DOI 10.1002/mnfr.200600279 Mol. Nutr. Food Res. 2007, *51*, 652–664

Review

# Cranberry and blueberry: Evidence for protective effects against cancer and vascular diseases

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Growing evidence from tissue culture, animal, and clinical models suggests that the flavonoid-rich fruits of the North American cranberry and blueberry (*Vaccinium* spp.) have the potential ability to limit the development and severity of certain cancers and vascular diseases including atherosclerosis, ischemic stroke, and neurodegenerative diseases of aging. The fruits contain a variety of phytochemicals that could contribute to these protective effects, including flavonoids such as anthocyanins, flavonols, and proanthocyanidins; substituted cinnamic acids and stilbenes; and triterpenoids such as ursolic acid and its esters. Cranberry and blueberry constituents are likely to act by mechanisms that counteract oxidative stress, decrease inflammation, and modulate macromolecular interactions and expression of genes associated with disease processes. The evidence suggests a potential role for dietary cranberry and blueberry in the prevention of cancer and vascular diseases, justifying further research to determine how the bioavailability and metabolism of berry phytonutrients influence their activity *in vivo*.

**Keywords:** Antioxidant / Blueberry / Cancer / Cranberry / Vascular disease

Received: December 15, 2006; revised: January 22, 2007; accepted: January 24, 2007

## 1 Introduction

This review examines studies exploring the possible health benefits of North American cranberry and blueberry fruit, emphasizing emerging evidence for their potential to ameliorate the effects of cancers and vascular diseases. North American cranberries and blueberries both wild and cultivated belong to the *Vaccinium* genus and are grown throughout the Eastern and Northeastern U.S., Pacific Northwest, Wisconsin, Michigan, and much of Canada including British Columbia, parts of Quebec and the Maritimes. Other members of the *Vaccinium* genus widely grown in Europe such as the bilberry or "European blueberry" (*Vaccinium myrtillus*) have been reviewed elsewhere [1, 2] and will not be addressed here, though many similarities exist between fruits in the *Vaccinium* genus with regard to phytochemical composition and biological effects.

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**Abbreviations: COX,** cyclooxygenase; **DP,** degrees of polymerization; **GAG,** glycosaminoglycan; **LDL,** low-density lipoprotein; **MMP,** matrix metalloproteinase; **ODC,** ornithine decarboxylase; **ORAC,** oxygen radical absorbance capacity; **PAC,** proanthocyanidin; **PPARα,** peroxisome proliferator activated receptor alpha; **QR,** quinone reductase; **ROS,** reactive oxygen species; **TNF-α,** tumor necrosis factor alpha; **VEGF,** vascular endothelial growth factor

## 2 Antioxidant activity of cranberries and blueberries

Cranberries and blueberries rank highly among fruits for both antioxidant quality and quantity [3], properties that can be attributed to the substantial content of flavonoids, tannins, and other phenolic acids in both fruits. The antioxidant properties of the phenolics in cranberry and blueberry fruit are likely to play a major role in their observed ability to decrease damage related to cardiovascular disease and aging as well as some of their reported antitumor activities. Some of the observed effects of cranberry linked to the prevention of oxidative processes include reduced lipoprotein oxidation [4, 5], and decreased oxidative damage in models of stroke [6] and vascular diseases of aging [7]. In addition to reducing the damage due to ischemic stroke [8], dietary blueberry has been linked to improved cognitive performance as a result of decreased oxidative stress in the brain [9] as well as increased serum antioxidant capacity [10]. These

and other effects on disease-linked processes will be considered in more detail in the sections that follow.

## 3 Phytochemical composition

The fruit of the North American Cranberry (*Vaccinium macrocarpon* Aiton) has a diverse phytochemical profile including three classes of flavonoids (flavonols, anthocyanins, and proanthocyanidins (PACs)); catechins; a variety of phenolic acids, among which the major is *p*-hydroxycinnamic acid; and triterpenoids of the ursane type. The major anthocyanins in cranberry, shown in Fig. 1, are galactosides and arabinosides of cyanidin and peonidin [11]. Anthocyanin content varies widely among cranberry cultivars, which averages between 25–65 mg *per* 100 g of ripe fruit at harvest [12]. Fruit of the Early Black cultivar is significantly higher in contents of both anthocyanins and PACs than other cranberry cultivars [13]. The primary flavonols in cranberries are shown in Fig. 2. Quercetin exists in several glycosidic forms with quercetin galactoside the most plentiful [4]. Myricetin galactoside and arabinoside are also found in the fruit. Cranberries are one of the leading fruit sources of quercetin, with the total flavonol content of cranberry fruit typically in the range of 20–30 mg *per* 100 g of fresh fruit weight.

Blueberry species common to North America include the lowbush or "wild" blueberry (*Vaccinium angustifolium* Aiton), the highbush or "cultivated" blueberry (*Vaccinium corymbosum* L.) grown primarily in the more temperate climates, and the rabbiteye blueberry (*Vaccinium ashei*) common in the southern US. Blueberries are ranked very highly among fruits and vegetables for their antioxidant capacity, with oxygen radical absorbance capacity (ORAC) values ranging between 14–45.9 μmol/g depending on variety [14]. Lowbush blueberries encompass many different wild genotypes, which on average contain higher levels of anthocyanins, total phenolics, and ORAC antioxidant capacity than cultivated highbush genotypes [15]. Blueberries are rich in chlorogenic acid. They also contain glycosides of anthocyanins petunidin, malvidin, delphinidin, peonidin, and cyanidin (Fig. 1), which are by far the most plentiful flavonoids. Reported anthocyanin content in highbush blueberries ranges from 120 to 208 mg *per* 100 g of fresh fruit [16–18]. Other polyphenolics include flavonol glycosides (Fig. 2) and catechins. A study of the oxygen radical scavenging capacity of highbush blueberries and other berries found that the eleven anthocyanins occurring in highbush blueberry accounted for over 50% of this activity [16].

Because of their superior antioxidant efficacy, the anthocyanins found in cranberries and blueberries may be expected to play a major role in the inhibition of oxidative processes linked to vascular diseases and cancer. Both cranberries and blueberries are also quite rich in PACs. The USDA database for PAC content of selected foods published in 2004 (http:/www.nal.usda.gov/fnic/foodcomp) reports that 100 g of cranberry fruit contains on average 45 mg of tannin dimers and trimers, 133 mg of oligomers in the range of 4–10 degrees of polymerization (DP) and nearly twice this mass of large polymers. Cranberries had the highest average PAC content of all fruit reported in the database, including apples, cherries, grapes, pears, peaches, currants, dates, blackberries, chokeberries, strawberries, and plums. For blueberries, the numbers are somewhat lower but they still appear among the top five. Lowbush (wild) blueberries are higher in PAC content, with 100 g of

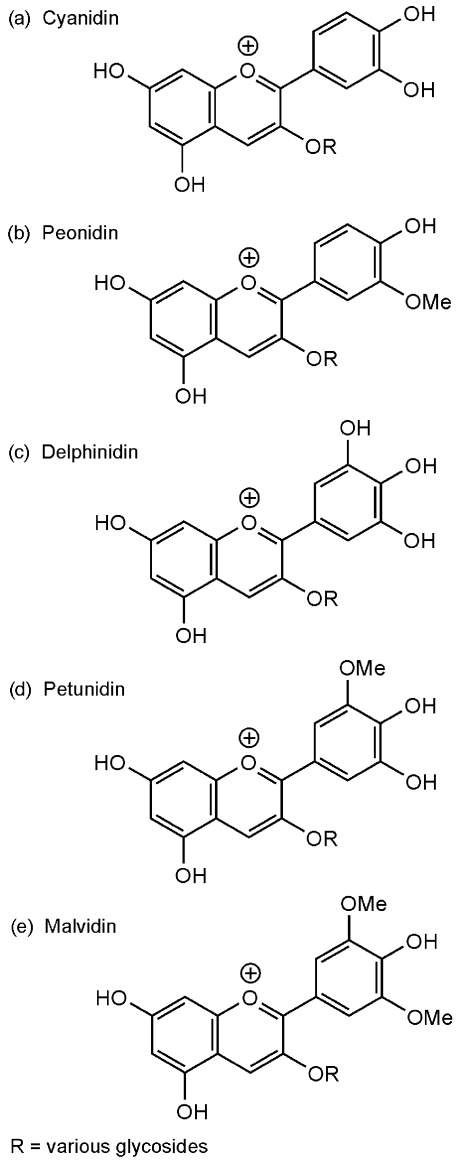


**Figure 1.** Anthocyanin glycosides in cranberry and blueberry fruit. Aglycones (a) and (b) occur in cranberries as both galactosides and arabinosides. Aglycones (a) through (e) occur in blueberries, mainly as glucosides, galactosides, or arabinosides.

 

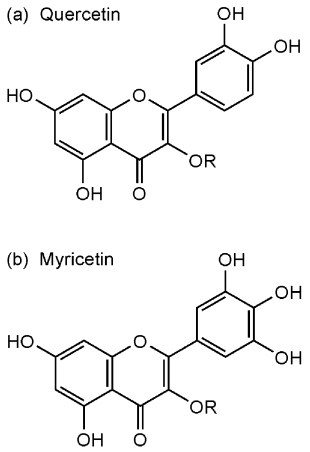


**Figure 2.** Flavonol glycosides in cranberry and blueberry fruit. The more plentiful flavonol quercetin (a) is found primarily as galactosides and arabinosides, but rhamnosides, xylosides, glucosides, and diglycosides have also been reported in cranberries.

fruit averaging 15 mg of dimers and trimers and 55 mg of oligomers in the 4–10 DP range. 100 g of highbush (cultivated) blueberries averaged around 10 mg dimers/trimers and 34 mg of PAC oligomers.

## 4 Evidence of anticancer activity and identification of active constituents

*In vitro* studies demonstrate that *Vaccinium* fruit extracts can prevent or decrease a number of processes involved in carcinogenesis. The anticancer properties of *Vaccinium* fruit have been the subject of investigation since the late 1990's. Several groups of phytochemicals plentiful in fruits of the *Vaccinium* genus can be expected to have an impact on cancer-related processes, particularly the flavonoids and the triterpenoids. The earliest report of potential anticancer activity appeared in 1996, in a study of several *Vaccinium* species. Extracts of cranberry, bilberry, and other fruits were observed to both inhibit ornithine decarboxylase (ODC) expression and induce the xenobiotic detoxification enzyme quinone reductase (QR) *in vitro* [19]. Early studies of cranberry effects on cellular models focused primarily on breast cancer. A Canadian study reporting that cranberry juice inhibited breast tumor growth appeared in 2000 [20]. This was followed up in 2004 by a study reporting that an extract of cranberry presscake inhibited proliferation of MCF-7 and MDA-MB-435 breast cancer cells [21].

In a 2000 study, lowbush (wild) blueberry fruits were evaluated for their antioxidant activity and ability to inhibit the initiation stage of chemically induced carcinogenesis based on induction of QR in Hepa 1c1c7 cells. A crude 70% acetone extract induced QR in a dose-dependent manner [22], suggesting potential anticarcinogenic activity.

More recently, *in vitro* studies have focused on identifying some of the phytochemical constituents responsible for the observed anticancer activity of these fruits. Many of the compounds in these fruits are likely contributors, including the flavonols, anthocyanins, PACs, catechins, various phenolic acids, triterpenoids, even stilbenes, although these are present in lesser quantities than the other constituents. The possible contributions of each of these subclasses of compounds will be considered.

### 4.1 Ursolic acid and related triterpenoids

Fractionation of whole fruit extracts makes it possible to examine the activities of each class of compounds or individual compounds separately in comparison to that of the whole extract and thus determine the relative contribution of the individual phytochemical's bioactivity. Prior to our interest in cranberry phytochemicals, we developed a bioactivity-guided fractionation approach to identification of antitumorigenic compounds from medicinal plants, for example ursane-type triterpenoids from plants used in traditional Peruvian medicine [23]. We employed a tumor growth inhibition assay developed by the National Cancer Institute [24] for the initial screening of inhibitory activity against several tumor cell lines. This approach was used to examine activities of not only whole cranberry fruit and juice extracts but also individual compounds and groups of compounds.

Using this approach, we determined that an ethyl-acetate extract of whole cranberry fruit inhibited growth of several tumor cell lines [4], and we subsequently isolated from this extract two phenolic esters of the pentacyclic triterpenoid ursolic acid, shown in Fig. 3. The esters, *cis* and *trans*-3-*O*-*p*-hydroxycinnamoyl ursolic acid, inhibited the growth of several types of tumor cells *in vitro* at micromolar concentrations [25]; particularly MCF-7 breast and also HT-29 colon, DU-145 prostate, H460 lung, ME180 cervical, and K562 leukemia cell lines. Quantitative analysis by LC-MS of cranberry fruit of different cultivars and a variety of cranberry products has shown that the ursolic acid content of whole cranberry fruit of different cultivars is substantial and comparable to the flavonoids, ranging from 60–110 mg *per* 100 g of fresh fruit [26] with a similar content found in sweetened, dried fruit. The hydroxycinnamate esters are present in whole cranberry fruit in quantities averaging about 15 mg *per* 100 g of fresh fruit [26].

Highbush blueberries also contain some ursolic acid in the peel. In a systematic study identifying the ethyl-acetate soluble constituents of highbush blueberry fruit, several sterols and triterpenoids, as well as phenolic acids were isolated [27]. Ursolic acid and its 19-hydroxy derivative pomolic acid (Fig. 3), both isolated from blueberry fruit, were reported to inhibit proliferation and DNA synthesis in the HL-60 leukemia cell line at μM concentrations [27]. A study of triterpenes and sterols isolated from the fruit of

 

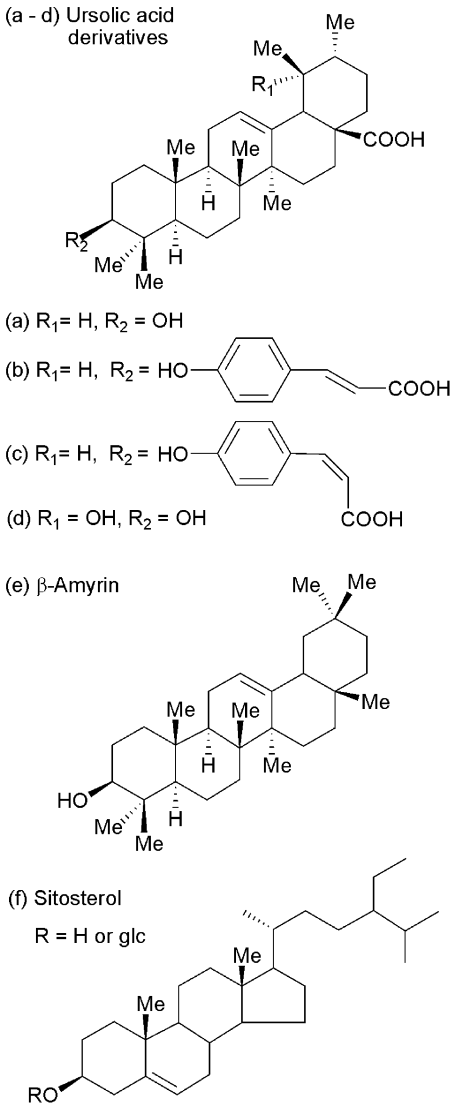


**Figure 3.** Triterpenoids and sterols in cranberry and blueberry fruit. (a), (b), (c), and (f) have been reported in cranberries; (a), (d), (e), and (f) have been reported in blueberries.

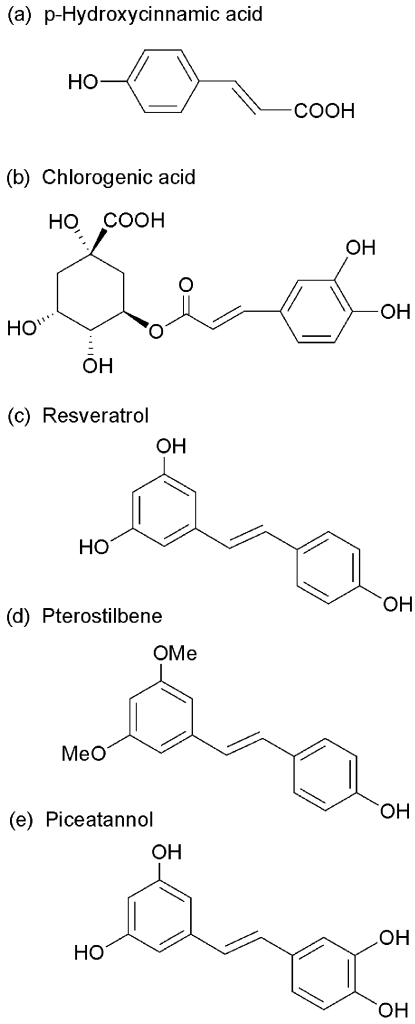


**Figure 4.** Phenolic acids and stilbenes occurring in cranberry and blueberry fruit. (a) is the major phenolic acid in cranberries; (b) is the major phenolic acid in blueberries. Stilbene (c) has been detected in both fruits; stilbenes (d) and (e) have been reported in blueberries.

"rabbiteye" blueberry (*V. ashei*) also found that ursolic acid, β-amyrin, and a glucoside of β-sitosterol inhibited the growth of HCT 116 human colon cancer cells and PC-12 adrenal pheochromocytoma cells at micromolar concentrations [28].

### 4.2 Stilbenes

Resveratrol, shown in Fig. 4, is a stilbene produced as a phytoalexin in grapes and found in red wine. Resveratrol has garnered considerable attention as an antitumor agent [29], as a likely contributor to the famed French Paradox [30] of cardiovascular disease, and more recently as a potential antiaging agent [31]. Studies of resveratrol's effects on cancer cells suggest that the compound can enhance sensitivity to tumor necrosis factor alpha (TNF-α), reducing NF-κB activation and thereby boosting apoptosis in those cells [32]. Blueberries of different varieties belonging to the *V. corymbosum*, *V. ashei*, and *V. angustifolium* species reportedly contain up to 860 ng of resveratrol *per* gram dry weight of fruit [33]. The same study found 900 ng of resveratrol *per* g dry weight in a sample of cranberry fruit from Nova Scotia (*V. macrocarpon*). Two analogs of resveratrol, pterostilbene and piceatannol (Fig. 4), were detected in rabbiteye (pterostilbene) and highbush (piceatannol) blueberries, in quantities ranging from 100–420 ng *per* gram fruit. Both pterostilbene and piceatannol have also been demonstrated to possess anticarcinogenic properties similar or superior to resveratrol [34, 35]. It has not yet been shown that the quantities present in blueberry fruit are

sufficient to induce beneficial effects, but it is hypothesized that they may act synergistically with other berry phytochemicals. An interesting associative effect of pterostilbene and the flavonol quercetin was demonstrated in a study which found that the combination inhibited growth and metastasis of B16M-F10 melanoma cells both *in vitro* and in mice, each acting by complementary pathways [36], suggesting possible synergistic effects from consumption of fruits containing both of these compounds.

### 4.3 Quercetin and its glycosides

Flavonoids from cranberries and blueberries, like those from other food sources can be expected to play a role in chemoprevention and may act synergistically with each other and with other phytochemicals. Each of the major flavonoid subclasses in the fruit has the potential to limit tumorigenesis. Of the flavonoids occurring in *Vaccinium* berries, the flavonol quercetin is perhaps the most well studied due to its occurrence in many other fruits and some vegetables. There are numerous reports of quercetin's ability to inhibit proliferation of cancer cell lines *in vitro*, including breast, colon, pancreas, and leukemia [37, 38]. Its mechanisms of chemopreventive action include induction of apoptosis, observed in HepG2 hepatoma and colorectal cells, with arrest of the HepG2 cell cycle in $G_1$ phase [38–40]; inhibition of epidermal growth factor (EGF) receptor expression and associated tyrosine kinase activity [37, 40]; reduced expression of Ras protein in colon cancer cells and primary colorectal tumors [41]; and increased expression of endogenous inhibitors of matrix metalloproteinases [42].

Quercetin is the major flavonol in cranberry fruit, present chiefly as the 3-*O*-galactoside, and is thus likely to be a significant contributor to cranberry's antitumor properties [43]. Tumor growth inhibition assays in our laboratories found that quercetin inhibited the growth of MCF-7 human breast adenocarcinoma, HT-29 human colon adenocarcinoma, and K562 human chronic myelogenous leukemia cell lines, with $GI_{50}$ in the range of 15–60 μg/mL; a level of activity similar to that of the ursolic acids [25].

### 4.4 PACs: Structure and activity

The role of PACs or condensed tannins in chemoprevention by food-based products is an area of growing interest. Grape seed PACs are perhaps the most well studied of these. Recently, grape seed PACs were reported to inhibit proliferation of a highly metastatic mouse mammary carcinoma cell line (4T1) both *in vitro* and in a mouse model [44]. There is growing evidence to suggest that condensed tannins may play a role in chemoprevention by *Vaccinium* fruit as well.

In 2002, a University of Illinois study revealed that extracts of whole cranberry containing PACs and other flavonoids inhibited ODC activity in mouse epithelial (ME-308) cells [45]. A subfraction also inhibited ODC in this cell line. Characterization of the active fraction revealed the presence of dimers and oligomers of catechin/epicatechin, monomeric catechins, and quercetin glycosides. ODC has an important role in the biosynthesis of polyamines involved in cellular proliferation.

A recent report from UCLA indicated that cranberry phenolic extracts prepared from water-soluble extracts of commercial cranberry powder effectively inhibited proliferation of several human tumor cell lines [46]. A total polyphenol extract containing a variety of flavonoids inhibited proliferation of two oral cancer cell lines (CAL27 and KB), four colon cancer cell lines (HT-29, HCT-116, SW480, and SW620), and three prostate cancer cell lines (RWPE-1, RWPE-2, and 22Rv1). In this study, anthocyanin and PAC subfractions were not as effective in the oral and colon cell lines as the total polyphenolic extract, but showed strong inhibition in the prostate cell lines. Studies demonstrating antiproliferative activity of cranberry extracts in MCF-7 and MDA-MB-435 breast cancer lines [21] implicate PACs as contributing to these activities.

A PAC fraction isolated from whole cranberry fruit was observed by us to selectively inhibit the growth of H460 human large cell lung carcinoma, HT-29 colon adenocarcinoma, and K562 chronic myelogenous leukemia cells in an eight tumor cell line panel. A subfraction retaining the activity of the parent fraction with some improvement in those three cell lines was isolated and characterized by us using MALDI-TOF-MS. The PAC subfraction contained oligomers composed primarily of four to seven epicatechin units with at least one or two A-type linkages between the units [47]. A representative structure of a cranberry tetramer is shown in Fig. 5.

In general, PACs or condensed tannins from different food sources vary in structure and composition, featuring various flavan-3-ols (catechin, epicatechin, and galloylated catechins) linked together in different ways. The PACs found in cranberry fruit are primarily dimers, trimers, and larger oligomers of epicatechin. These molecules may contain two types of linkages between epicatechin units. The B-type ($4\beta \rightarrow 8$) linkage is also found widely in PACs from sources other than *Vaccinium* fruit (apples, grape seed, cacao). Cranberry PACs also have a significant occurrence of the A-type linkage, which features two linkage sites between the units: $4\beta \rightarrow 8$ and $2\beta \rightarrow O \rightarrow 7$ interflavanoid bonds. Therefore, three-dimensional structures within this group of molecules are diverse. Even among the smaller PACs such as PACs from cranberry juice that inhibit adherence of P-fimbriated *E. coli*, at least three different trimer structures exist [48]. Our MALDI-TOF MS analysis of PACs from whole cranberry fruit with tumor antiproliferative activity showed the presence of oligomers of up to 12 DP and as many as four A-type linkages, most with exclusively epicatechin units but some with epigallocatechin unit masses detected [47].

 

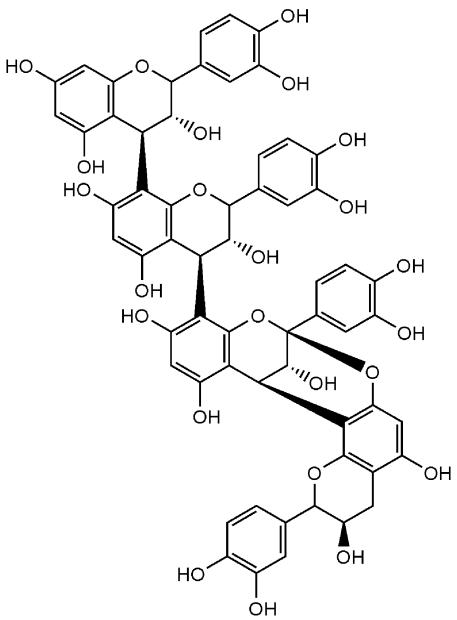

**Figure 5.** An A-linked PAC tetramer composed of epicatechin units is representative of the condensed tannin oligomers occurring in cranberry fruits.

Further exploration of fractions from wild blueberries with QR-inducing activity [22] showed that compounds in the fruit were active in models of various stages of carcinogenesis. The blueberry phytosterols fraction inhibited initiation based on QR induction; a fraction rich in anthocyanins and flavan-3-ols inhibited promotion by ODC and cyclooxygenase (COX), and proliferation of hepatocytes was limited by a fraction containing PACs [49]. Wild and cultivated blueberry PAC fractions also demonstrated antiproliferative effects on two models of prostate cancer: an androgen-sensitive cell line (LNCaP) and a more aggressive androgen-insensitive cell line (DU145) [50]. Growth inhibition was more pronounced in the androgen-sensitive cell line, with significant inhibition of LNCaP at 20 μg/mL, suggesting potential protection against early-stage prostate cancer.

The structure of PACs in wild blueberries was determined by mass spectral and NMR methods in a study investigating antiproliferative and antiadhesion properties of PAC-rich fractions. Antiproliferative activity against LNCaP prostate and Hepa1c1c7 liver cancer cell lines was most pronounced in fractions containing an average DP of 5.65 [51]. Oligomers ranged in size from 3 to 8 DP in fractions obtained in the study. Fractions with lower DP exhibited bacterial antiadhesion activity but little tumor inhibition. The authors suggest that $^{1}$H NMR data from this study indicate a small percentage of PACs with A-type linkages in blueberries, compared to the percentage of A-type typically found in cranberries.

### 4.5 Anthocyanins

The potential chemopreventive mechanisms of anthocyanins have been summarized elsewhere [52] and include effects on signal transduction, apoptosis, epidermal growth factor receptor, and COX activity in addition to antioxidative properties. Anthocyanins have been implicated in the observed antiangiogenic properties of extracts prepared from a mixture of berries including both cranberries and blueberries [53, 54]. Mixed anthocyanin-rich extracts inhibited the induction of vascular endothelial growth factor (VEGF) by both hydrogen peroxide and tumor necrosis factor (TNF-α) which suggests that the antioxidant and anti-inflammatory properties of these compounds may act to limit angiogenesis. Endothelioma cells treated with this extract also exhibited decreased hemangioma formation and tumor growth [55].

A study of whole polyphenolic extracts and subfractions prepared from three rabbiteye blueberry varieties (*V. ashei*) found that the anthocyanin and tannin subfractions of the blueberry polyphenolics inhibited proliferation of HT-29 and Caco-2 human colon cancer cells at concentrations ranging from 15 to 50 μg/mL for the anthocyanins and 70 to 100 μg/mL for the tannins. The most plentiful anthocyanins in these fruits by weight are delphinidin, petunidin, and malvidin. DNA fragmentation experiments indicated that treatment with the anthocyanin fractions significantly increased apoptosis levels in both cell lines [56]. Further studies by the same group on the absorption of blueberry anthocyanins by human intestinal cells found a transport efficiency averaging 3–4% across Caco-2 monolayers, a lower efficiency than those exhibited by more lipophilic flavonoids, but an indication that a portion of the anthocyanins could be absorbed in the colon [57]. Another study in which MCF-7 breast and HT-29 colon cancer cells were treated with ethanol/water extracts of ten fruits found blueberry as effective as the other fruits, though its vitamin C and carotenoid contents were relatively low [58]. The authors suggest that the antiproliferative activity may be due to other compounds in blueberry fruit. In this study, the anthocyanin extracts were less effective than the whole extract. However, studies of anthocyanin-rich extracts of other fruits including bilberry report growth inhibition of HT-29 cells at extract concentrations of 10–75 μg/mL [59]. A recent study of six anthocyanin-rich fruits at the University of California, Los Angeles (UCLA) including blueberries and cranberries found that aqueous methanol berry extracts inhibited proliferation of human oral, prostate, breast, and colon cancer cell lines in a dose-dependent manner [60].

### 4.6 Possible mechanisms of anticancer activity

The mechanisms of anticancer activity by cranberry and blueberry are still in the early stages of investigation, though much can be inferred from studies of individual

 

phytochemicals found in these fruits. Tumor inhibition by cranberry is likely to involve synergistic activities between the cranberry phytochemicals discussed above including the flavonols (mainly quercetin), PACs, anthocyanins, and ursolic acid. Some possible mechanisms of action for which *in vitro* evidence exists include induction of apoptosis in cancer cells, reduction of invasion and metastasis by inhibition of matrix metalloproteinases, inhibition of ODC expression and activity, inhibition of angiogenesis, and inhibition of inflammatory processes including COX activity. Similar mechanisms have been proposed for blueberry phytochemicals.

Apoptosis may play a key role in cranberry's antiproliferative activity; an event which may be induced in tumor cells by quercetin and other compounds in the fruit. *In vitro* studies employing breast tumor models treated with cranberry extract have reported dose-dependent induction of apoptosis coupled with cell cycle arrest. An antiproliferative fraction from cranberry presscake induced apoptosis in MDA-MB-435 breast tumor cells as determined by Annexin-V staining [21], with cells arrested in both G1 and G2 phase. An 80% aqueous acetone extract of whole cranberry fruit increased apoptosis in MCF-7 cells by 25% at 50 mg/mL concentration with significant arrest in the G1 phase [61].

We used a fluorescent TUNEL assay method (Terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling) to evaluate the ability of whole polyphenolic cranberry extract to induce apoptosis in tumorigenic (MCF-7) *versus* nontumorigenic (MCF-10A) breast cell lines. At the highest concentration tested (250 μg/mL) the cranberry extract increased the rate of apoptosis to 92% in MCF-7 cells, while no significant increase in baseline apoptosis rates was observed in MCF10A cells (Griffin *et al.*, unpublished results; presented at the American Society for Cell Biology Conference, 2005). In the UCLA study of six anthocyanin-rich fruits, blueberry extract was among those found to increase the percentage of cells undergoing apoptosis in the HT-29 colon cancer cell line, an activity the authors suggest may be due to anthocyanin content. The cranberry extract inhibited tumor proliferation, but did not increase apoptosis compared to the control in this model [60].

PACs and other flavonoids from cranberries and blueberries demonstrate some promise toward limiting processes involved in tumor invasion and metastasis, including blocking the expression of matrix metalloproteinases (MMPs), which are involved in remodeling of the extracellular matrix [62]. We found that whole cranberry polyphenolic extract at 100 μg/mL inhibits the expression of matrix metalloproteinases MMP-2 and MMP-9 in the DU-145 prostate tumor cell line. A cranberry PAC fraction also showed MMP inhibition in the DU-145 cell line, although its activity was slightly less than that of the whole fruit extract [47], suggesting that other flavonoids in the fruit also contribute to the observed MMP inhibition. Purified ursolic acid hydroxycinnamate esters from cranberry fruit were also evaluated by us and found to strongly inhibit expression of both MMP-2 and MMP-9 at micromolar concentrations [63].

Wild blueberry extracts exhibit similar potential to interfere with metastasis-linked events. A flavonoid-rich extract of highbush blueberry (*V. angustifolium*) inhibited expression of MMP-2 and MMP-9 in the DU-145 prostate tumor model [64]. This activity was attributed in large part to the PACs, based on activity of a PAC fraction compared to crude and anthocyanin-enriched fractions. Treatment with blueberry fractions also increased expression of tissue inhibitors of metalloproteinase expression (TIMP-1). The activity of blueberry anthocyanins was affected by protein kinase C (PKC) and mitogen-activated protein (MAP) kinase inhibitors, suggesting involvement of these pathways in the observed down-regulation of MMPs [65].

## 5 Berries and vascular disease

The phenolic antioxidants in cranberry and blueberry fruits may play an important role in inhibiting events that occur in the progression of cardiovascular diseases including atherosclerosis and stroke as well as some neurodegenerative diseases of aging; primarily by reducing oxidation of lipoproteins, improving serum antioxidant status and lipid levels, and mitigating the effects of oxidative stress and inflammation on the vascular system.

### 5.1 Atherosclerosis and associated events

Atherosclerosis is a condition affecting the coronary arteries in which gradual uptake of oxidized lipoproteins by the endothelium and the resulting inflammatory response leads to deposition of plaques in the arterial walls and eventual restriction of blood flow which can aggravate or produce hypertension and eventually cause irreparable damage to the heart. It is a complex process of events and for many years cardiovascular disease has been a leading cause of death in the US and UK. Emerging scientific evidence has indicated that consumption of a diet rich in foods and beverages containing flavonoids may decrease the risk of developing atherosclerosis, due to the ability of these compounds to inhibit low-density lipoprotein (LDL) oxidation, platelet aggregation and adhesion, and inflammatory response of the vascular tissues, while also inducing endothelium-dependent vasodilation [66].

The accumulation of oxidatively modified LDLs in the intima and their uptake by macrophages are early events in atherosclerosis that could be lessened by the presence of antioxidant species that reduce initial damage to LDLs and counteract the effects of oxidative enzymes produced by the macrophages [66]. Cranberry compounds have been demonstrated through *in vitro* models to inhibit the oxidation of lipoproteins. A University of Wisconsin study reported

 

inhibition of copper-induced lipoprotein oxidation by juice pressed from cranberry fruit [67]. Cranberry flavonoids were subsequently investigated for their antioxidative properties. Cyanidin-3-galactoside and several quercetin glycosides isolated from cranberry fruit were evaluated for their free-radical scavenging activity by the diphenyl picryl hydrazyl (DPPH) assay and found to possess antioxidant activity comparable to vitamin E [4]. The compounds in this study were then evaluated for their ability to inhibit the $Cu^{2+}$ catalyzed oxidation of LDL and very low-density lipoprotein (VLDL) isolated from human plasma. The activities of cyanidin-3-galactoside, free quercetin, and quercetin-3-xyloside in preventing LDL damage were superior to that of vitamin E, with the flavonoids inhibiting LDL and VLDL oxidation by 50% at concentrations of 2–3 μM [4]. Porter and coworkers [5] reported similar activity for cranberry PACs, which were observed to associate with lipoproteins and prevent copper-induced oxidation. These studies support a potential role of LDL protection by cranberry flavonoids provided they are present in the plasma.

Encouragingly, clinical studies indicate that the plasma antioxidant capacity of humans improves with consumption of blueberry and cranberry products, which has implications for cardiovascular disease. Two studies of blueberry consumption and serum antioxidant status showed that addition of a 100 g of blueberry supplement or 100 g of freeze-dried blueberry powder to a high-fat diet significantly increased serum antioxidant capacity, with ORAC values increasing by 8.5% over the first hour after consumption. The majority of the blueberry anthocyanin glycosides were also detectable in the serum [10, 68]. Metabolism of anthocyanin glycosides is thought to involve absorption of both intact anthocyanin glycosides and aglycone forms in the intestine, followed by methylation and glucuronidation [69]. The form taken by the supplementation (*i.e.* powder, fruit, or juice) may affect the degree of absorption and circulation in the plasma.

In a 2000 study of juice supplementation, healthy female volunteers were given 500 mL of cranberry or blueberry juice after an overnight fast. Analysis of blood samples found a significant increase in the plasma antioxidant capacity of individuals after consuming cranberry juice, with the maximum effect obtained 1–2 h after consumption [70]. Antioxidant capacity of the plasma was evaluated by measuring its ferric reducing antioxidant potential value. The researchers observed that despite its higher phenolic content and antioxidant potential, blueberry juice consumption did not produce the same increase in plasma antioxidant content and activity. This was in contrast to the boost in serum antioxidant capacity observed when blueberry supplementation accompanied a high-fat meal. The researchers suggest that the higher vitamin C content of the cranberry juice compared to blueberry juice may be responsible for the greater effect observed for cranberry [70]. The same group found in a 2006 study that consumption of 750 mL/day of cranberry juice for 2 wk did not affect the levels of anthocyanins, total phenols, total cholesterol, triglycerides, HDL, or LDL in the plasma; nor did consumption affect the levels of antioxidative enzymes including glutathione peroxidase, catalase, or superoxide dismutase, in healthy female volunteers [71]. However, a Canadian clinical study of 21 male volunteers aged 30–46 found that approximately the same dosage of cranberry juice produced both a 6.5% average increase in antioxidant capacity and an average decrease of nearly 10% in the levels of circulating oxidized LDL in the plasma after a 2-wk treatment [72], suggesting some protection of LDL against oxidation. An *in vitro* model study of cranberry extracts with LDL-protective properties suggests that cranberry may lower lipid levels by inducing expression of hepatic LDL receptors. Cranberry treatment caused a dose-dependent increase in cholesterol uptake by HepG2 cells [73] that was significant at concentrations of 15 μg/mL or more.

A very interesting finding highlighting the nature of blueberry compounds with potential to improve blood lipids and their mechanisms of action is the ability of blueberry component pterostilbene to lower plasma lipoprotein and cholesterol levels. Hypercholesterolemic hamsters fed a diet containing 25 ppm pterostilbene showed a 29% decrease in plasma LDL levels, a 7% increase in plasma HDL, and a significant improvement in LDL/HDL ratio [74]. The observed effects are thought to arise through activation of peroxisome proliferator activated receptor alpha (PPARα), since pterostilbene demonstrated an induction of PPARα superior to that of the known cholesterol-lowering drug ciprofibrate. PPARα induction *in vivo* leads to a sequence of events that ultimately reduce triglycerides, and increase production of apolipoprotein expression, boosting plasma HDL.

Another possible mechanism toward lowering plasma lipids involves the binding and excretion of bile acids. Increased binding and excretion by dietary fiber and other phytonutrients is thought to lower cholesterol levels by stimulating the liver to further convert cholesterol into bile acids [75]. A recent *in vitro* study of the bile acid binding capacity of several anthocyanin-rich fruits found that blueberries were the most effective, binding at approximately 47% the level of cholestyramine, a cholesterol-lowering drug [76].

Endothelial cells lining the vasculature both in the cardiovascular system and in the brain are constantly in contact with reactive oxygen species (ROS) generated by monocytes and neutrophils. They respond in a variety of ways including expression of TNF-α and production of proinflammatory cytokines and adhesion molecules, events which play a key role in early atherogenesis and plaque rupture [77] as well as brain tissue damage leading to neurological dysfunction [78]. The ability of hydroxycinnamic acids and anthocyanins in blueberry and cranberry fruit to mitigate endothelial vulnerability to oxidation and inflamma-

 

tion was investigated in a microvascular endothelial cell model. Blueberry and cranberry anthocyanins and hydroxycinnamic acids protected membrane lipids from oxidation at concentrations of 10 or 100 μg/mL. The anthocyanins, tested at 100 μg/mL, were particularly effective at reducing up-regulation of inflammatory mediators IL-8, monocyte chemoattractant protein-1 (MCP-1), and intracellular adhesion molecule-1 (ICAM-1) induced by TNF-α. All phenolic fractions at 100 μg/mL increased the population of viable cells surviving exposure to hydrogen peroxide treatment [7]. These studies suggest a protective role of *Vaccinium* berry flavonoids against both oxidative and inflammatory stress in the vascular system.

Recent research suggests that compounds in wild blueberries can alter the composition of glycosaminoglycans (GAGs), a structurally diverse group of macromolecules produced by vascular endothelial and smooth muscle cells. GAGs participate in organization of the extracellular matrix and by their interaction with other molecules, regulate functions as diverse as cell proliferation, migration and adhesion, cell signaling, blood coagulation, and lipoprotein metabolism [79]. An abundance of oversulfated GAGs has been linked to chronic inflammation and the progression of atherosclerosis [80]. A feeding study employing Sprague-Dawley rats investigated the effect of wild blueberry consumption over 13 wk on the composition and abundance of GAGs in the aorta. The study found the concentration of GAGs in the aortas of blueberry-fed rats significantly higher than the control, with an overall decrease in the sulfation level of GAG disaccharides, which may be expected to affect the structure of the extracellular matrix, lipoprotein lipase activity, and signal transduction in vascular endothelial and smooth muscle cells. We have recently observed that polyphenolic extracts of whole cranberries reduce the expression of matrix metalloproteinases involved in remodeling of the extracellular matrix in aortic smooth muscle cells (Neto *et al.*, unpublished results; presented at ACS National Meeting, 2005) suggesting the potential to decrease abnormal proliferation and migration of vascular cells. The nature of the compounds responsible for this activity and their mechanisms of action are under investigation.

### 5.2 Antioxidants in the brain: Ischemic stroke and vascular diseases

Evidence for the ability of cranberry and blueberry extracts to protect brain cells from oxidative stress is growing. Recent studies have focused on the neuroprotective properties of these berries, using models of stroke, cognitive and motor functioning, and other diseases of aging.

Brain damage by ischemic stroke occurs in part from oxidative stress to neurons, induced by ROS produced during reperfusion which are not adequately inactivated by endogenous or exogenous antioxidant systems. Tissue hypoxia followed by reperfusion therefore leads to the cell damage associated with stroke [81]. A Canadian study recently found that rats fed on a 6-wk diet enriched with extracts of lowbush blueberry (*V. angustifolium*), suffered much less neuronal cell death upon induced stroke [8]. The possible identity of the compounds responsible for this *in vivo* protection was investigated in tissue culture, using isolated rat neurons challenged with a 6 h incubation of oxygen–glucose deprived medium, to simulate a stroke, or 100 μM hydrogen peroxide, to induce oxidative stress/reperfusion injury. The whole blueberry polyphenol mixture added to cells produced a concentration-dependent reduction in both apoptotic and necrotic cell death (MacKinnon *et al.*, unpublished results presented at the 50th Annual Congress of the Society for Medicinal Plant Research, 2002). Enriched fractions of anthocyanins and PACs also provided neuroprotection, although they were less effective than the polyphenolic mixture. A 2005 study employing rats fed a blueberry, spinach or spirulina diet for 4 wk found that blueberry diet reduced apoptosis induced by ischemia and reperfusion, with a significant reduction of cerebral infarction and an accompanying improvement in poststroke locomotor behavior compared to the control group [82].

A similar tissue culture study was conducted by us in collaboration with the Canadian group to investigate whether cranberry (*V. macrocarpon*) also provides neuroprotection. Using the same *in vitro* rat neuron model, whole cranberry extract was found to reduce hydrogen-peroxide-induced necrosis by 48% relative to the control and also reduced necrosis in oxygen and glucose deprived neurons by up to 42% [6]. Cranberry treatment reduced apoptosis in both the ischemia and reperfusion model cultures by up to 50 and 36% respectively. Examination of the activity of flavonoid subfractions revealed that the cranberry anthocyanins and flavonols were the major contributors to neuroprotection (Neto *et al.*, unpublished results; presented at ACS National Meeting, 2005). The effect of dietary cranberry supplementation in a rat model of stroke is currently under investigation. Taken together, these models suggest that the flavonoid-rich fruits of *Vaccinium* have the potential ability to reduce stroke damage and improve recovery.

The ability of flavonoids to protect neurons from oxidative stress and associated diseases of aging such as Alzheimer's disease and vascular dementia may be linked to increased glutathione and decreased calcium influx despite high intracellular levels of ROS [83]. The increased vulnerability to oxidative stress associated with the aging brain may also result from increased sphingomyelin and metabolites among membrane lipids and changes in the distribution of certain neuronal muscarinic receptor subtypes [84]. This vulnerability may be mitigated by a diet containing fruits and vegetables high in antioxidants. Due to their high ORAC activity relative to other foods [14], blueberry, spinach, and strawberry were chosen for a study of the ability of short-term dietary supplementation to reverse age-

 

related deficits in neuronal, motor, and cognitive function [9]. Using a 19-month old Fischer 344 rat as a model of aging, the study treated the animals for 8 wk and then conducted a variety of age-sensitive psychomotor tests and a Morris water-maze evaluation of working memory, prior to killing and analyzing the brains for markers of neurodegenerative activity. Blueberry-supplementation was the most effective of the three foods, actually reversing the age-related deficits in neuronal and behavioral parameters [9]. The improvement may be due in part to the ability of blueberry flavonoids to scavenge ROS, but other mechanisms including increased membrane fluidity and decreased inflammatory response may also play a role [85].

## 6 Conclusion

Emerging evidence suggests that phytochemicals from blueberries and cranberries (particularly PACs, anthocyanins, quercetin, ursolic acid, and stilbenes) are likely to have a mitigating effect on oxidative processes involved in the development of vascular diseases and some cancers. Protection against vascular disease by berry phytonutrients is likely to involve antioxidant mechanisms including prevention of oxidative LDL damage and cellular necrosis or apoptosis induced by oxidative stress; other potential mechanisms include decreased expression of inflammatory cytokines, increased LDL uptake, and changes in molecular structure in the extracellular matrix. Most *in vitro* studies have employed μg/mL concentrations of berry extracts or compounds that are much higher than the concentrations expected *in vivo*. However, some of the activities observed in tissue culture are supported by evidence from *in vivo* studies, for example the ability of dietary blueberry to decrease brain damage induced by stroke and improve cognitive function in a rat model of aging. Small clinical trials have furnished some conflicting results regarding the effect of dietary cranberry and blueberry on atherosclerosis-linked markers such as blood lipid levels and serum antioxidant status, pointing out the continuing need for additional human studies involving dietary berry supplementation.

The evidence for anticancer activity has largely been provided by tissue culture and molecular studies, which suggest a variety of mechanisms and targets for the myriad phytochemicals in these fruits. Potential chemopreventive activities include reduced proliferation, increased apoptosis and cell cycle arrest in tumor cells, and modulation of the expression and activity of metastasis-linked matrix metalloproteinases, VEGF, ODC, and QR. The efficacy of these fruits against tumor development *in vivo* will depend largely on the bioavailability of phytochemicals to the various tissues, a topic that is yet to be thoroughly researched. A key area for future study is the identity and biodistribution of metabolites of berry phytochemicals, since it is the metabolites that may be capable of producing biological effects *in vivo*. There is a clear need for well-designed studies to examine the effects of dietary cranberry and blueberry on animal models of breast and colon cancer as well as those which examine prostate tumor growth and metastasis. Design of such studies should pay close attention to chemical composition in order to maximize the diversity of available phytochemicals because several compounds in the fruit may be capable of producing complementary biological effects.

*The author gratefully acknowledges the assistance of UMass Dartmouth science librarian Elizabeth Winiarz in obtaining the references used herein.*

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