenin are compared in terms of their EC50 values.[17] These studies confirmed that the conjugation between the A and B rings allocates a resonance effect of the aromatic nucleus that generates a stable flavonoid radical.

Another structure that distinguishes the antioxidant activity is O-methylation. The differences in antioxidant capacity within flavonoids are mostly caused by differences in both hydrophobicity and molecular planarity. Wen, et al[18] showed that methylated flavones were much metabolically steady and have better intestinal absorption through human colon adenocarcinoma (Caco-2) cell monolayers in comparison with their unmethylated analogues, suggesting that methylation preserve these compounds from hepatic biotransformation. A corroborative *in vitro* study using human oral SCC-9 cancer cells suggested that 5,7-dimethoxyflavone and 5,7,4-trimethoxyflavone were both have higher inhibitory activity than the equivalent unmethylated analogues.[19] This is due not only to the greater hepatic metabolic stability but also to better intestinal absorption than that of unmethylated compounds. Thus, obstructing the free hydroxyl group by methylation discharge the effect of metabolizing enzymes, and subsequently improves the antioxidant activity.

Therapeutic applications of antioxidant by flavonoids

Cardiovascular protective effect of flavonoids resembles in their antioxidant activity. There is cumulative evidence that strongly linked the oxidative stress to cardiovascular diseases, such as myocardial infarction, myocardial ischemia or re-perfusion, and atherosclerosis as well as hypertension and heart failure. Numbers of studies have indicated that flavonoid intake gives positive effect on cardiac performance. Recently, Liu, et al[20] in their *in vivo* study using rats, found that quercetin increases the production of antioxidant enzyme activity, such as glutathione-peroxidase (GSH-Px), glutathione reductase (GR), superoxide dismutase (SOD), and catalase (CAT). The authors described that quercetin prevents lipid peroxidation and subsequently helps to preserve membrane integrity. This is further supported by reduced levels of cardiac markers such as creatine kinase (CK), aspartate aminotransferase (AST), and lactate dehydrogenase (LDH) in the quercetin treated group.[20]

Furthermore, a study also showed that consumption of catechin dramatically increased the endothelial function and endothelium-dependent arterial vasodilation in renal transplant case through the stimulation of endothelial nitric oxide synthase (eNOS) isoform.[21] Reduction of free radicals prevent NO inactivation, thus more NO are available. This results highlighted the importance of ROS scavenging action of cathecin. Flavonoids as a strong antioxidant have also been used in certain study related to diabetes mellitus. Kaempferol, one of the most important flavonols, has been shown to have protective effect on HIT-T15 pancreatic beta cells from oxidative damage. This compound is reported to scavenge ROS produced by glucose toxicity in type 2 diabetes.[22]

Flavonoids are also responsible for the stimulation of antioxidant enzymes. Its ability to trigger the generation of antioxidant enzymes in human body was tested in a study by Soto, et al.[23] They studied the effects of silymarin on antioxidant enzymes in alloxan-induced diabetic rats, and found that flavonoids stimulate the generation of these enzymes. Certain studies have also linked disrupted iron metabolism to neurodegenerative diseases. Iron has been found to accumulate in degenerative neuronal sites, induces aggregation and deposition of peptides such as amyloid-β peptide (Aβ) and α-synuclein in the brain, linking this metal compound to numbers of diseases including Alzheimer, Parkinson, and Huntington diseases.[24] A comprehensive review of neuroprotective actions from green tea for prevention or treatment of Alzheimer's and Parkinson diseases has been discussed by Weinreb, et al.[25]

The ability of flavonoids to interfere with cancer treatment has been tested in a series of flavonoids compound. Samy, et al[26] compared the effect of cyclophosphamide (CYC), luteolin, and luteolin in combination with CYC against 7,12-dimethylbenz(a)anthracene (DMBA) induced mammary carcinogenesis in Wistar rats. CYC showed the greatest capacity in minimizing tumor number and volumes, but the researchers stated that long-term administration is toxic to the rats as seen by a significant decrease of body weight. Whereas, luteolin combined with CYC express lower anti-

tumor potential, but able to eliminate the toxic effect. The quantity of antioxidant enzymes, such as SOD, CAT and glutathione peroxidase (GPX) in multiple organs (liver, kidney and breast) were lessen by 50 to 80% in rats, but were reversed to normal by the combination treatment. Similarly, Hussein and Khalifa[27] observed enhancement of hepatic antioxidant status [GPX, gamma glutamyl transferase (c-GT) and glutathione-S-transferase (GST)], with an improvement in reduced glutathione (GSH) and serum total protein with concomitant significant reductions in tumor markers arginase and a-L-fucosidase. Reduction of liver enzymes [AST, alanine aminotransferase (ALT), alkaline phosphatase (ALP), and GST, glucose- 6-phosphate dehydrogenase (G6PD)], direct and total bilirubin was also observed in rats with N-nitrosodiethylamine (NDEA) induced hepatocarcinogenesis upon intragastric administration of ellagitannins.

In conclusion, studies evaluating the antioxidant properties prompted by flavonoids have currently expanded to a wider range of therapeutic applications. In this context, the mechanisms underlying the antioxidant properties of flavonoids have been concisely described. This manuscript focuses on the physicochemical characteristics of flavonoids including the free hydroxyl groups, 4-carbonyl group, the C2-C3 double bond in relation to their antioxidant activity which strongly supported its biological activities. To point out, flavonoids serve as a potent treatment for oxidative stress.

Conflict of interest

The authors affirm no conflict of interest in this study.

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