Antioxidant Flavonoids: Structure, Function and Clinical Usage

Alan L. Miller, ND

Abstract

Flavonoids occur in most plant species, and account for a significant percentage of the chemical constituents of some; e.g. dried green tea leaves contain approximately 30% flavonoids by weight. Flavonoids have been shown to have antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, and vasodilatory activity. The potent antioxidant activity of flavonoids—their ability to scavenge hydroxyl radicals, superoxide anions, and lipid peroxy radicals—may be the most important function of flavonoids, and underlies many of the above actions in the body. Oxidative damage is implicated in most disease processes, and epidemiological, clinical, and laboratory research on flavonoids and other antioxidants suggest their use in the prevention and treatment of a number of these. Catechin and its derivatives, oligomeric proanthocyanidins, quercetin and quercetin chalcone, Ginkgo flavone glycosides, silymarin, and others can be utilized in preventative and treatment protocols for cardiovascular disease, cancer, inflammatory conditions, asthma, periodontal disease, liver disease, cataracts and macular degeneration.
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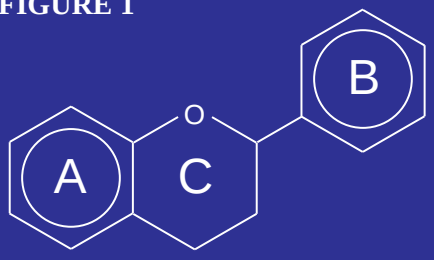
Introduction

Flavonoids, or bioflavonoids, are a ubiquitous group of polyphenolic substances which are present in most plants, concentrating in seeds, fruit skin or peel, bark, and flowers. A great number of plant medicines contain flavonoids, which have been reported by many authors as having antibacterial, anti-inflammatory, antiallergic, antimutagenic, antiviral, antineoplastic, anti-thrombotic, and vasodilatory actions. The structural components common to these molecules include two benzene rings on either side of a 3-carbon ring (see Figure 1). Multiple combinations of hydroxyl groups, sugars, oxygens, and methyl groups attached to these structures create the various classes of flavonoids: flavanols, flavanones, flavones, flavan-3-ols (catechins), anthocyanins, and isoflavones. Flavonoids have been shown in a number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions, and lipid peroxy radicals.

Free radicals, including the superoxide radical ($O_2^{\cdot -}$), hydroxyl radical (·OH), hydrogen peroxide (H_2O_2) , and lipid peroxide radicals have been implicated in a number of disease processes, including asthma,[1,2] cancer,[3] cardiovascular disease,[4,5] cataracts,[6,7] diabetes,[8,9] gastrointestinal inflammatory diseases,[10,11] liver disease,[12] macular degeneration,[13,14] periodontal disease,[15] and other inflammatory processes. These radical oxygen species (ROS) are

FIGURE 1



produced as a normal consequence of biochemical processes in the body and as a result of increased exposure to environmental and/or dietary xenobiotics. ROS are also beneficial components of the immune response, hepatic cytochrome P450-mediated detoxification, and regulation of smooth muscle tone.[1] It is an imbalance in these oxidant versus antioxidant processes (oxidative stress) that is thought to cause the subsequent cellular damage which leads to the disease processes named above. The body's antioxidant systems, including superoxide dismutase, catalase, and glutathione, should keep the oxidative processes 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.[16]

The mechanism of free-radical damage includes ROS-induced peroxidation of polyunsaturated fatty acids in the cell membrane lipid bilayer, which causes 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.[17,18]

Epidemiological Studies

Two recent epidemiological studies reveal an inverse correlation between dietary flavonoid intake and coronary heart disease mortality. A Finnish study of 5133 men and women found that those with the highest intake of flavonoids (mostly from onions and apples) had a reduced risk for coronary disease.[19] A Dutch study (The Zutphen Elderly Study) of 805 men also noted an inverse relationship between dietary flavonoid intake and heart disease, with the majority of dietary flavonoids coming from tea, onions, and apples.[5]

LDL Cholesterol Oxidation

Oxidation of low-density lipoproteins (LDL) is considered by many sources to be a very important component of the development of atherosclerotic lesions.[4,5,17,19-22] Circulating monocytes scavenge oxygen-modified LDL molecules with a very high affinity—up to ten times greater than "native LDL."[4] These monocytes/macrophages penetrate into the subendothelial space and become the first stage of atherogenesis, the so-called "fatty streak." Antioxidants which interrupt this process can be very helpful in the process of preventing and/or treating cardiovascular disease.

A number of flavonoids, including quercetin, morin, gossypetin, chrysin, myricetin, rutin, catechin and its derivatives, and the oligomeric proanthocyanidins (OPCs), have been shown in in vitro studies to inhibit the oxidation of LDL.[18,20-24] Oxidation of LDL is used as a model of the anti-lipid peroxidation activity of flavonoids, as the LDL molecule has an outer phospholipid layer similar to cell membranes. The mechanism by which flavonoids inhibit LDL is not totally known, but it is thought that they reduce free radical formation, protect LDL-α-tocopherol or regenerate oxidized LDL-α-tocopherol, and/or sequester metal ions which participate in oxidation reactions.[18,22]

The French Paradox

The French diet, on average, contains a greater amount of saturated fat than the average diet in other countries; however, the French have a lower incidence of cardiovascular disease. This has been termed the "French Paradox." Initial research into this phenomenon noted that the intake of moderate amounts of alcohol, specifically wine, can reduce the risk of coronary heart disease by at least 40%.[25] It was thought that the alcohol content or some other component of red wine was responsible for this vascular protection. Subsequent studies of the antioxidant effects of red wine have shown that red wine and isolated red-wine polyphenols inhibit the oxidation of LDL cholesterol, interrupting the first step of atherogenesis.[21,23] Fuhrman et al. also noted in their 1995 study of red wine and LDL oxidation that the red wine polyphenols associated themselves within the LDL fraction, and resulted in a reduction in LDL oxidation (initiated by copper ions). This was illustrated by a reduction of 73%, 46%, and 54%, respectively, in lipid peroxides, TBARS (thiobarbituric acid substances—an indication of lipid peroxidation), and conjugated dienes (another measure of oxidation). The authors also noted that white wine, which contains only a fraction of the polyphenol content of red wine, actually increased the susceptibility of LDL to undergo oxidation, most likely due to the inability of the small polyphenol content to overcome the pro-oxidant properties of alcohol.[23]

Inflammation

Inflammation is both a free-radical-generated and free-radical-producing process. The enzymes cyclooxygenase and lipoxygenase act on arachidonic acid in cell membranes, oxidizing arachidonic acid and forming potent pro-inflammatory metabolites, including prostaglandins, leukotrienes, and thromboxanes. Many flavonoids, including quercetin, rutin, baicalein, kaempferol, curcumin, silymarin, and green tea polyphenols exhibit inhibition of cyclooxygenase and lipoxygenase in vitro,[26-29] which seems to be related to their antioxidant activity. The cardiovascular protectant effects and antineoplastic effects of these flavonoids might be due to their ability to inhibit these enzymes and the resultant formation of arachidonic acid metabolites.

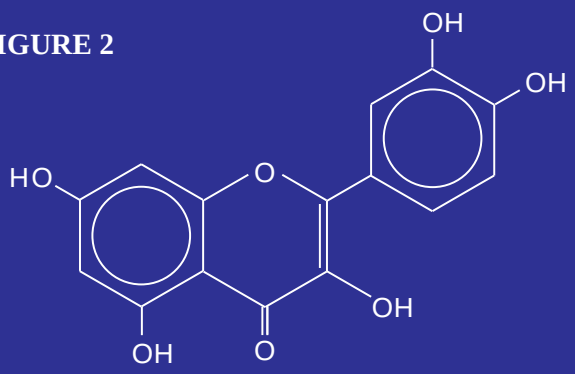
Green Tea Extract

Green tea contains catechin-based flavonoids, including catechin and epicatechin, and their gallic acid esters. These and other flavonoids make up approximately 30% of the weight of dried tea leaves. Epigallocatechin gallate is the flavonoid in the highest proportion in green tea. Black tea contains more free gallic acid, which is a strong antioxidant; however, the gallic acid esters of catechins, which are found in greater quantity in green tea, are more potent free radical scavengers.[30]

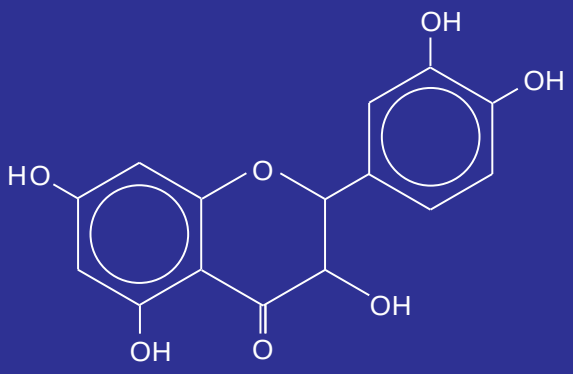
In a 1992 study, Ho and Chen found that, of the eight teas tested, all four green teas but only one black tea inhibited the induction of fatty acid oxidation, showing that green tea is a more consistent antioxidant, while with black tea the gallic acid content, and thus the antioxidant activity, depends on the method of manufacture/fermentation. Partially-fermented teas had much lower antioxidant activity than the other teas.[30]

Epidemiologic studies of people who drink a large amount of green tea suggest that green tea consumption is protective for gastrointestinal cancers. Green tea polyphenols have shown promise in vitro as antineoplastic substances, due to their ability to scavenge oxidative initiators of neoplasia.[31]

FIGURE 2



Quercetin



Dihydroquercetin

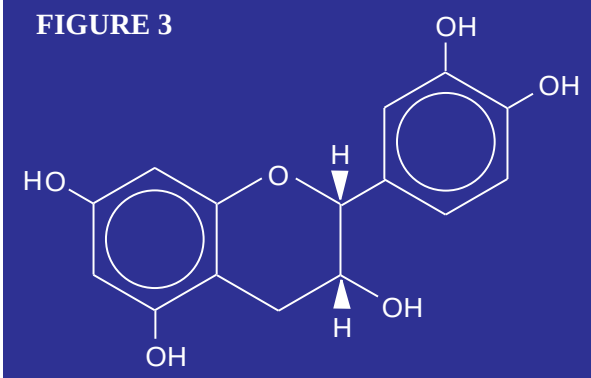
In a study of flavonoid antioxidant activity in the aqueous phase, epicatechin gallate (ECG), epigallocatechin gallate (EGCG), and quercetin scored the highest, followed by epigallocatechin (EGC), gallic acid, epicatechin, catechin, rutin, and dihydroquercetin. It is interesting to note here that the only difference between quercetin and dihydroquercetin is the double bond between the #2 and #3 carbons on the center (C) ring (see Figure 2). The absence of this double bond significantly reduces the antioxidant activity of the flavanol. To illustrate this, epicatechin, which also lacks this double bond, has an antioxidant activity which is only 53% of quercetin's. Epicatechin can increase its antioxidant activity with the addition of another hydroxyl group on the B ring (forming epigallocatechin), and further with the addition of gallic acid (with its three hydroxyl groups, forming epigallocatechin gallate) on the C ring, to the point where it is equivalent to quercetin (see Figure 3).[24]

Quercetin and Quercetin Chalcone

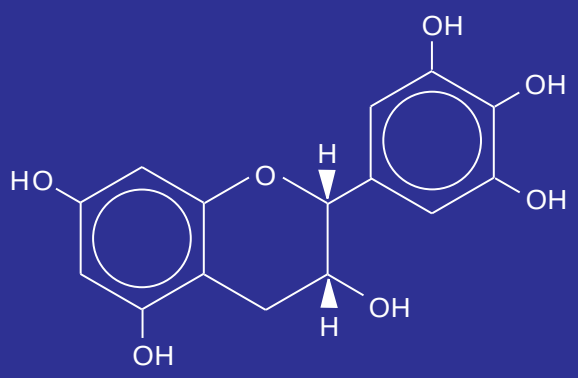
Quercetin chalcone (QC), a novel flavonoid, is quercetin with an opened C ring and the oxygen found in the C-ring of quercetin converted into a hydroxyl group (see Figure 4). QC also retains the C-ring double bond from quercetin, and should retain the antioxidant properties of quercetin as well. In fact, with the addition of the extra hydroxyl group in the C ring, quercetin chalcone could be a more potent antioxidant than quercetin. Quercetin chalcone is also more water soluble than quercetin (unpublished data), which should increase QC's absorption and bioavailability over quercetin.

Absorption from oral supplementation has been a major challenge in quercetin supplementation. Absorption of quercetin from an oral dose was estimated at less than 1% in a 1975 human pharmacokinetic study.[32] A more recent study of quercetin absorption in ileostomy patients showed 24% absorption; however, the researchers measured the absorption of quercetin by the difference between the amount ingested and the amount recovered in the ileostomy bag, assuming that if it was not detected in the ileostomy effluent, it was absorbed. The amount of quercetin or its conjugates recovered in the urine was minimal, but did increase as the amount recovered in the ileostomy bag decreased. The researchers were concerned about degradation of the quercetin in the stomach and small intestine, and tested this by placing quercetin in a solution of gastric juices in vitro, which showed no loss of quercetin. However, there is still a possibility that the quercetin was degraded in vivo by GI secretions or by small intestinal bacteria, thus going undetected in the effluent. [33]

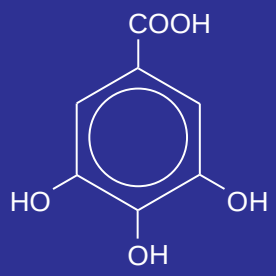
FIGURE 3



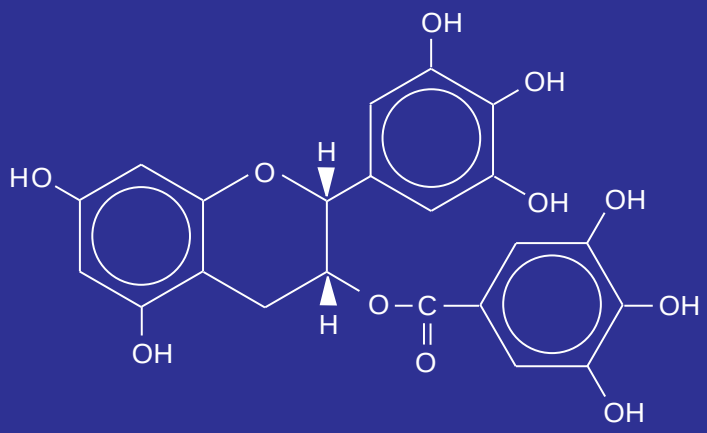
Epicatechin



Epigallocatechin (EGC)



Gallic Acid



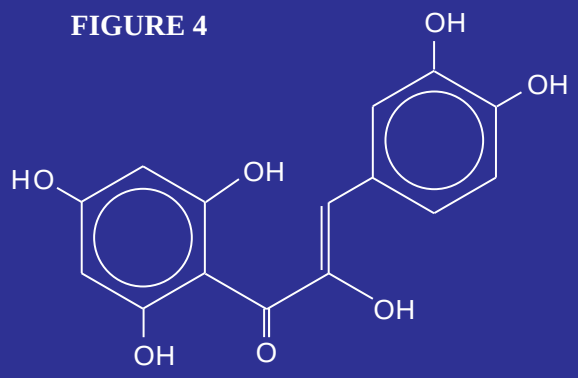
Epigallocatechin gallate (EGCG)

Oligomeric Proanthocyanidins

Oligomeric proanthocyanidins (OPCs, pycnogenols) are oligomeric flavonoids, usually dimers and trimers, based on the flavan-3- ol, or catechin, molecule, sometimes attached to gallic acid. These molecules are found in the bark of pine trees, in grape seeds and skins, in peanut skins, cranberries, tea, and other sources. Commercial sources of OPCs include grape seeds and pine bark. Research on OPCs began in the late 1940's, when French researcher Jacques Masquelier discovered the vascular protective qualities of OPCs. In 1986, Masquelier, after having devised an efficient method of extracting OPCs from pine bark and grape seeds, found that OPCs have strong antioxidant effects, and patented OPCs extracted using his methods as antioxidant substances. It is now known that the antioxidant effects of OPCs contribute to their vascular protective activity. OPCs also cause more efficient cross-linking of collagen, which strengthens collagenous structures. OPCs' antioxidant activity also spares vitamin C so that vitamin C can be utilized in collagen synthesis. Another benefit of OPCs sparing effect on vitamin C is that vitamin C is able to efficiently participate in the synthesis of bile acids from cholesterol. Cholesterol 7α-hydroxylase, the rate-limiting enzyme for bile acid synthesis from cholesterol, is an ascorbic acid-dependent enzyme. Vitamin C-deficient guinea pigs show a decreased activity of this enzyme and concomitant cholesterol accumulation in plasma, liver,

and arteries, and a decreased HDL:total cholesterol ratio. Human trials have shown a decreased total cholesterol and increased HDL:total cholesterol ratio after moderate vitamin C supplementation of 500 mg/day.[34-36]

FIGURE 4



Quercetin Chalcone

In vitro testing of the antioxidant activity of OPCs by Masquelier and his colleagues indicated that the catechin-catechin-gallic acid dimer, proanthocyanidin B2-3-O-gallate "was the strongest antioxidant substance in the extract, and was found to be 20 times stronger as an antioxidant than vitamin E." Masquelier says this compound is present in the grape seed extract, but is absent from the pine bark extract.

Ginkgo Biloba Extract

Ginkgo biloba is the oldest living tree species, having survived thousands of years. It is used worldwide in the treatment of cerebrovascular insufficiency, peripheral vascular disease, and depression, especially in the elderly.

Standardized Ginkgo biloba extracts (GBE) typically contain 24% Ginkgo flavone glycosides (flavonoids, Ginkgo heterosides) and 6% terpenes. It has been proposed that antioxidant mechanisms underlie some of the therapeutic effects of GBE, as a growing body of evidence points toward free radical and lipid peroxidation reactions as participants in peripheral and central vascular diseases and neuronal damage.[37,38] Researchers at the University of Tokushima in Japan found that the flavonoid constituents of GBE—quercetin, myricetin, kaempferol, and rutin—scavenge radical oxygen species. The authors suggest that these flavonoids, particularly quercetin and myricetin, are the beneficial constituents of GBE in preventing free radical-induced neuronal damage.[34] Potent peroxyl radical scavenging activity was noted in a 1995 in vitro study, along with reduced LDL oxidative modification.[37]

Diabetics characteristically exhibit signs of oxidative stress in the retina, resulting in thickened basement membranes and altered retinal vessel permeability. In animal models, GBE improved retinal functioning by decreasing oxidative retinal stress.[8,39]

Recent theories regarding the etiology of macular degeneration, the most common cause of blindness in the elderly population, and for which there are no adequate allopathic medical treatments, center on free radical damage to the retina.[14,40] Studies of antioxidant status and macular degeneration note that elderly subjects with a high antioxidant index (ascorbic acid, alpha-tocopherol, and beta-carotene status) have a reduced risk for development of macular degeneration.[41-42] A preliminary double-blind study on the use of Ginkgo extract in macular degeneration showed a statistically significant improvement in long distance visual acuity with GBE vs. placebo. The authors state that GBE's antioxidant activity was responsible for this change.[13]

Cataracts, another common cause of visual impairment, have been associated with free radical damage in numerous studies. Flavonoids have not been studied as a

preventative or treatment for cataracts as yet; however, oxygen radicals do seem to play a role in the formation of cataracts, and subjects with lower antioxidant status have been shown to have a greater incidence of cataract formation.[7,43,44]

Clastogenic factors (CFs)—long-term, persistent superoxide ion-induced DNA damage—are found commonly in the plasma of people irradiated by accident or therapeutically, and are thought to be responsible for chromosomal abnormalities many years after exposure. CFs were found in a high percentage of Chernobyl salvage workers. In a recent study, ten of these workers who exhibited a high amount of CFs were given GBE for two months (40 mg TID), and showed no signs of clastogenic activity following the treatment period.[45]

Ginkgo extract has also been found to have a protective effect against lipid peroxidation in hepatic microsomes secondary to cyclosporin A treatment. Cyclosporin A is an immunosuppressive drug with a small therapeutic window; i.e., the difference between therapeutic blood levels and toxic levels is quite small. It is lipid-soluble, and thus concentrates in fatty tissue, causing lipid peroxidation. Ginkgo was shown to inhibit this lipid peroxidation in a dose-dependent manner in an in vitro study.[46]

Silymarin

Silybum marianum, or milk thistle, is commonly used in the treatment of hepatic damage caused by toxic exposure and viral hepatitis, and has been shown to be a potent hepatoprotective agent. Silybum contains a number of flavonoids, the most abundant being silymarin, which is actually a mixture of three flavonoids; silibin, silydianin, and silychristine. These flavonoids have been shown to inhibit lipid peroxidation, iron-induced hepatic toxicity, and acetaminophen-induced lipid peroxidation and liver damage. Silymarin's antioxidant activity has been linked to all of these hepatoprotective effects.[47-50]

Conclusions

Flavonoids have been studied since the 1940s, and their antioxidant activity is undisputed at this point. With the immense volume of research being released every year regarding the effects of radical oxygen species on human health, the role of flavonoid antioxidants cannot be ignored. For example, cardiovascular disease and cancer, the two leading causes of mortality in the United States, can be significantly impacted by the ingestion of antioxidants, including flavonoid-rich foods or supplemental standardized extracts. In fact, almost every disease process has some component of oxidative damage. Green tea, onions, apples, grapes, Ginkgo, and silybum are just a few of the many thousands of plants that contain flavonoid antioxidants. And the list of flavonoids keeps growing, as more are being discovered each year. Hopefully, more clinical research on flavonoids will be forthcoming, as this is an area which is lacking. However, there is enough epidemiological, clinical, and laboratory research on flavonoids, and on antioxidants in general, to make some conclusions about the clinical use of flavonoids, and to warrant their use in the prevention and/or treatment of cardiovascular disease, cancer, inflammatory conditions, asthma, periodontal disease, liver disease, cataracts and macular degeneration.

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