

THE HIERARCHY OF FLAVONOID ANTIOXIDANT POTENTIALS/2003

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(This information is provided to the professional health practitioner as an overview of the current research on flavonoids. The practitioner may apply this information as per his/her interpretation of the research provided. This document serves as both an introduction to the chemistry/pharmacology of flavonoids and demonstrates a positive correlation between certain health benefits and flavonoid intake.)

Supplementing the diet with plant constituents can assist the body in preventing and treating disease. Current potentials now being demonstrated in phytotherapy research regarding the therapeutic strengths of plants have long been recognized by traditional herbal medicine. At present, the antioxidant and free radical scavenging effects of plant constituents are of great research interest. The nearly ubiquitous secondary metabolites in plants, fruits, vegetables known as flavonoids are increasingly attracting the attention of the research community. Flavonoids are found in seeds, nuts, grains, spices and different medicinal plants as well as wine (particularly red wine), tea and (at lower levels) beer.¹⁶ Flavonoids are also found in coffee.)

The presence of flavonoids in vegetables and fruits is thought to be one of the reasons for the beneficial influence on human health of these components of the diet.¹ They are among the commonest of the secondary metabolites found in plants, possessing a wide spectrum of physiological effects. Flavonoids have been referred to as “nature’s biological response modifiers” because of their anti-inflammatory, anti-allergic, antiviral and anti-cancer properties.¹² Flavonoids appear to modify the body’s reaction to other compounds such as allergens, viruses and carcinogens as evidenced by their anti-inflammatory, anti-allergic, antiviral and anticancer properties. Flavonoids are involved in immune function, gene expression, capillary and cerebral blood flow, platelet aggregation, liver function, enzyme activity and collagen, phospholipid, cholesterol and histamine metabolism.

Flavonoids are a sub-category of the plant phenolics, very widely found constituents characterized by the same basic structural element: an aromatic ring bearing one or more hydroxyl groups. Over 4,000 different types of flavonoids have been described to date and total daily intake in the U.S. has been estimated to be approx 1 g, although this is probably an over-estimation.^{4,5} Several epidemiological studies provide support for a protective effect of the consumption of fresh fruits and vegetables against cancer^{20,21}, heart disease^{22,23} and stroke^{24,25}. Epidemiologic studies have indicated an inverse association between the intake of selected flavonoids and cancer, coronary heart disease, and stroke among older adults. Many flavonoids are also capable of binding to metal ions, which prevent these metals from acting as catalysts that enhance free radical production in the body.

Given the complexity of this family of compounds, scientists still have a great deal to learn in terms of the exact mechanisms by which these compounds may offer protection against disease.⁶

The antioxidant activity of flavonoids is generally more potent and effective against a broader range of oxidants than traditional antioxidant nutrients like vitamins C and E, beta carotene, selenium and zinc.¹² In vitro tests show the free radical scavenging effects of OPC’santhocyanidins are 20 times greater than Vitamin C and 50 times greater than Vitamin E.⁶⁵

Synergy: Based on extensive data, it appears that a combination of antioxidants will provide greater antioxidant protection than any single nutritional antioxidant. Mixtures of antioxidant nutrients appear to work together harmoniously to produce the phenomena of synergy.¹²

Bioflavonoids, also known as flavonoids are phenolic compounds (many of which have phytoestrogenic effects) that occur naturally in many plants. Nutrient databases as well, remain inadequate to provide references for age-related intake of this class of antioxidant compounds. Flavonoids are an extremely complex group of compounds. They are divided into six categories:

Flavonols – found in onions, kale and broccoli. Quercetin is an example.

Flavones – found in greens, including thyme and parsley

Flavonones – found in citrus fruits

Isoflavones – found predominately in legumes, include genistein found in soy beans

Catechins – found in tea and apples

Tannins: Anthocyanidins/Proanthocyanidins(PCOs)/gallic acid phenolics – Give certain plants their dark purple/blue color. These are found in grapes, cherries, blueberries, plums and other colored fruits. Herbs include Hawthorne and Bilberry.

(*Flavones and flavonols(Quercetin) are the most widely occurring flavonoids.

Flavonoids are found in ferns and higher plants where they occur both in the free state and as glycosides. The flavonoids are mainly yellow compounds and contribute to the yellow colors of flowers and fruits where they are present as glycosides, dissolved in the cell sap. An exception to the color rule are the anthocyanins which are red, violet or blue depending on the pH of the cell sap. Flavonoids act as catalysts in the light phase of photosynthesis and/or as regulators of iron channels involved in phosphorylation.¹⁷

They can also function as stress protectants in plant cells by scavenging ROS produced by the photosynthetic electron transport system.¹⁸ Furthermore, because of their UV absorbing properties, flavonoids protect plants from the UV radiation of the sun and scavenge UV generated ROS.¹⁹

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The first suggestion of biological activity of flavonoids was presented by Szent-Gyorgi in 1938, who isolated both flavonoids and Vitamin C from paprika. He reported that citrus peel flavonoids were effective in preventing capillary bleeding and fragility associated with scurvy.⁶³ Szent-Gyorgyi called bioflavonoids “vitamin P” and suggested they were crucial for the integrity of the small blood vessels and as treatment for the skin disease purpura. Since then, the following activities have been described: Anti-inflammatory, anti-hepatotoxic, anti-tumoral, anti-microbial, antiviral, enzyme inhibiting, antioxidant and improvement of central vascular system effects including reducing the susceptibility of LDL to oxidation, as well as improving other parameters associated with free radical damage. Many herbal remedies contain constituents of which are mainly flavonoids and whose alleged effect is ascribed to these compounds. In old folk medicine, a cure for cancer and arthritis was proposed called the "grape cure". In this therapy, grapes were consumed exclusively. Grape skins are very rich in anthocyanins which are potent antioxidants and connective tissue stabilizers.²

Current studies confirm the European blueberries, both wild and cultivated are one of the richest sources of plant-derived antioxidants. Some studies have found that the antioxidant potency blueberries may vary depending on what type of cultivated berry is tested. Also, the antioxidant efficacy in vivo of flavonoids has been less thoroughly documented, possibly due to the limited knowledge on their pharmacokinetics. □

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Vitamin C connection: Flavonoids are usually found alongside vitamin C in nature. An important recommendation is to supplement with a flavonoid-rich extract to provide extra antioxidant protection. Studies have shown that supplementing with Vitamin C *alone* may not be as effective as supplementing with Vitamin C with flavonoids. Flavonoids may correspondingly increase the amount of Vitamin C in tissues, by preventing the breakdown of Vitamin C. Flavonoid molecules are hydroxyl radical scavengers. The proposed mechanism is via flavonoids which positively affect valencies and oxidation states of iron and copper stabilizing them to produce less free radicals. One study showed that Flavonoids prevent the breakdown of Vitamin C by chelating trace copper ions, scavenging free radicals and preventing rancidity of oils, similar to the way EDTA binds trace metals in a chelation protocol. □

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Coronary heart disease and cancer are areas where flavonoids may be of importance for preventative health. □

1. Protecting the Endothelium of the blood vessels: Coronary artery disease

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Key points:□

- The incidence of coronary mortality is higher among populations with low dietary intake of flavonoids.
- The effects may be mediated through prevention of oxidation of low density lipoproteins, but other mechanisms could be involved.□
- Flavonoids offer an explanation for the suggested beneficial effect of fruits and vegetables in coronary heart disease.

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Atherosclerosis, hypertension and stroke (decrease platelet aggregation).

Many flavonoids support the health of the body's circulatory system by helping maintain proper vascular permeability, integrity and resiliency.□Dark skinned berries contain bioflavonoids which are termed anthocyanins. These water soluble flavonoids are important in stabilizing and strengthening capillary beds and in strengthening and protecting our blood vessels from damage from metabolic waste products carried by the bloodstream en route to the liver for detoxification or to the kidneys for removal.²

Cholesterol :□Anthocyanins decrease the LDL peroxidation that causes atherosclerotic plaques in the arteries.□The antioxidant potential of flavonoids is based on their ability to increase the resistance of isolated LDL to copper oxidation in vitro.□This approach stems from the oxidative theory of atherogenesis, which states that it is not LDL or VLDL that is atherogenic but the oxidized form of these lipoproteins.^{26,32}□The French have plasma cholesterol levels comparable with the Americans and the fat intakes are comparable as well, but in spite of this the French have a far lower incidence of coronary disease.□This has been termed "the French paradox" and has been related to the high French consumption of red wine which contains flavonoids like quercetin and myricetin.□Oxidation of the LDL (Low Density Lipoprotein) is an important factor in the development of atherosclerosis, which is a very complex process.□In vitro experiments have shown that flavonoids inhibit the oxidation of LDL, which prevents the formation of foam cells triggered by cellular (macrophages) which is what causes the atherosclerotic plaques.□Scavenging of free radicals participating in oxidative processes may thus be an explanation for some of the observed effects of the flavonoids.□Other mechanisms such as protection of alpha-tocopherol have also been suggested.□In addition, quercetin reduces the cytotoxic effect of oxidized LDL.□It is also of interest to note that 15-lipoxygenase has been suggested to participate in the oxidation of LDL and several flavonoids inhibit this enzyme.^{1,28,29}

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2.□Anti-Aging and Alzheimers:□With the new insights concerning the role of free radicals in aging, auto-immune disease and many other health issues, the anti-oxidant potential of flavonoid containing herbs suggests exciting therapeutic possibilities.□These properties prevent the formation of free radicals and the leukotrienes that promote inflammation. Animal studies have shown that blueberry-enriched diets can delay the appearance of age-related decline in mental and behavioral function.□Harmon originally proposed that normal aging results from random deleterious damage to tissues by free radicals produced and accumulated during normal aerobic metabolism.⁸□There is also an age-related two to three-fold increase in the concentration of oxidatively damaged proteins.□Increases with age in lipid peroxidation reactions are indicated by such alkane hydrocarbon exhalation.□The ratio of redox couples such as glutathione (GSH: oxidized GSH(GSSG), NADPH:NADP+, and NADH:NAD+ tend to shift toward more pro-oxidant values during aging.^{9,10,11}□

The cascade of reactions resulting from the pairing of one of the long pair electrons of oxygen is interrupted by enzymatic systems such as superoxide dismutases, catalase and glutathione peroxidase which□reduce peroxides and later on hydroperoxides.□Many flavonoids and polyphenols react with free radicals to prevent the degradations linked to their intense activity on membrane phospholipids.□It has been hypothesized that flavonoid capacity to modify membrane-dependent processes (free radical induced membrane lipoperoxidation) , along with their ability to positively affect the intima in blood vessels may decrease cerebral aging damage.⁴⁴□In Alzheimers Disease, free radicals generate the beta amyloid protein which is what causes the plaques in the brain. The beta amyloid protein precursor is formed in part in the blood vessels. (Scientific American Nov 1991). It is possible that the intake of anthocyanin's may be useful in slowing down or preventing Alzheimer's DZ.^{2,7}□

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3. Addictions: Bioflavonoids may have an impact on addictive behaviors by modifying the damage produced by the addictive behavior and decreasing the severity of withdrawal symptoms.²

4. Anticarcinogens:

Flavonoids have profound effects on the function of immune and inflammatory cells as determined by a large number and variety of in vitro and some in vivo observations. These ubiquitous dietary chemicals may have significant in vivo effects on homeostasis within the immune system and on the behavior of secondary cell systems comprising the inflammatory response.⁴⁴ They have potential anticarcinogenic activity as evaluated by in vitro screening tests.

Flavonoids inhibit carcinogenesis by several mechanisms. Ample evidence indicates that selected flavonoids can affect (usually inhibit) secretory processes, mitogenesis and cell-cell interactions including possible effects on adhesion and the elaboration of effects of cytokines and cytokine receptors. Research on blueberry extracts has shown inhibition of human cervical and breast cancer cells, but only in vitro not in vivo at this time. Recent studies indicate that flavonoids have the ability to inhibit tumor growth in vitro.¹³ Ellagic acid has been described as an anti-carcinogen in the Merck manual. We know cancer incidence is reduced by consuming fruits and vegetables and this acid can be found in blackberries, boysenberries, loganberries, marionberries and particularly high amounts in red raspberries.^{2,8} However, the association between flavonoid intake and cancer protection is (at present) weak. According to some epidemiological studies, there is no evidence that flavonoid intake is protective against some types of cancer.²⁸ Only one study has shown that the consumption of flavonoids is inversely correlated with lung cancer where a weak inverse relationship was observed between flavonoids and cancer by Knekt and co-workers.²⁷

FAA (flavone acetic acid), when combined with IL-2 results in positive outcomes. One benefit is IL-2 does not exhibit its usual severe toxicity.⁶³ In mice, kidney cancer treated with FAA and IL-2 resulted in "up to 80% long term survivors". The two substances appear to work by stimulating NK cells and the production of tumor necrosis factor. When these two products were combined in humans, there were nearly 60 % more long-term survivors than when either drug was used alone.^{64,65} How all the below referenced anti-carcinogenic effects are mediated is not yet clear, but one important mechanism may be the capacity of flavonoids to stimulate or inhibit protein phosphorylation and thereby regulate cell function.⁴⁵

Enzymes involved in cell activation:

Flavonoids have been shown to affect a variety of enzymes involved in cell activation.

- Phospholipase A2 (PLA2) inhibition³⁶
- T Lymphocyte stimulation through inhibition of T cell protein tyrosine kinase (PTK)³⁷
- Immunosuppressive effects on anti-CD28 monoclonal antibody stimulation of T cell proliferation, IL-2 formation and the expression of IL-2 receptors.³⁸
- Inhibition of protein kinase C (PKC)³⁹
- Inhibition of growth and cell cycle in normal leukocytes and HL-60 cells⁴⁰.

This multitude of effects upon enzyme systems points to an influence on immune cell function.

- Suppression of Class II histocompatibility antigens in peripheral blood monocytes⁴⁶
- Inhibition of lymphocyte proliferation in response to phytoimitogens and antigens.⁴⁷
- Inhibition of thymidine transport in lymphocytes⁴⁸
- Effects on tumor cell immunity (Flav's cause enzymatic alterations in tumor cells)⁴⁹
- Stimulation of IgE formation⁵⁰
- Inhibition of cytotoxic lymphocyte formation⁵¹
- Inhibition of cell proliferation⁵²
- Multiple immune and inflammation-related properties⁵³
- Reduction of Delayed Type hypersensitivity (DTH)⁵⁴
- Inhibition of TPA-induced inflammation⁵⁵
- Inhibition of tumor promotion in mice⁵⁶

- Inhibition of mitogen-stimulated immunoglobulin secretion of IgG, IgM and IgA in vitro⁵⁷
- Effects on cytoskeletal structure and function⁵⁸
- Inhibition of PHA-induced cell proliferation and IL-2 formation⁵⁹
- Blockage of LTB4 generation⁶⁰
- Phosphorylation of PKT P56 inhibition⁶¹
- Reduction of LPS-induced activation of nuclear factor Kappa B⁶²

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Possible mechanisms of flavonoids in cancer prevention:

1. Stabilization of Collagen: □The base membrane surrounds capillaries and protects against tumor growth and invasion. □Flavonoids strengthen this membrane and inhibit collagen breakdown which in turn may inhibit tumor invasion and metastasis

2. Flavonoids induce the production of TGF beta-1. □Transforming growth factor is a cytokine that can regulate or down-regulate some growth factors, particularly in cancer promotion and initiation.⁶⁵

3. □Hyaluronidase inhibition: □Hyaluronidase is an enzyme that takes part in the inflammation process; it triggers the release of mast cells and histamines. □Certain flavonoids (specifically PCO's =proanthocyanidin oligomers like pycnogenol) have been shown to inhibit this process.⁶⁴ □

4. □Flavonoids bind with Laminin: □Laminin is a glycoprotein in the extracellular matrix that regulates invasion. □Laminin can bind to tissue plasminogen activators and reduce tumor activation and invasion.⁶³

5. □Respiratory: □Flavonoids prevent free radicals from causing tissue inflammation in the lungs. □Flavonoids are effective in the treatment of asthma, emphysema, and COPD. ² □ Some flavonoids are selective inhibitors of 5-lipoxygenase, which initiates the biosynthesis of leukotrienes, compounds considered important in mediating release, inflammation and immediate-type hypersensitivity pathophysiological changes in tissues undergoing allergic reactions:

- Mast cell and basophil roles in late phase allergic reactions inhibited³⁶
- Inhibition of the release of histamine and other mediators^{37,38,39}
- Histamine-releasing factor inhibited by quercetin⁴⁰
- Lipoxygenase-inhibition activity^{41,42,43}

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6. □Dermatology: □Anthocyanins are used to treat skin diseases specifically dermatological hypersensitivity reactions/inflammations. □Collagen may be degraded during the process of inflammation and some flavonoids may protect the skin from this process. □Two flavonoids extracted from bilberries and a third one from grapes were demonstrated to protect collagen against non-enzymatic proteolytic activity.¹⁵ □Other positive bioflavonoid research centers around atopic dermatitis, chronic urticaria, eczema and scleroderma (connective tissue disease where there is thickening of the skin and internal connective tissue problems).²

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7. □Macular degeneration/Cataracts/Glaucoma: □Anthocyanins have an affinity for blood vessels of the retina, especially the macula, and improve circulation to the retina. □Flavonoids improve the integrity of the blood vessels, decrease leakiness and reduce breakage of capillaries.^{33,34} □Supplementation with anthocyanosides from bilberry, rutin, or other flavonoids has been reported to reduce excessive capillary permeability in diabetics, which may reduce the risk of retinal hemorrhage.³⁵ □

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8. □Diabetic retinopathy: □Bioflavonoids support microvascular abnormalities of DM by improving the integrity of the blood vessels, decreasing leakiness and breakage of capillaries and improving circulation to the retina. □Flavonoids inhibit aldose reductase, the enzyme involved in glycosylation, preventing sorbitol from entering the cell which causes swelling/complications in diabetes.³⁴ □

9. □Venous insufficiency/varicose veins/hemorrhoids: □Bioflavonoids reduce capillary fragility which reduces venous insufficiency.^{30,31} □

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10. □Edema/Lymphedema: □Flavonoids have been shown to reduce fluid retention and capillary permeability. (Flavonoids –Tx of Fluid Retention/Christie) □

Anthocyanins improve the integrity of the capillary mesh, peripheral edema (ankle swelling) for women who have heart problems, pregnancy, and helps overall circulation.²

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11. □Anti-viral/antibacterial: □

Naturally occurring flavonoids can inhibit reverse transcriptases. □Amentoflavone, scullerein & quercetin are inhibitors of avian myeloblastosis RT, Rous-associated virus 2 RT, Maloney murine leukemia virus RT.⁵⁸

HIV RT and HIV-1 replication inhibited by baicalein.^{59,60}

Gardenin A, myricetin, morin, quercetin and fisetin inhibited activity of HIV-1 proteinase, it also decreased measurable levels of HTLV-1 p19 gag protein by 70% without adversely affecting the cells. □Baicalein, quercetin, quercetagenin and myricetin were potent inhibitors of the reverse transcriptases from Rauscher murine leukemia virus and HIV. □Significant activity at 1-2 um/ml. Quercetin, quercetagenin also inhibitors of DNA polymerase beta and DNA polymerase 1 respectively.⁶¹

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12. □G.I.: □Bioflavonoids stabilize mast cells in the gut to decrease intestinal permeability. □

In one in vitro study examining the effects of the flavonoids flavone, flavanone and quercetin on the production of acid by parietal cells in the gut, H⁺/K⁺-ATPase was also inhibited. □This action was found to be related to the ability of the flavonoids to complex ATP. □Also flavone and flavanone stimulated prostaglandin E2 production in isolated gastric mucosal cells. □All 3 flavonoids were found to inhibit H. pylori growth in a dose dependent manner with inhibition beginning at concentrations of 10 mg or more.¹⁴

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13. □Menorrhagia/Metrorrhagia: □Flavonoids decrease capillary fragility and may also decrease abnormal/excess bleeding in females.

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14. □Acute Viral Hepatitis: □Flavonoids may accelerate recovery, however they may also exacerbate *chronic active hepatitis*.

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Possible Contraindications: □

1. Phenolic and salicylate contents of blueberries can cause reactions in certain patients and limitation of these berries in diets is recommended. □Certain toxic patients with heavy metal poisoning or compromised liver function should use caution in taking □berries containing the above chemical components. □

2. □One study has shown a potential concern from high doses of blueberries killing beneficial intestinal probiotic bacteria due to blueberries' potent antibacterial effects.

3. □Flavonoids may exacerbate chronic active hepatitis.

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