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Potential Synergy of Phytochemicals in Cancer Prevention: Mechanism of Action[1]

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ABSTRACT Epidemiological studies have consistently shown that regular consumption of fruits and vegetables is strongly associated with reduced risk of developing chronic diseases, such as cancer and cardiovascular disease. It is now widely believed that the actions of the antioxidant nutrients alone do not explain the observed health benefits of diets rich in fruits and vegetables, because taken alone, the individual antioxidants studied in clinical trials do not appear to have consistent preventive effects. Work performed by our group and others has shown that fruits and vegetable phytochemical extracts exhibit strong antioxidant and antiproliferative activities and that the major part of total antioxidant activity is from the combination of phytochemicals. We proposed that the additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for these potent antioxidant and anticancer activities and that the benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in whole foods. This explains why no single antioxidant can replace the combination of natural phytochemicals in fruits and vegetables to achieve the health benefits. The evidence suggests that antioxidants or bioactive compounds are best acquired through whole-food consumption, not from expensive dietary supplements. We believe that a recommendation that consumers eat 5 to 10 servings of a wide variety of fruits and vegetables daily is an appropriate strategy for significantly reducing the risk of chronic diseases and to meet their nutrient requirements for optimum health. J. Nutr. 134: 3479S–3485S, 2004.

KEY WORDS: • *diet and cancer* • *fruits* • *vegetables* • *antioxidant* • *cancer prevention* • *phytochemicals*

Epidemiological studies have consistently shown that a high dietary intake of fruits and vegetables as well as whole grains is strongly associated with reduced risk of developing chronic diseases, such as cancer and cardiovascular disease (CVD), which are the top 2 causes of death in the United States and in most industrialized countries (1–3). It is estimated that one third of all cancer deaths in the United States could be avoided through appropriate dietary modification (3–5). This suggests that change in dietary behavior, such as increasing consumption of fruits, vegetables, and whole grains, and related lifestyles is a practical strategy for significantly reducing the incidence of cancer.

In 1982 the National Academy of Sciences of the United States included guidelines in their report on diet and cancer, emphasizing the importance of fruits and vegetables (6). The value of adding citrus fruits, carotene-rich fruits and vegetables, and cruciferous vegetables to the diet for reducing the risk of cancer was specifically highlighted. In 1989 a report from the National Academy of Sciences on diet and health recommended consuming 5 or more servings of fruits and vegetables daily for reducing the risk of both cancer and heart disease (7). The Five-a-Day program was developed as a tool to increase public awareness of the health benefits of fruits and vegetable consumption and to promote adequate intakes of known vitamins. Plant-based foods, such as fruits, vegetables, and whole grains, which contain significant amounts of bioactive phytochemicals, may provide desirable health benefits beyond basic nutrition to reduce the risk of chronic diseases (8).

Phytochemicals

The "phyto-" of the word phytochemicals is derived from the Greek word *phyto*, which means plant. Therefore, phytochemicals are plant chemicals. Phytochemicals are defined as bioactive nonnutrient plant compounds in fruits, vegetables, grains, and other plant foods that have been linked to reducing the risk of major chronic diseases. It is estimated that >5000 individual phytochemicals have been identified in fruits, vegetables, and grains, but a large percentage still remain unknown and need to be identified before we can fully understand the health benefits of phytochemicals in whole foods

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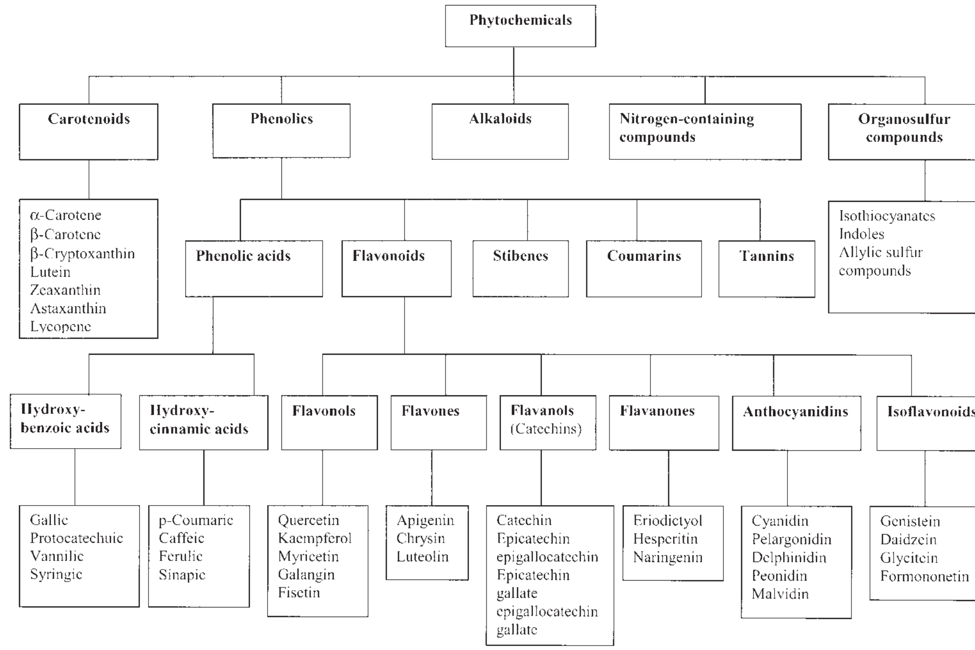


FIGURE 1 Classification of dietary phytochemicals.

(8). However, more and more convincing evidence suggests that the benefits of phytochemicals in fruits and vegetables may be even greater than is currently understood, because the oxidative stress induced by free radicals is involved in the etiology of a wide range of chronic diseases (9).

Phytochemicals can be classified as carotenoids, phenolics, alkaloids, nitrogen-containing compounds, and organosulfur compounds (**Fig. 1**). The most studied of the phytochemicals are the phenolics and carotenoids.

Phenolics. Phenolics are compounds possessing one or more aromatic rings with one or more hydroxyl groups and generally are categorized as phenolic acids, flavonoids, stilbenes, coumarins, and tannins (Fig. 1). Phenolics are the products of secondary metabolism in plants, providing essential functions in the reproduction and the growth of the plants; acting as defense mechanisms against pathogens, parasites, and predators, as well as contributing to the color of plants. In addition to their roles in plants, phenolic compounds in our diet may provide health benefits associated with reduced risk of chronic diseases. Among the 11 common fruits consumed in the United States, cranberry has the highest total phenolic content, followed by apple, red grape, strawberry, pineapple, banana, peach, lemon, orange, pear, and grapefruit (10). Among the 10 common vegetables consumed in the United States, broccoli possesses the highest total phenolic content, followed by spinach, yellow onion, red pepper, carrot, cabbage, potato, lettuce, celery, and cucumber (11). It is estimated that flavonoids account for approximately two thirds of the phenolics in our diet and the remaining one third are from phenolic acids.

Flavonoids. Flavonoids are a group of phenolic compounds with antioxidant activity that have been identified in fruits, vegetables, and other plant foods and that have been linked to reducing the risk of major chronic diseases. More than 4000 distinct flavonoids have been identified. They commonly have a generic structure consisting of two aromatic rings (A and B rings) linked by 3 carbons that are usually in an oxygenated heterocycle ring, or C ring (**Fig. 2**). Differences in the generic structure of the heterocycle C ring classify them as flavonols, flavones, flavanols (catechins), flavanones, anthocyanidins, and isoflavonoids (Fig. 1 and **Fig. 3**). Flavonols (quercetin, kaempferol, and myricetin), flavones (luteolin and apigenin), flavanols (catechin, epicatechin, epigallocatechin, epicatechin gallate, and epigallocatechin gallate), flavanones (naringenin), anthocyanidins, and isoflavonoids (genistein) are common flavonoids in the diet (Fig. 1 and **Fig. 4**). Flavonoids are most frequently found in nature as conjugates in glycosylated or esterified forms but can occur as aglycones, especially as a result of the effects of food processing. Many different glycosides can be found in nature; >80 different sugars have been discovered bound to flavonoids (12). Anthocyanidins give the red and blue colors in some fruits and vegetables.

Human intake of all flavonoids is estimated at a few hundred milligrams (13) to 650 mg/d (14). The total average intake of flavonols (quercetin, myricetin, and kaempferol) and flavones (luteolin and apigenin) was estimated as 23 mg/d, of which quercetin contributed ~70%; kaempferol, 17%; myricetin, 6%; luteolin, 4%; and apigenin 3% (15).

Phenolic acids. Phenolic acids can be subdivided into two major groups, hydroxybenzoic acids and hydroxycinnamic acids (**Fig. 5**). Hydroxybenzoic acid derivatives include *p*-hydroxybenzoic, protocatechuic, vannilic, syringic, and gallic acids. They are commonly present in the bound form and are typically a component of a complex structure like lignins and hydrolyzable tannins. They can also be found in the form of sugar derivatives and organic acids in plant foods.

Hydroxycinnamic acid derivatives include *p*-coumaric, caffeic, ferulic, and sinapic acids (Fig. 5). They are mainly present in the bound form, linked to cell-wall structural components,

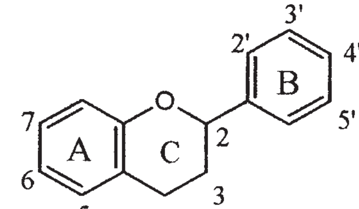


FIGURE 2 The generic structure of flavonoids.

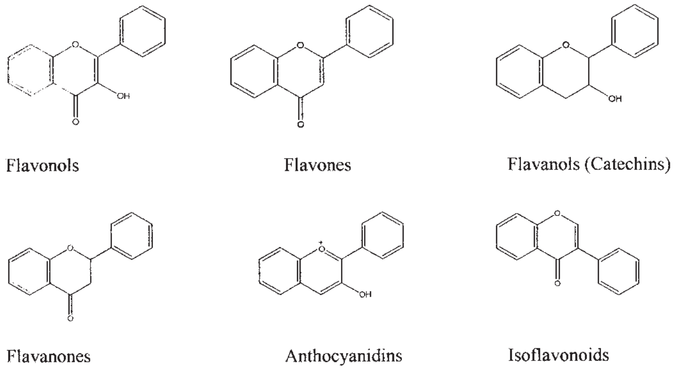


FIGURE 3 Structures of main classes of dietary flavonoids.

such as cellulose, lignin, and proteins through ester bonds. Ferulic acids occur primarily in the seeds and leaves of plants, mainly covalently conjugated to mono- and disaccharides, plant-cell-wall polysaccharides, glycoproteins, polyamines, lignin, and insoluble carbohydrate biopolymers. Wheat bran is a good source of ferulic acids, which are esterified to hemicellulose of the cell walls. Free, soluble-conjugated, and bound ferulic acids in grains are present in the ratio of 0.1:1:100 (16). Food processing, such as thermal processing, pasteurization, fermentation, and freezing, contributes to the release of these bound phenolic acids (17).

Caffeic, ferulic, *p*-coumaric, protocatechuic, and vannilic acids are present in almost all plants. Chlorogenic acids and curcumin are also major derivatives of hydroxycinnamic acids

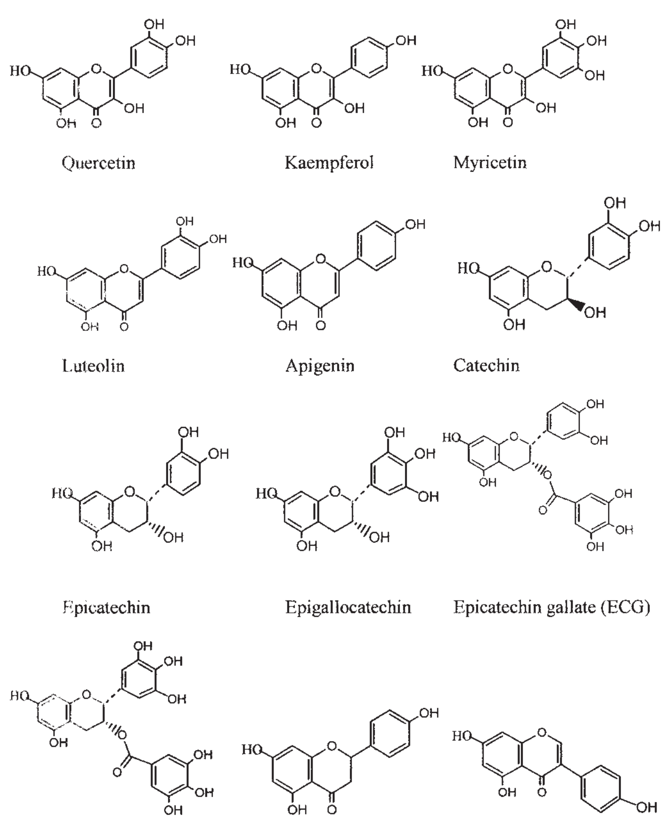
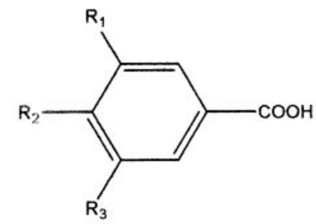


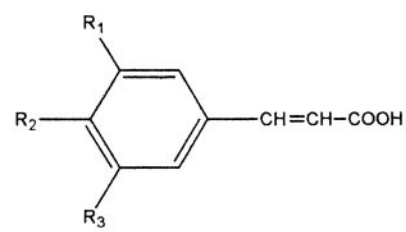
FIGURE 4 Chemical structures of common dietary flavonoids.

(a) Benzoic acid



Benzoic acid Derivatives	Substitutions		
	R_1	R_2	R_3
p-Hydroxybenzoic	H	OH	H
Protocatechuic	H	OH	OH
Vannilic	CH_3O	OH	H
Syringic	CH_3O	OH	CH_3O
Gallic	OH	OH	OH

(b) Cinnamic acid



Cinnamic acid Derivatives	Substitutions		
	R_1	R_2	R_3
p-Coumaric	H	OH	H
Caffeic	OH	OH	H
Ferulic	CH_3O	OH	H
Sinapic	CH_3O	OH	CH_3O

FIGURE 5 Structures of common phenolic acids: (*a*) benzoic acid and derivatives; (*b*) cinnamic acid and derivatives.

present in plants. Chlorogenic acids are the ester of caffeic acids and are the substrate for enzymatic oxidation leading to browning, particularly in apples and potatoes. Curcumin is made of two ferulic acids linked by a methylene in a diketone structure and is the major yellow pigment of mustard.

Carotenoids. Carotenoids are nature's most widespread pigments and have also received substantial attention because of both their provitamin and antioxidant roles. More than 600 different carotenoids have been identified in nature. They occur widely in plants, microorganisms, and animals. Carotenoids have a 40-carbon skeleton of isoprene units (**Fig. 6**). The structure may be cyclized at one or both ends, may have various hydrogenation levels, or may possess oxygen-containing functional groups. Lycopene and β-carotene are examples of acyclized and cyclized carotenoids, respectively. Carotenoid compounds most commonly occur in nature in the all-*trans*

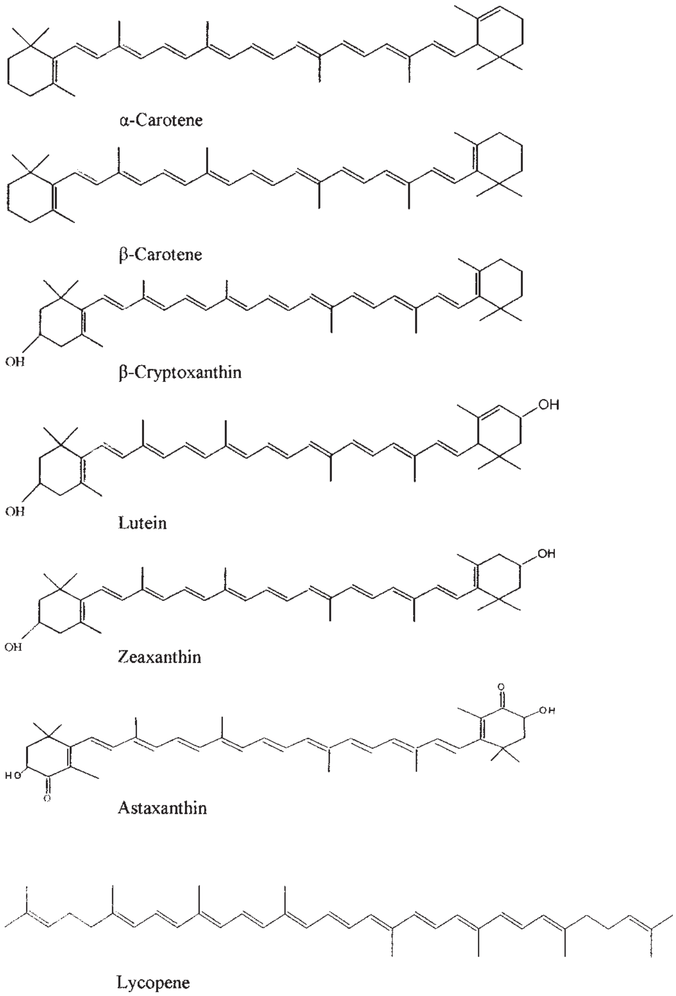


FIGURE 6 Chemical structures of common dietary carotenoids.

form. The most characteristic feature of carotenoids is the long series of conjugated double bonds forming the central part of the molecule. This gives them their shape, chemical reactivity, and light-absorbing properties. β-Carotene, α-carotene, and β-cryptoxanthin are able to function as provitamin A. Zeaxanthin and lutein are the major carotenoids in the macular region (yellow spot) of the retina in humans.

Orange vegetables and fruits, including carrots, sweet potatoes, winter squash, pumpkin, papaya, mango, and cantaloupe, are rich sources of the carotenoid β-carotene. Tomatoes, watermelons, pink grapefruits, apricots, and pink guavas are the most common sources of lycopene; 85% of American lycopene intake comes from processed tomato products such as ketchup, tomato paste, and tomato soup.

Carotenoid pigments play important functions in photosynthesis and photoprotection in plant tissues. The photoprotection role of carotenoids originates from their ability to quench and to inactivate reactive oxygen species such as singlet oxygen formed from exposure of light and air. This photoprotection role is also associated with its antioxidant activity in human health. Carotenoids can react with free radicals and become radicals themselves. Their reactivity depends on the length of the chain of conjugated double bonds and the characteristics of the end groups. Carotenoid radicals are stable by virtue of the delocalization of the unpaired electron over the conjugated polyene chain of the molecules. This delocalization also allows addition reactions to occur at many sites on the radical (18). Astaxanthin, zeaxanthin, and lutein are excellent lipid-soluble antioxidants that scavenge free radicals, especially in a lipid-soluble environment. Carotenoids at sufficient concentrations can prevent lipid oxidation and related oxidative stress.

Role of phytochemicals in the prevention of cancer

Cells in humans and other organisms are constantly exposed to a variety of oxidizing agents, some of which are necessary for life. These agents may be present in air, food, and water, or they may be produced by metabolic activity within cells. The key factor is to maintain a balance between oxidants and antioxidants to sustain optimal physiological conditions. Overproduction of oxidants can cause an imbalance, leading to oxidative stress, especially in chronic bacterial, viral, and parasitic infections (19). Oxidative stress can cause oxidative damage to large biomolecules such as lipids, proteins, and DNA, resulting in an increased risk for cancer and CVD (9,19,20). To prevent or slow the oxidative stress induced by free radicals, sufficient amounts of antioxidants need to be consumed. Fruits, vegetables, and whole grains contain a wide variety of antioxidant compounds (phytochemicals), such as phenolics and carotenoids, and may help protect cellular systems from oxidative damage and also may lower the risk of chronic diseases (10,11,16,21–23).

Strong epidemiological evidence suggests that regular consumption of fruits and vegetables can reduce cancer risk. Block et al. (24) reviewed ~200 epidemiological studies that examined the relationship between intake of fruits and vegetables and cancer of the lung, colon, breast, cervix, esophagus, oral cavity, stomach, bladder, pancreas, and ovary. In 128 of 156 dietary studies, the consumption of fruits and vegetables was found to have a significant protective effect. The risk of cancer was 2-fold higher in persons with a low intake of fruits and vegetables than in those with a high intake. Significant protection was found in 24 of 25 studies for lung cancer. Fruits were significantly protective in cancer of the esophagus, oral cavity, and larynx. Fruits and vegetable intake was protective for cancer of the pancreas and stomach in 26 of 30 studies and for colorectal and bladder cancer in 23 of 38 studies. A prospective study involving 9959 men and women in Finland showed an inverse association between the intake of flavonoids and incidence of cancer at all sites combined (25). After a 24-y follow-up, the risk of lung cancer was reduced by 50% in the highest quartile of flavonol intake. Consumption of quercetin from onions and apples was found to be inversely associated with lung cancer risk (26). The effect of onions was particularly strong against squamous-cell carcinoma. Boyle et al. (27) showed that increased plasma levels of quercetin after a meal of onions was accompanied by increased resistance to strand breakage by lymphocyte DNA and decreased levels of some oxidative metabolites in the urine.

Carcinogenesis is a multistep process, and oxidative damage is linked to the formation of tumors through several mechanisms (19,20). Oxidative stress induced by free radicals causes DNA damage, which, when left unrepaired, can lead to base mutation, single- and double-strand breaks, DNA cross-linking, and chromosomal breakage and rearrangement (20). This potentially cancer-inducing oxidative damage might be prevented or limited by dietary antioxidants found in fruits and vegetables. Studies to date have demonstrated that phytochemicals in common fruits and vegetables can have complementary and overlapping mechanisms of action (**Table 1**), including antioxidant activity and scavenging free radicals; regulation of gene expression in cell proliferation, cell differ-

TABLE 1

Proposed mechanisms by which dietary phytochemicals may prevent cancer

Antioxidant activity
Scavenge free radicals and reduce oxidative stress
Inhibition of cell proliferation
Induction of cell differentiation
Inhibition of oncogene expression
Induction of tumor suppress gene expression
Induction of cell-cycle arrest
Induction of apoptosis
Inhibition of signal transduction pathways
Enzyme induction and enhancing detoxification
Phase II enzyme
Glutathione peroxidase
Catalase
Superoxide dismutase
Enzyme inhibition
Phase I enzyme (block activation of carcinogens)
Cyclooxygenase-2
Inducible nitric oxide synthase
Xanthine oxide
Enhancement of immune functions and surveillance
Antiangiogenesis
Inhibition of cell adhesion and invasion
Inhibition of nitrosation and nitration
Prevention of DNA binding
Regulation of steroid hormone metabolism
Regulation of estrogen metabolism
Antibacterial and antiviral effects

entiation, oncogenes, and tumor suppressor genes; induction of cell-cycle arrest and apoptosis; modulation of enzyme activities in detoxification, oxidation, and reduction; stimulation of the immune system; regulation of hormone metabolism; and antibacterial and antiviral effects (10,11,28,29).

Health benefits of phytochemicals in whole foods—food synergy

The hypothesis that dietary antioxidants lower the risk of chronic disease was developed from epidemiological studies. These have consistently shown that consumption of whole foods, such as fruits, vegetables, and whole grains, is strongly associated with reduced risk of chronic diseases, especially cancer and CVD. Therefore, it is reasonable for scientists to identify the bioactive compounds responsible and hope to find the magic bullet to prevent those chronic diseases. The key question here is whether a purified phytochemical has the same health benefit as the compound when its source is a food or a mixture of foods. It is now widely believed that the actions of the dietary supplements alone do not explain the observed health benefits of diets rich in fruits, vegetables, and whole grains, because, taken alone, the individual antioxidants studied in clinical trials do not appear to have consistent preventive effects (30–32). The isolated pure compound either loses its bioactivity or may not behave the same way as the compound in whole foods. For example, numerous investigations have shown that the risk of cancer is inversely related to the consumption of green and yellow vegetables and fruits. Because β-carotene is present in abundance in these vegetables and fruits, it has been extensively investigated as a possible cancer-preventive agent. However, the role of carotenoids as anticancer supplements has recently been questioned as a result of several clinical studies (30,33–35). In one study, the incidence of nonmelanoma skin cancer was unchanged in patients receiving a β-carotene supplement (33). In other studies, smokers gained no benefit from supplemental β-carotene with respect to lung cancer incidence and may even have suffered a significant increase in lung cancer and total mortality (30,35). In The Heart Outcomes Prevention Evaluation (HOPE) Study, patients at high risk for cardiovascular events were given 400 IU vitamin E or a placebo daily for 4.5 y. No difference was found in deaths from cardiovascular causes, myocardial infarctions, or deaths from CVD or strokes between the 2 groups (32). In the Cambridge Heart Antioxidant Study (CHAOS), patients with CVD were given 400 IU or 800 IU α-tocopherol or a placebo for a median of 510 d. α-Tocopherol intake was associated with a significantly reduced risk of myocardial infarction; however, it insignificantly increased risk of cardiovascular death (31). Vitamin E supplementation had no effect on the end points of death, myocardial infarction, or stroke for the patients who had recently suffered a myocardial infarction (36). Vitamin C supplements also failed to lower the incidence of cancer or CVD (37,38).

Phytochemical extracts from fruits and vegetables were recently shown to have potent antioxidant and antiproliferative effects, and the combination of phytochemicals from fruits and vegetables was proposed to be responsible for the potent antioxidant and anticancer activity of these foods (10,11,39). The total antioxidant activity of phytochemicals in 1 g apples with peel is equivalent to 83.3 μmol vitamin C equivalents; to put it another way, the antioxidant value of 100 g apples is equivalent to 1500 mg vitamin C (39). This is far higher than the total antioxidant activity of 0.057 mg vitamin C (the amount of vitamin C in 1 g apples with peel) that is equivalent to 0.32 μmol vitamin C equivalent. In other words, vitamin C in apples contributes <0.4% of its total antioxidant activity. Thus, most of the antioxidant activity comes from phytochemicals, not vitamin C. The natural combination of phytochemicals in fruits and vegetables is responsible for its potent antioxidant activity. Apple extracts also contain bioactive compounds that inhibit tumor cell growth in vitro. Phytochemicals in apples with peel (50 g/L on a wet basis) inhibit colon cancer cell proliferation by 43%. However, this was reduced to 29% when apple without peel was tested (39).

Different species and varieties of fruits, vegetables, and grains have different phytochemical profiles (10,11,16,23). The combination of orange, apple, grape, and blueberry displayed a synergistic effect in antioxidant activity (**Fig. 7**). The dose–response curve of antioxidant activity was shifted to the left after the combination of 4 fruits. The median effective dose (EC_{50}) of each fruit after combination was 5 times lower than the EC_{50} of each fruit alone, suggesting synergistic effects

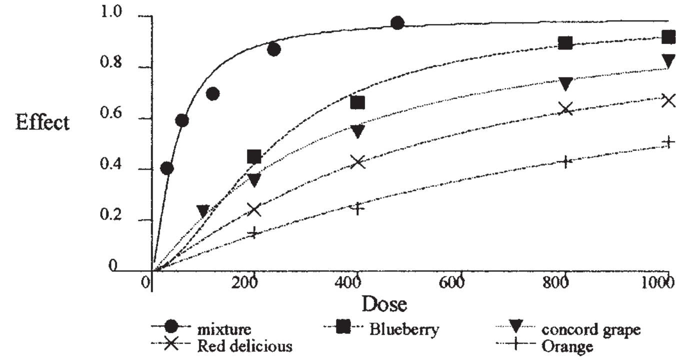


FIGURE 7 Dose–response of antioxidant activity of orange, apple, grape, blueberry and 4-way combination.

after the combination of the 4 fruits. Therefore, consumers should obtain their phytochemicals from a wide variety of fruits, vegetables, and whole grains for optimal health benefits. In 2003 Temple and Gladwin (40) reviewed > 200 cohort and case-control studies that provided risk ratios concerning intake of fruits and vegetables and risk of cancer. They concluded that cancer prevention is best achieved by consumption of a wide variety of fruits and vegetables, although one group of fruits and vegetables may dominate for a particular cancer. To improve their nutrition and health, consumers should be obtaining antioxidants from their diet and not from expensive dietary supplements, which do not contain the balanced combination of phytochemicals found in fruits and vegetables and other whole foods. More importantly, obtaining antioxidants from dietary intake by consuming a wide variety of foods is unlikely to result in consumption of toxic quantities, because foods originating from plants contain many diverse types of phytochemicals in various quantities. Fruits and vegetables eaten in the recommended amounts (5–10 servings of fruits and vegetables per day) are safe. Furthermore, health benefits from the consumption of fruits and vegetables extend beyond lowering the risk of developing cancers and CVD: benefits also include preventive effects on other chronic diseases such as cataracts, age-related macular degeneration, central neurodegenerative diseases, and diabetes.

The additive and synergistic effects of phytochemicals in fruits and vegetables have been proposed to be responsible for their potent antioxidant and anticancer activities. The benefit of a diet rich in fruits and vegetables is attributed to the complex mixture of phytochemicals present in these and other whole foods (10,11,39). This partially explains why no single antioxidant can replace the combination of natural phytochemicals in fruits and vegetables in achieving the observed health benefits. Thousands of phytochemicals are present in whole foods. These compounds differ in molecular size, polarity, and solubility, which may affect the bioavailability and distribution of each phytochemical in different macromolecules, subcellular organelles, cells, organs, and tissues. This balanced natural combination of phytochemicals present in fruits and vegetables cannot simply be mimicked by pills or tablets.

Research progress in antioxidant and bioactive compounds has boosted the dietary supplement and nutraceutical industries. The use of dietary supplements is growing, especially among baby-boomer consumers. However, many of these dietary supplements have been developed based on the research results derived from biochemical and chemical analyses and studies, in vitro cell culture studies, and in vivo animal experiments and not from human intervention studies. The health benefits of natural phytochemicals at the low levels present in fruits and vegetables does not mean that these compounds are more effective or safe when they are consumed at a higher dose, even in a pure dietary supplement form. Generally speaking, higher doses increase the risk of toxicity. The basic principle of toxicology is that any compound can be toxic if the dose is high enough, and dietary supplements are no exception. Therefore, a thorough understanding of the efficacy and the long-term safety of many dietary supplements needs further investigation.

It is also important to differentiate the pharmacological dose from the physiological (or nutritional) dose. Pharmacological doses are used clinically to treat specific diseases in certain situations and require a doctor's prescription; physiological (or nutritional) doses are used to improve or maintain optimal health, such as in dietary supplements or in foods. In the case of antioxidant nutrients, the proper physiological (or nutritional) dose should follow the recommended dietary allowances. The pharmacological dose is not equal to the physiological (or nutritional) dose and, in some cases, can be toxic for long-term use. Currently, there are no recommended dietary allowances for phytochemicals. Therefore, it is not wise to take megadoses of purified phytochemicals as dietary supplements before the appearance of strong supporting scientific evidence.

Summary

Dietary modification by increasing the consumption of a wide variety of fruits, vegetables, and whole grains daily is a practical strategy for consumers to optimize their health and to reduce the risk of chronic diseases. Use of dietary supplements, nutraceuticals, and functional foods is increasing as industry is responding to consumers' demands. However, more information about the health benefits and the possible risks of dietary supplements is needed to ensure their efficacy and safety. Phytochemical extracts from fruits and vegetables have strong antioxidant and antiproliferative activities, and the major part of total antioxidant activity is from the combination of phytochemicals. The additive and synergistic effects of phytochemicals in fruits and vegetables are responsible for their potent antioxidant and anticancer activities. The benefit of a diet rich in fruits, vegetables, and whole grains is attributed to the complex mixture of phytochemicals present in these and other whole foods. This explains why no single antioxidant can replace the combination of natural phytochemicals in fruits and vegetables and achieve their health benefits. Therefore, the evidence suggests that antioxidants are best acquired through whole food consumption, not from expensive dietary supplements. Further research on the health benefits of phytochemicals in whole foods is warranted.

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