Critical Reviews in Food Science and Nutrition
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/bfsn20

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Version of record first published: 06 Aug 2010.

To cite this article: J. Côté , S. Caillet , G. Doyon , J.-F. Sylvain & M. Lacroix (2010): Bioactive Compounds in Cranberries and their Biological Properties, Critical Reviews in Food Science and Nutrition, 50:7, 666-679

To link to this article: http://dx.doi.org/10.1080/10408390903044107

Critical Reviews in Food Science and Nutrition, 50:666–679 (2010)

ISSN: 1040-8398
DOI: 10.1080/10408390903044107

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Bioactive Compounds in Cranberries and their Biological Properties

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Cranberries are healthy fruit that contribute color, flavor, nutritional value, and functionality. They are one of only three fruits native to America. Over the past decade, public interest for the North American cranberry (Vaccinium macrocarpon) has been rising with reports of their potential health benefits linked to the numerous phytochemicals present in the fruit—the anthocyanins, the flavonols, the flavan-3-ols, the proanthocyanidins, and the phenolic acid derivatives. The presence of these phytochemicals appears to be responsible for the cranberry property of preventing many diseases and infections, including cardiovascular diseases, various cancers, and infections involving the urinary tract, dental health, and Helicobacter pylori-induced stomach ulcers and cancers. Recent years have seen important breakthroughs in our understanding of the mechanisms through which these compounds exert their beneficial biological effects, yet these remain to be scientifically substantiated. In this paper these characteristics, as well as the antioxidant, radical scavenging, antibacterial, antimutagen, and anticarcinogen properties of cranberry major bioactive compounds are explained.

Keywords anthocyanins, flavonoids, proanthocyanidins, phenolic acids, antibacterial, antioxidant, antimutagen

INTRODUCTION

Cranberries are healthy fruit that contribute color, flavor, nutritional value, and functionality. They are one of the only three native Northern American fruits. The North American cranberry (*Vaccinium macrocarpon*) is recognized by the US Department of Agriculture, USDA, as the standard for fresh cranberries and cranberry juice cocktail. The European variety, grown in parts of central Europe, Finland, and Germany, is known as *Vaccinium oxycoccus*. This is a smaller fruit with anthocyanins and acid profiles slightly different to that of the North American variety (Girard and Sinha, 2006).

Phenolic acids, flavonoids, and tannins are phytochemicals ubiquitous in food of plant origin (Lugasi and Hovari, 2000). They constitute one of the most abundant groups of natural metabolites and are now recognized for their important contribution to both human and animal diet and health (Vattem et al., 2005a; Vattem and Shetty, 2005). These phytochemicals occur in virtually every plant and in all parts of the plant. Their quantitative distribution can vary between different organs of the plants and within different populations of the same plant species, and for this reason they are often used for the plant taxonomical classification (Robards and Antolovich, 1997; Merken and Beecher, 2000a). The predominant bioactive compounds found in cranberries are the flavonols, the flavan-3-ols, the anthocyanins, the tannins (ellagitannins and proanthocyanidins), and the phenolic acid derivatives. These phytochemicals are commonly associated with the fruit organoleptic (sensory) qualities and have also shown diverse biological properties and physiological activities in animals (Robards and Antolovich, 1997; Kren and Martinkove, 2001). Numerous clinical trials and epidemiological studies have established an inverse correlation between the intake of phenolic-rich fruits and vegetables and the occurrence of diseases such as inflammation, cardiovascular disease, cancer, and aging-related disorders (Willet, 2001).

The objective of this paper is to provide an overview of the main bioactive compounds of cranberry, their characteristics, and their biological properties. This paper is organized in two parts; the first part presents the main phytochemical composition of cranberry. In the second part the antioxidant, radical scavenging, antibacterial, antimutagen, and anticarcinogen properties of cranberry are reviewed in detail.

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Table 1 Polyphenols in different cranberry products based on serving size (Vinson et al., 2008)

Cranberry Products	Total polyphenols (as mg catechin/serving size)
Frozen (97.5 g, 1/2 cup)	632
Dried (40 g, 1/3 cup)	352
Sauce (57 g, 1/4 cup)	170
Jellied sauce (57 g, 1/2″ thick, approx 8 slices per can)	122
Cocktail, 27% juice (240 mL)	176
Juice, 100% (240 mL)	540

CRANBERRY BIOACTIVE COMPOUNDS

Cranberries and cranberry products are known for their elevated concentration in total polyphenols (Vinson et al., 2008). On a fresh weight basis, the order of the amount of total polyphenols in cranberry foods is as follows: dried > frozen > sauce > jellied sauce. On a serving size basis for all cranberry poducts, the order is: frozen > 100% juice > dried > cocktail > sauce > jellied sauce (Table 1).

More specifically, cranberries are rich in anthocyanins, in tannins (ellagitannins and proanthocyanidins), and have significant concentrations of the flavonoids—flavonols and flavan-3-ols (Neto, 2007; Ruel and Couillard, 2007; USDA, 2004; 2007). Recently, many authors have also reported the presence of certain phenolic acid derivatives in cranberries: hydroxycinnamic acid, HCA, and hydroxybenzoic acid, HBA (Neto, 2007; Ruel and Couillard, 2007). Although these phenolic acids occur naturally in their free forms, they are generally considered as structural moieties in polyphenol compounds, i.e. they are embedded within a polyphenolic structure (Seeram and Heber, 2007).

Flavonoids are defined as naturally-occurring organic species that possess two six-carbon aromatic centers, called the A and B rings, and a three-carbon bridge, called the C ring which forms a phenol bridge with oxygen (Robards and Antolovich, 1997; Haslam, 1998; Gee and Johnson, 2001). They are further classified according to the degree of unsaturation and oxidation of their three-carbon heterocycle segment (Fig.1). Additional structural complexities are introduced by the common occurrence of glycosylation, where one or more of the hydroxyl groups of the flavonoid aglycone are bound to a sugar or sugars by an acid-labile hemiacetal bond to form what are called *O*-glycosides (Robards and Antolovich, 1997; Merken and Beecher, 2000a; Escarpa and Gonzalez, 2001; Rice-Evans, 2001; Cuyckens and Claeys, 2004). This glycosylation step has a profound effect on the economy of the plant, as the glycosylated flavonoids have greater sap solubility and more plant mobility than the parent aglycone. This greater water solubility also permits their storage in the cell vacuole where they are commonly found (Harborne, 1964; Shahidi and Naczk, 1995; Robards and Antolovich, 1997; Cuyckens and Claeys, 2004). Studies have shown that glycosylated flavonoids can be better absorbed than aglycons (Hollman and Katan, 1997) and tend to form more stable aroma precursors (Mayorga et al., 2001). Glucose is the sugar most commonly encountered and glycosylated to flavonoids, although galactose, rhamnose, arabinose, and xylose are not unusual. Glycosylation with mannose, fructose, glucoronic, and galacturonic acids is exceptionally rare. Disaccharide and more complex carbohydrates have also been found in association with flavonoids—the more common are rutinose (rhamnosyl-($\alpha 1 \rightarrow 6$)-glucose) and neohesperidose (rhamnosyl-($\alpha 1 \rightarrow 2$)-glucose), but occasionally tri- and tetrasaccharides can be found (Shahidi and Naczk, 1995; Robards and Antolovich, 1997; Cuyckens and Claeys, 2004, Rijke et al., 2006). The stereochemistry of the glycosidic linkages is another important characteristic of glycosylated flavonoids. In principle, any of the hydroxyl groups can be glycosylated but certain positions seem favored. For example, the 3- and 7-hydroxyls in flavonols and flavan-3-ols, and the 3- and 5- hydroxyl in anthocyanidins are common glycosylation sites (Robards and Antolovich, 1997; Cuyckens and Claeys, 2004). The linkage can either be *O*-glycosyl or *C*-glycosyl, but glycosylated flavonoids usually occur as *O*-glycosyl (Bohm, 1998; Rijke et al., 2006). The *C*-glycosylflavonoids are characterized by having one or two sugar units directly linked to the aromatic nucleus through carbon-carbon bonds, whereas the *O*-glycosylation can occur at any of the hydroxyl groups although certain ones, such as 3-*O*-glycosyl and 7-*O*-glycosyl, seem favored (Stafford, 1990; Bohm, 1998).

Anthocyanins

Anthocyanins are generally found in fruit and more specifically in red, purple, and blue berries (Nijveldt et al., 2001; Higdon, 2007). Their concentrations in food tend to increase as fruit ripens in response to climatic factors (light, temperature) (Bohm, 1998). The anthocyanin content of a cranberry averages 95 mg/100 g for a ripe fruit at harvest, with reports of anthocyanin content as high as 124 mg/100 g of fresh fruit weight (USDA, 2007). The concentration can also vary quite significantly among cranberry cultivars—the early black cultivar appears to be significantly higher in anthocyanins compared to other cranberry cultivars (Neto, 2007).

Anthocyanins consist of an anthocyanidin molecule bound to one or more sugar moieties (Robards and Antolovich, 1997). The six most common anthocyanidins are cyanidin, delphinidin, malvidin, perlagonidin, petunidin, and peonidin and their chemical structures are shown in Fig. 2 (Robards and Antolovich, 1997; Merken and Beecher, 2000a). Glycosylation of anthocyanidins almost always occurs at the C_3 position with glucose, arabinose, and galactose being the most common sugar moieties (Strack and Wray, 1994; Robards and Antolovich, 1997). In fact, the formation of anthocyanidin 3-*O*-glucosides is considered a necessary step in anthocyanins biosynthesis (Robards and Antolovich, 1997). Anthocyanins with sugars at both the C_3 and C_5 positions and 3,7-diglycosides do also occur as they are considered more stable than C_3 *O*-glycosylanthocyanins (Strack and Wray, 1994; Bohm, 1998). Anthocyanins are

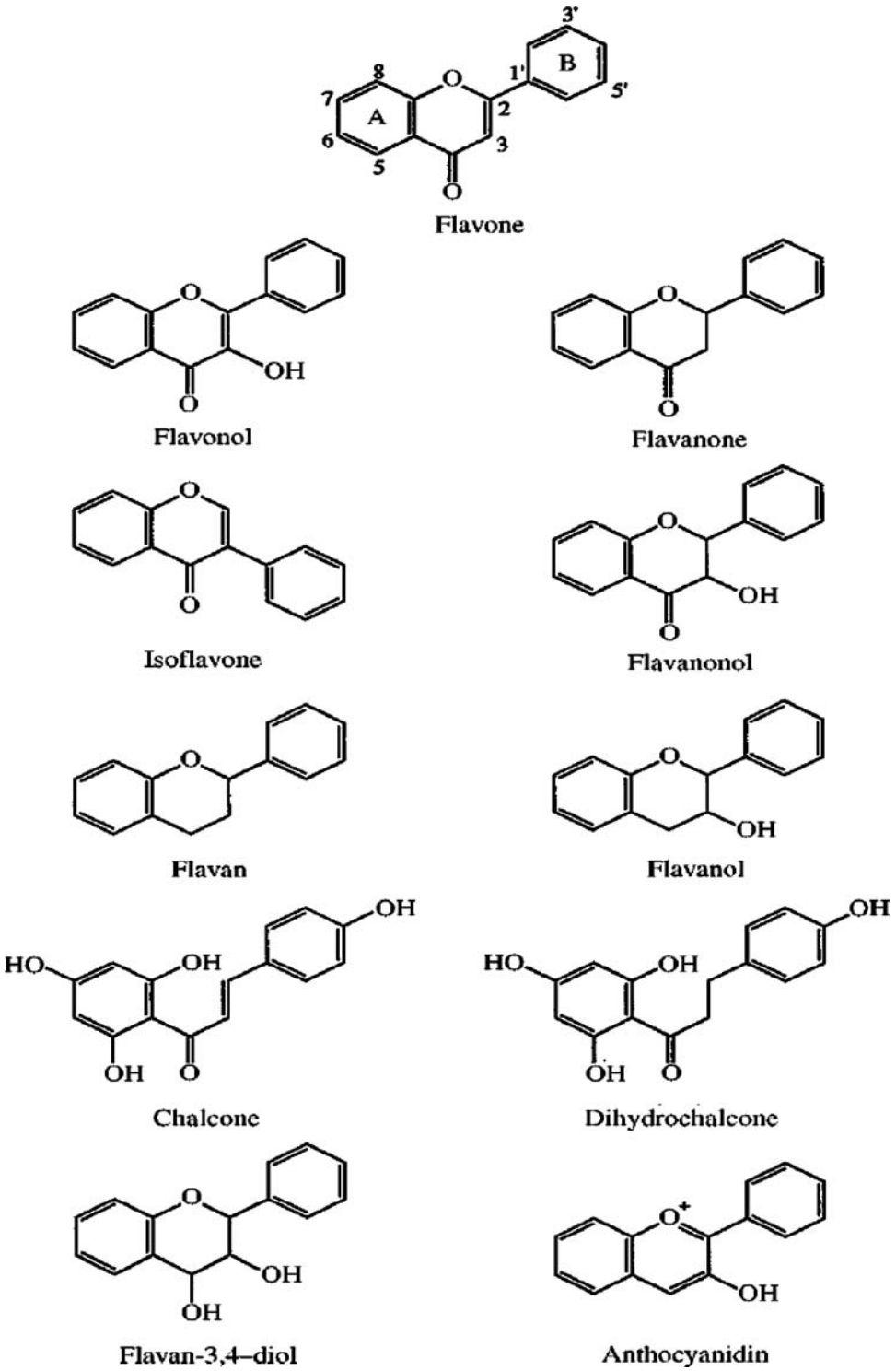
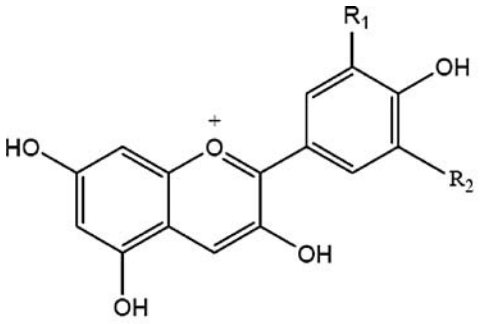


Figure 1 Structure of the various classes of flavonoids. Reproduced with permission from Kevin Robards (Robards and Antolovich, 1997).

frequently found acylated with aromatic and aliphatic acids such as *p*-coumaric acid, caffeic acid, ferulic acid, gallic acid, acetyl malonic acid, malic acid, etc. (Bohm, 1998; Iwashina, 2000). In acidic medium, the aglycone can exist in cationic form with numerous mesomeric forms (Robards and Antolovich, 1997).

The major anthocyanins in cranberry are galactosides and arabinosides of cyanidin and peonidin (Macheix et al., 1990; Neto, 2007). However, the following anthocyanins were also detected at low concentrations: cyanidin 3-*O*-glucoside, peonidin 3-*O*-glucoside, peonidin 3-*O*-galactoside, peonidin



Anthocyanidin	R_1	R_2
Cyanidin	H	OH
Delphinidin	OH	OH
Malvidin	OMe	OMe
Pelargonidin	H	H
Peonidin	H	OMe
Petunidin	OH	OMe

Figure 2 Molecular structure of anthocyanidins. Reproduced with permission from Joanne Holden (USDA, 2007).

3-*O*-arabinoside, peonidin 3,5-digalactoside, delphinidin 3-*O*-glucoside, delphinidin 3-*O*-galactoside, delphinidin 3-*O*-arabinoside, pelagonidin 3-*O*-galactoside, pelagonidin 3-*O*-arabinoside, petunidin 3-*O*-galactoside, malvidin 3-*O*-galactoside, and malvidin 3-*O*-arabinoside (Macheix et al., 1990; Wu and Prior, 2005; Lin and Harnly, 2007).

Flavonols

Flavonols are found in abundance in Ericaceae fruits such as cranberry, blueberry, and bilberry (Robards and Antolovich, 1997; King and Young, 1999; Nijveldt et al., 2001; Heinonen, 2007). They are known to be concentrated mainly in the skin of fruits (Hawker et al., 1972; Wildanger and Herrmann, 1973; Price et al., 1999). On a weight basis, the cranberry is one of the leading fruit sources of flavonols. According to Neto (2007) and the database for the flavonoid content of selected foods (USDA, 2007), the average flavonol content in cranberry is 20–30 mg/100 g fresh fruit weight, although contents as high as 48 mg/100 g were reported.

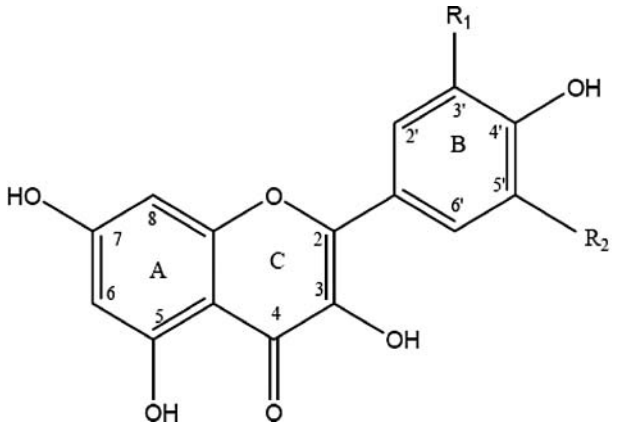
As shown in Fig. 3, the most predominant type of flavonols includes isorhamnetin, kaempferol, myricetin, and quercetin. The flavonols are characterized by the absence of any substituant at the C_3 position. Because of the double bond present in their central aromatic ring, they are renowned for their planar structure (Bohm, 1998; Iwashina, 2000; Nijveldt et al., 2001).

Approximately 75% of the cranberry flavonols consist of quercetin glycosides (quercetin 3-*O*-galactoside), although other flavonols have also been reported in the fruit at lower concentrations: quercetin, quercetin 3-*O*-glucoside, quercetin 3-*O*-xyloside, quercetin 3-*O*-arabinopyranoside, quercetin 3-*O*-arabinofuranoside, quercetin 3-*O*-rhamnoside, myricetin, myricetin 3-*O*-galactoside, myricetin 3-*O*-xylopyranoside, myricetin 3-*O*-arabinopyranoside, myricetin 3-*O*-arabinofuranoside, myricetin 3-*O*-rhamnoside, kaempferol 3-glucoside, and kaempferol derivative (Macheix et al., 1990; Häkkinen et al., 1999; Zheng and Wang, 2003; Chen and Zuo, 2007; Lin and Harnly, 2007; Neto, 2007).

Flavonols are often found in nature as acylated derivatives, involving linkages between aliphatic and aromatic acids (hydroxybenzoic, gallic, *p*-coumaric, caffeic, ferulic, and sinapic acids) and the sugar hydroxyls (Stafford, 1990; Bohm, 1998). The predominant type of glycosylated flavonols in fruits are 3-*O*-monoglycosides with the sugars occurring in the following order—glucose > galactose > rhamnose > glucuronic acid.

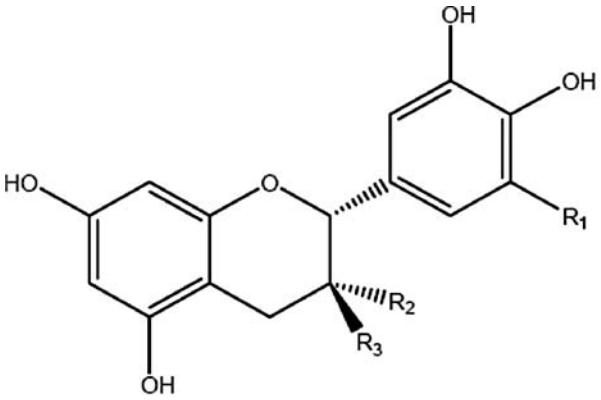
Flavan-3-ols and Tannins

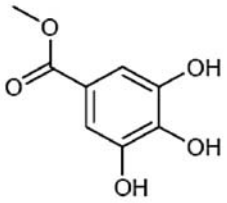
Flavan-3-ols are important constituents of fruits, and their presence has been reported in cranberry (Macheix et al., 1990; Lin and Harnly, 2007). They share the same flavonoid molecular structure with flavonols, but as shown in Fig. 4, they lack the C_4



Flavonol	R_1	R_2
Isorhamnetin	OMe	H
Kaempferol	H	H
Myricetin	OH	OH
Quercetin	OH	H

Figure 3 Molecular structure of flavonols. Reproduced with permission from Joanne Holden (USDA, 2007).





Gallate

Flavan-3-ol	R_1	R_2	R_3
(+)-Catechin (C)	H	H	OH
(+)-Catechin-3-gallate (CG)	H	H	Gallate
(-)-Epicatechin (EC)	H	OH	H
(-)-Epicatechin-3-gallate (ECG)	H	Gallate	H
(-)-Epigallocatechin (EGC)	OH	OH	H
(-)-Epigallocatechin-3-gallate (EGCG)	OH	Gallate	H
(+)-Gallocatechin (GC)	OH	H	OH
(+)-Gallocatechin-3-gallate (GCG)	OH	H	Gallate

Figure 4 Molecular structure of flavan-3-ols. Reproduced with permission from Joanne Holden (USDA, 2007).

carbonyl group (Iwashina, 2000; Escarpa and Gonzalez, 2001). In contrast to other flavonoids, they are known to occur naturally in plant food as aglycons of catechin and epicatechin as they are soluble enough to be maintained in the vacuole without needing glycosylation. The flavan-3-ol content of a cranberry averages 7 mg/100 g for a ripe fruit at harvest, with content as high as 11 mg/100 g fresh fruit weight being reported (USDA, 2007).

Through reactions catalyzed by light, heat, and oxygen, flavan-3-ols tend to combine with esters of gallic acid and ellagic acid to form compounds refered to as hydrolyzable tannins, such as catechingallate, epigallocatechin, epicatechin gallate, gallocatechin, and epigallocatechin gallate, as shown in Fig. 4 (Robards and Antolovich, 1997; Merken and Beecher, 2000a; Escarpa and Gonzalez, 2001; Higdon, 2007; Szajdek and Borowska, 2008).

Flavan-3-ols can also form oligomeric and copolymeric compounds with a high degree of polymerization through *O*- and *C*-glycosyl linkages with sugars and other *C*- substitutions through acylation and complexation with anthocyanins and noncyanic flavonoids such as phenolic acids or metal ions (Stafford, 1990; Bohm, 1998; Iwashina, 2000; Lazarus et al., 2001; Dixon et al., 2005; Higdon, 2007; Szajdek and Borowska, 2008). Cranberries have a high content of oligomeric and polymeric pigments, also referred to as condensed non-hydrolyzable tannins or proanthocyanidins, which have structures similar to those found in wine (Krueger et al., 2004). According to the USDA Database for the proanthocyanidin content of selected foods (USDA, 2004), the proanthocyanidin content of raw cranberries can average 410 mg/100 g fresh fruit weight, although contents as high as 505 mg/100 g were reported. The proanthocyanidin concentration appears to be significantly higher in cranberry fruit of the early black cultivar, compared to most other cranberry cultivars (Neto, 2007). These tannins are generally located in vacuoles of intact plant cells. The seed coats of many plant species also accumulate proanthocyanidins, which frequently cause poorly-soluble, brown or red-brown pigmentation once the seeds have matured (Leung et al., 1979; Shirley et al., 1992; 1995; Nozzolillo and Ricciardi, 1992; Marles et al., 2003).

In proanthocyanidins, the successive flavan-3-ol monomeric units are generally linked together by C-C interflavan bonds occuring between the C_4 position of the "upper" unit and the C_6 or C_8 position of the lower unit ($4\beta \rightarrow 6$ or $4\beta \rightarrow 8$) (Hammerstonne et al., 2000; Gu et al., 2004; Dixon et al., 2005). This type of condensed tannin, as shown in Fig. 5, is also called the B-type proanthocyanidin oligomer; they differ only in the arrangement of (+)-catechin and (–)-epicatechin starter and

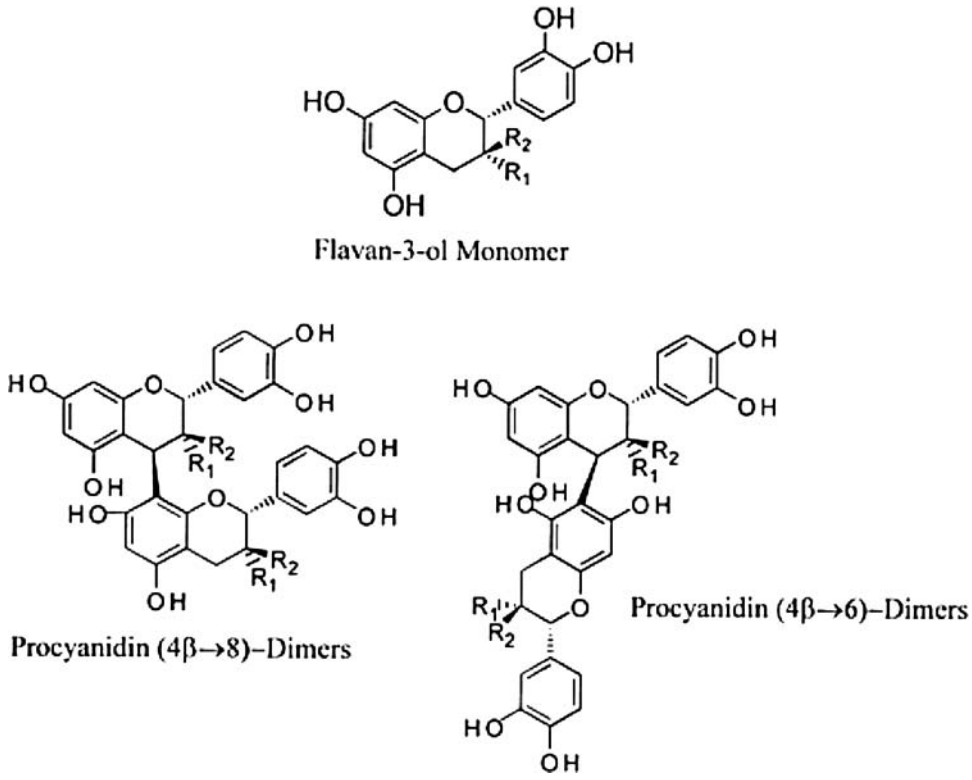


Figure 5 Representative structures of flavan-3-ol monomers and their dimers. When R_1 = OH and R_2 = H, the monomer is (−)-epicatechin. When R_1 = H and R_2 = OH, then the monomer is (+)-catechin. Reproduced with permission from John F. Hammerstone (Hammerstone et al., 2000).

extension units. B-type proanthocyanidins containing gallic acid esters have also been reported (Hammerstonne et al., 2000). Their formation appears to be under strict enzymatic control because the different types of dimers are characteristic of specific plant species: proanthocyanidin B_1, for example, is found in cranberry, whereas B_3 is found mainly in strawberry and B_4 in raspberry and blackberry (Fletcher et al., 1977). Another structural variation is known to occur, although less frequently, in condensed tannins where flavan-3-ol units are linked together by a mixture of C-C bonds and interflavonoid bonds by C-O oxidative coupling between both C_2 and C_4 of the upper unit and the oxygen at C_7 and positions C_6 or C_8, respectively, of the lower unit ($4\beta \rightarrow 8$ and $2\beta \rightarrow O \rightarrow 7$). Shown in Fig. 6, these condensed tannins are referred to as A-type proanthocyanidin oligomers (Porter, 1994; Foo et al., 2000a; Gu et al., 2004; Neto, 2007). Due to the complexity of this conversion, A-type proanthocyanidins are not encountered as frequently in nature in comparison to B-type proanthocyanidins (Lazarus et al. 2001; Dixon et al., 2005). Cranberry proanthocyanidins tend to occur as tetramers to decamers, but also as polymers of epicatechin units, with the two types of linkages between epicatechin units, the B-type and the less common A-type (USDA, 2004; Howell et al., 2005;Neto, 2007). Proanthocyanidin characterization in cranberry juice cocktail has shown the presence of a series of polyflavan-3-ol oligomers composed of 4-10 repeating unit structures of epicatechin and epigallocatechin with one or more A-type interflavanyl linkages (Howell et al., 2005). The presence of epicatechin-anthocyanin dimers, trimers, and tetramers was also detected in organic cranberry juice and early black cultivar fruit (Neto et al., 2008).

Phenolic Acids

Phenolic acids contribute to the characteristic and unique flavor of berries (Vattem et al., 2005a; Vattem and Shetty, 2005). This family of compounds includes derivatives of hydroxycinnamic acid, HCA, and of hydroxybenzoic acid, HBA. They both have very similar molecular structures composed of a backbone phenol ring, although HCA has an additional ethylenic chain attached to the aromatic ring. Compounds classified in these two families of acid derivatives can otherwise differ by the number and position of hydroxyl and methyl groups attached to the phenol ring. The presence of HCA derivatives in plant foods is more frequent than that of the HBA derivatives (Macheix and Fleuriet, 1998; Escarpa and Gonzalez, 2001).

The HCA derivatives are the most widely distributed group of phenolic compounds (Shahidi and Naczk, 1995; Escarpa and Gonzalez, 2001). Liquid chromatography combining mass spectrometer analysis of various cranberry cultivars allowed the detection of hydroxycinnamate esters in the whole fruit in quantities averaging 15–20 mg/100 g fresh fruit (Kondo, 2006). The HCA derivatives reported in cranberry includes compounds such as ferulic acid, *p*-coumaric acid, coumaroylglucose,

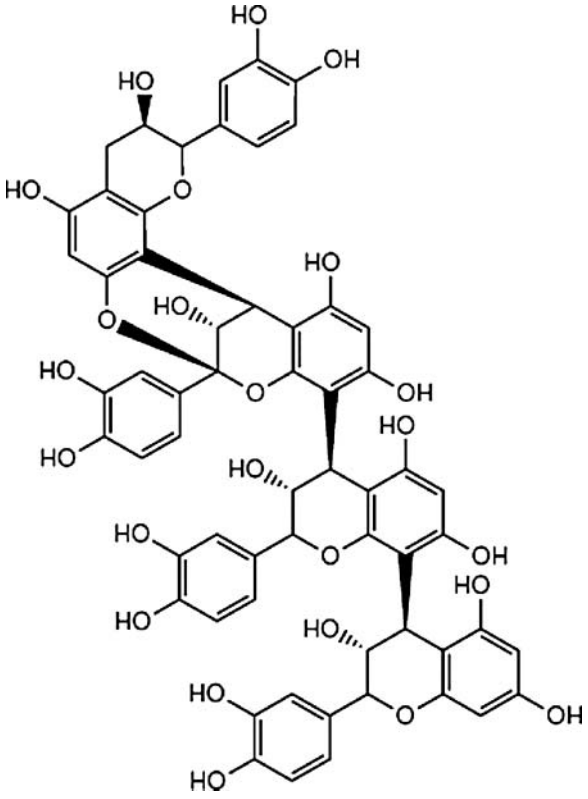


Figure 6 Structure of a typical cranberry proanthocyanidin tetramer composed of epicatechin units with one A-type linkage. Reproduced with permission from Catherine C. Neto (Neto, 2007).

feruloylglucose, glycosylated sinapic acid, caffeoylglucose, and diglucoside of caffeoylglucose, (Macheix et al., 1990; Häkkinen et al., 1999). As shown in Fig. 7, this class of phenolic compounds consists of an aromatic ring onto which a three-carbon side-chain is attached (Harborne, 1998; Sakakibara et al., 2003). They rarely occur in free form; they are instead associated to other types of compounds. When they do occur as simple phenolic acid, it is generally after they have gone through brutal processes, such as contamination by microorganism or technological processing (freezing, sterilization, fermentation) (Macheix et al., 1990; Shahidi and Naczk, 1995). Ferulic, *p*-coumaric, caffeic, and sinapic acids are often found combined with sugars by means of glycosidic linkage, or with organic acids such as quinic acid (Harborne, 1964; Escarpa and Gonzalez, 2001). Common glycosylated HCA derivatives include esters *p*-coumaroylglucose and caffeoylglucose (Shahidi and Naczk, 1995).

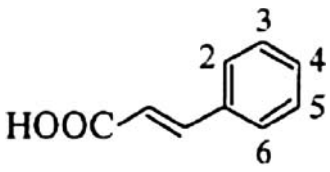


Figure 7 Molecular structure of phenylpropanoids. Reproduced with permission from Kazuki Kanazawa (Sakakibara et al., 2003).

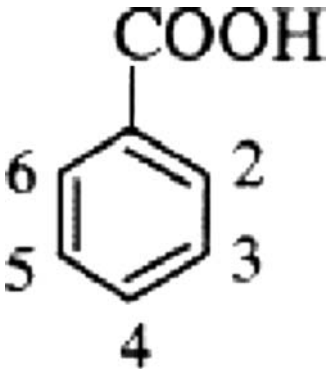


Figure 8 Molecular structure of hydroxybenzoic acid derivatives. Reproduced with permission from Kazuki Kanazawa (Sakakibara et al., 2003).

The concentration of HBA derivatives is generally low in food of plant origin, the exception being for the majority of berries, whose content in protocatechuic, ellagic, and gallic acids is very high (Shahidi and Naczk, 1995; Tomas-Barberan and Clifford, 2000). The presence of ellagic and p-hydroxybenzoic acids was reported in cranberries by Häkkinen et al. (1999), whereas Zheng and Whang (2003) reported the presence of vanillic acid. HBA derivatives are the simplest structure within the congregation of polyphenolic compounds, with their six-carbon structure shown in Fig. 8 (Macheix and Fleurinet, 1998; Escarpa and Gonzalez, 2001). They are also found glycosylated—glycosides of 4-hydroxybenzoic, protocatechuic, vanillic, and syringic acids were reported in plant food products (Shahidi and Naczk, 1995; Tomas-Barberan and Clifford, 2000). Due to reactions catalyzed by light, heat, and oxygen, ellagic acid esters readily combine with flavan-3-ols to form hydrolyzable tannins called ellagitannins. Monomeric ellagitannins can further polymerize and form oligomeric and polymeric ellagitannins. These complex tannins can form the structural components of the plant cell wall (lignin) and cell membrane (Marwan and Nagel, 1986; Chen et al., 2001). High contents of ellagitannins have been reported in cranberry (Kähkönen et al., 2001; Lei et al., 2001; Vattem and Shetty 2003; Kaponen et al., 2007).

BIOLOGICAL PROPERTIES OF CRANBERRY BIOACTIVE COMPOUNDS

Cranberries (*Vaccinium macrocarpon*) have been identified with beneficial properties toward bacterial infections involving the urinary tract disorders, dental decay, as well as stomach ulcers and cancers (Heinonen, 2007). Although berry phenolics are potent in vitro antioxidants, they exert in vivo biological activities beyond antioxidation and can have complementary and overlapping mechanisms of action (Seeram and Heber, 2007). In vitro experimental systems showed that berry phenolics possess antioxidant and free radical-scavenging activities, but also metal chelation, antiproliferative, anticarcinogenic, antibacterial, anti-inflammatory, antiallergenic, and antiviral properties

(Lee, 2000; Merken and Beecher, 2000a; 2000b; Nijveldt et al., 2001; Higdon, 2007). This would explain why they exhibit such strong physiological activities against infections, cancerous mutations and cancers, as well as against allergies, inflammation, virus, hypertension, arthritis, and AIDS (Robards and Antolovich, 1997; Merken and Beecher, 2000a).

Antimicrobial Properties

Juice of the American cranberry (*Vaccinium macrocarpon*) has long been consumed for the prevention of urinary tract infections. Its ability to protect the urinary tract from adherence of urinopathogenic bacteria such as *Escherichia coli* and other pathogens, has led doctors to recommend drinking cranberry juice as a treatment for various urinary tract infections and prostatitis. For more than a decade, the tannic components of cranberry have been proposed to inhibit bacterial adherence to the epithelial cells by competing for the bindings of both these fimbriae. In a 2008 Cochrane clinical study review, the authors Jepson and Craig (2008) concluded that there was some evidence that cranberry juice may decrease the number of symptomatic urinary tract infections over a 12 month period, particularly for women with recurrent urinary tract infections. All this knowledge has led to the first ever health claim on berry phenolics issued by the French Food Safety Authority in April 2004: "cranberry proanthocyanidins, in a daily dose of 36 mg, help reducing the adhesion of certain *E. coli* bacteria to the urinary tract" (Heinonen, 2007).

In the past decade, cranberry antimicrobial activity was demonstrated toward other groups of illness-causing pathogenic bacteria, including *Helicobacter pylori*, *Salmonella*, *Staphylococcus aureus*, *Escherichia coli*, and *Campylobacter*, to name a few. Recent in vitro studies indicated that the presence of high molecular weight components in cranberry could also inhibit the siallylactose-specific (S fimbriae) adhesion of *Helicobacter pylori* strains to immobilized human mucus, erythrocytes and cultured gastric epithelial cells (Burger et al., 2000; Burger et al., 2002; Vattem et al., 2005b; Neto, 2007). This action against the *Helicobacter pylori* is thought to have a potential inhibitory effect on the development of peptic ulcers and gastric cancer (Neto, 2007). Puupponen-Pimia et al. (2005) have also reported a significant inhibitory effect of various concentrations of cranberry extracts (*Vaccinium oxycoccus*) against *Salmonella enteric* sv. Typhimurium and *Staphylococcus aureus*. When freeze-dried berry powder of 2 mg/mL was used, both pathogenic strains of bacteria were clearly affected, *Staph. Aureus* stronger than *Salm.* Sv. Typhimurium. The effect of anthocyanin-and proanthocyanin-rich fractions isolated from cranberry juice were studied for their antibacterial activity by Leitao et al. (2005). *Staphylococcus aureus* was the only strain to exhibit some susceptibility to four out of the 10 anthocyanin-rich fractions tested, whereas a variable susceptibility of *S. aureus*, *Enterococcus feacalis*, and *Micrococcus luteus* to procyanidin-rich fraction was observed.

Cranberry has also showed potential inhibitory effect against bacteria involved in dental caries and periodontal diseases (Bodet et al., 2008). Dental caries are caused principally by *Streptococcus mutans* infection but also by *Actinomyces spp.* and *Lactobacillus spp.* (Beighton, 2005). During the formation of dental caries by *Steptococcus mutans*, the microorganism synthesizes extracellular glucans from sucrose using glucosyltransferases. The glucans can then promote the accumulation of the streptococci and other microorganism on the tooth surface and help in forming a biofilm to eventually carry out glycolysis of multiple carbohydrates efficiently. Duarte et al. (2006) have investigated the influence of cranberry components on the activities of glucosyltransferase B adsorbed onto a saliva-coated hydroxyapatite surface. Their results indicated that compared to the fractions (flavonols, proanthocyanidins, anthocyanins), the crude cranberry extract showed the highest percentage of inhibition of glucosyltransferase activity. Cranberry phenolic compounds may also play an important role against periodontal diseases, a group of inflammatory disorders initiated by specific gram-negative bacteria, and that leads to connective tissue destruction. The gingivite (gum inflammation) and parodontite (progressive destruction of tooth support—parodontal ligament and alveolar bone) are mainly caused by infections by *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* (Sorsa et al., 1990). Proteolytic enzymes, including matrix metalloproteinases and elastase, produced by resident and inflammatory cells in response to periodontopathogens and their products, play a major role in gingival tissue destruction (Palcanis et al., 1992; Sorsa et al., 2004). Recent in vitro studies have demonstrated the efficient inhibitory effect of cranberry fractions on the lipopolysaccharide-induced metalloproteinases and elastase (Bodet et al., 2006; 2007; 2008). Certain extracts obtained from cranberry juice were also shown to be effective in decreasing the congregation and salivary concentration of *Streptococcus mutans* which causes tooth decay (Weiss et al., 1998; 2002).

Adherence of pathogens to the host tissue is one of the most important prerequisite steps for bacteria colonization and subsequent infection. Inability of the pathogen to bind to the cell surface causes the microorganism to be washed away without causing any infection. Investigations into the mechanism of adherence to host tissue has led to an understanding that these are mediated by specific glycoprotein receptors called fimbriae or lectins on the bacterial cell surface which can specifically bind to sugars present on the mucosal or intestinal cell surfaces of the host tissue (Zafriri et al., 1989; Sharon and Ofek, 2002). This type of binding, however, occurs only via specific types of fimbriae-mediated adhesion—type 1 fimbriae (mannose sensitive) and type P fimbriae [α-Gal(1$\rightarrow$4) β-Gal], which is mannose resistant (Zafriri et al., 1989; Sharon and Ofek, 2002).

Over the last decade, specific bioactive components of cranberry were proposed to inhibit bacterial adherence to the epithelial cells. The polymeric tannins and in particular, the proanthocyanidins consisting primarily of epicatechin tetramers and pentamers with at least one A-type linkage, seem to be

the protecting element against pathogenic bacteria (Foo et al., 2000b; Heinonen, 2007; Seeram and Heber, 2007).

Several mechanisms could explain the effect of the A-type proanthocyanidin in the growth inhibition of bacteria—the destabilization of cytoplasmic membrane, the permeabilization of cell membrane, the inhibition of extracellular microbial enzymes, the direct actions on microbial metabolism, and the deprivation of the substrates required for microbial growth (Heinonen, 2007). Proanthocyanidins are known to bind metals and form complexes involving their *o*-diphenol groups, a property often viewed as imparting negative traits with regards to the bioavailability of essential mineral micronutrients, especially iron and zinc (House, 1999). Iron depletion can severely limit bacterial growth, and it has been suggested that the ability to bind iron represents another mechanism through which proanthocyanidins inhibit bacterial activity (Dixon et al., 2005).

Antioxidant and Radical-Scavenging Properties

Oxidative stress is a widely used and ill-defined term that refers to the imbalance in the rate at which the intracellular content of reactive oxygen species (ROS) increases relative to the capacity of the cell to dispose of these free radicals, most likely due to insufficient antioxidant reserves or intake. Free radicals or ROS are produced continuously in humans through a number of normally occurring cellular events, such as energy production and detoxification of the body, or are generated by exhaustive exercise. They are also found in atmospheric pollution and tobacco smoke. Free radicals have the capacity to alter the integrity of large biomolecules such as lipids, proteins, and DNA, resulting in an increased risk of inflammatory diseases, cardiovascular disease, some cancers, diabetes, Alzheimer's disease, cataracts, and age-related functional decline (Ruel and Couillard, 2007; Seeram and Heber, 2007).

In biology, compounds that can retard or prevent the effects of oxidation have been broadly considered as antioxidants, including compounds that either inhibit specific oxidizing enzymes or react with oxidants before they damage critical biological molecules (Ruel and Couillard, 2007). Effective antioxidants are radical scavengers that break down radical chain reactions caused by reactive oxygen/nitrogen species or that can inhibit the reactive oxidants from being formed in the first place (Huang et al., 2005). These reactions are based on a compound's ability to donate hydrogen as well as to stabilize the resulting antioxidant radical by electron delocalization (resonance) and/or intramolecular hydrogen bonding or by further oxidation. The loss of hydrogen may take place by donation of an electron followed by deprotonation (Frankel and Meyer, 2000; Higdon, 2007).

Considerable in vitro evidence exists, showing that cranberry phenolics are powerful antioxidants. However, the great diversity of methods applied to studying both the radical-scavenging activity and the antioxidant activity has resulted in great differences in the outcome (Heinonen, 2007). Still, cranberry phenolics appear to have free radical-scavenging properties against superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$), and can also inhibit lipid peroxidation, as well as protein and lipid oxidation in liposomes (Wang and Jiao, 2000; Wu et al., 2004; Seeram and Heber, 2007). This could explain why they prevent the oxidation of bulk lipids. The formation of methyl linoleate hydroperoxides for example, was inhibited over 90% by cranberry phenolics at concentrations as low as 500 μg/mL (Heinonen, 2007).

Antioxidant activity will differ depending on the phenolic molecular structure (Bravo, 1998; Ruel and Couillard, 2007). Flavonoids with adjacent dihydroxy substituents on the B ring, for example, have been shown to be effective in the scavenging of radicals. In the group of flavonoids, this catechol unit is found in the flavonol quercetin, the anthocyanidin cyanidin, the flavan-3-ol catechins, and the proanthocyanidin procyanidin (Zheng and Wang, 2003). Despite their apparent lack of effect in bulk lipids, anthocyanins exhibit good free radical-scavenging activities and are powerful antioxidants in lipid-containing hydrophilic environments such as emulsified lipids, liposomes, and LDL (Heinonen, 2007). Indeed, anthocyanin isolates and anthocyanin-rich mixtures of bioflavonoids were shown to provide protection from lipid peroxidation (Ramirez-Tortosa et al., 2001; Acquaviva et al., 2003; Lazze et al., 2003). However, the antioxidant effect of anthocyanins will vary significantly across species and cultivars of cranberries. Dimeric and oligomeric B-type procyanidins possess greater scavenging activity toward peroxyl radicals than catechins, which is probably due to the presence of their interflavonoid linkage that increases the electron delocalization capacity of the phenyl radical (Ursini et al., 2001). Polymerized chains of catechin monomers that form procyanidin trimers usually have decreased ability to prevent radical damage in the lipid system, but their antioxidant activity is increased in the aqueous phase (Plumb et al., 1998).

In cranberry, anthocyanins are among the principal antioxidant constituents, although hydroxycinnamates such as chlorogenic acid, flavonols, and proanthocyanidins are also effective antioxidants (Heinonen, 2007). Indeed, Zheng and Wang (2003) have reported that the anthocyanins contributed 54.2% of the antioxidant activity of cranberries, whereas the flavonols contributed 34.6% of the observed antioxidant activity. When Porter et al. (2001) compared the antioxidant activity of six fractions of cranberry phenolic compounds, cinnamic acid derivatives, anthocyanins, flavonols, and proanthocyanidins, they found that the fractions containing proanthocyanidin trimers through heptamers and pentamers through nonamers, more efficiently inhibited the oxidation of low-density lipoproteins. Eighty-seven percent of the formation of methyl linoleate hydroperoxide was inhibited by a 100 ppm catechin-proanthocyanidin-rich fraction obtained from cranberry (Määttä-Riihinen et al., 2005). According to Yan et al. (2002), the cranberry extract composed primarily of flavonol glycosides showed the best inhibition of oxidative processes measured by DPPH assay (EC_{50} at 30-40 μg/mL) compared to anthocyanin-rich cranberry extract or to crude phenolic cranberry extract. From the

compounds isolated from cranberry extract by He and Liu (2006), quercetin, 3,5,7,3',4'-pentahydroxyflavonol-3-O-β-D-glucopyranoside, 3,5,7,3',4'-pentahydroxyflavonol-3-O-β-D-galactopyranoside, and 3,5,7,3',4'-pentahydroxyflavonol-3-O-α-L-arabinofuranoside, showed potent antioxidant activities, with lower median effective concentration, EC_{50}, values of approximately 10 μM.

Although it was initially hypothesized that one of the biological effects of cranberry phenolics was related to their antioxidant activity, more and more studies suggest that another variety of mechanisms accounts for their health properties (Neto, 2007). According to a review of 93 intervention studies concerning the relevance of phenolics antioxidant protection in humans, the ingestion of cranberry juice was reported to increase plasma antioxidant activity and decrease the formation of lipid-oxidation products (Heinonen, 2007). Despite these positive results, studies indicate that even with very high flavonoid intakes, plasma and intracellular flavonoid concentrations in humans tend to be 100 to 1000 times lower than concentrations of other antioxidants, such as ascorbate or glutathione. Moreover, most circulating flavonoids are actually flavonoid metabolites, some of which have lower antioxidant activity than the parent flavonoids. For these reasons, the relative contribution of cranberry phenolics to plasma and tissue antioxidant function in vivo is likely to be relatively minor (Higdon, 2007). Although it was initially hypothesized that the biological effects of phenolic compounds would be related to their antioxidant activity, more and more studies suggest that their health properties may involve another variety of mechanisms (Neto, 2007).

Antimutagen and Anticarcinogen Properties

Current evidence available from cell culture experiments suggests that many of the biological effects of cranberry phenolic compounds are related to their ability to modulate cell signalling pathways. Cells are capable of responding to a variety of different stresses or signals by increasing or decreasing the availability of specific proteins. The complex chain of events that leads to changes in the expression of specific genes is known as cell signalling pathways or signal transduction pathways. These pathways regulate numerous cell processes, including growth, proliferation, and death (apoptosis). Effective signal transduction requires proteins known as kinases that catalyze the phosphorylation of target proteins at specific sites. Cascades involving specific phosphorylations or dephosphorylations of signal transduction proteins ultimately affect the activity of transcription factors—proteins that bind to specific response elements on DNA and promote or inhibit the transcription of various genes (Higdon, 2007).

It is believed that, through this mechanism, phenolics could interfere in several steps leading to the development of malignant tumors, including the inactivation of carcinogens and the inhibition of mutant gene expression. Many studies have also shown that they can activate detoxification enzymatic systems (Phase II) and prevent oxidative damage to the DNA, an important factor in the age-related development of some cancers (Mitscher et al., 1996; Bravo, 1998; Halliwell, 1999; Vattem et al., 2005a; Vattem and Shetty, 2005). Apoptosis or programmed cell death is another major mechanism through which phenolics could suppress cancer (Ramos et al., 2005). Numerous studies with cell cultures have shown that phenolics, through their effect on cell signalling pathways and by selectively inhibiting kinases, may alter growth factor signalling by inhibiting receptor phosphorylation or blocking receptor binding by growth factors (Higdon, 2007). Reports on quercetin in particular have demonstrated its ability to inhibit cancer cell line proliferation in vitro, including breast, colon, pancreas, and leukemia. When looking at the effect of quercetin on cancer cell proliferation, studies observed apoptosis in HepG2 hepatoma and colorectal cells, with the arrest of the HepG2 cell cycle in G1 phase; inhibition of the epidermal growth factor receptor expression and associated tyrosine kinase activity; reduced expression of Ras protein in colon cancer cells and primary colorectal tumors; increased expression of endogenous inhibitors of matrix metalloproteinases; and phytoestrogenic activity involving interaction with the estrogen α- and β-receptors of human mammary MCF-7 cells (Neto, 2007). Anthocyanins have been shown to be a major contributor toward the induction of apoptosis (Seeram and Heber, 2007). Anthocyanin isolates and anthocyanin-rich mixtures of bioflavonoids may also provide protection from DNA cleavage and enzyme inhibition (Ramirez-Tortosa et al., 2001; Acquaviva et al., 2003; Lazze et al., 2003). A refined bioassay designed to assess the potential antitumor activity of compounds showed how a complex mixture containing (–)-epicatechin, (+)-catechin, dimers of gallocatechin and epigallocatechin, and a series of proanthocyanidin oligomers could inhibit phorbol ester-mediated ornithine decarboxylase (Dixon et al., 2005). Ellagic acid has also been shown to be a potent anticarcinogen agent. One of the main mechanisms by which ellagic acid is proposed to have anticancer benefits is by modulating the metabolism of environmental toxins, thereby preventing initiation of carcinogenesis induced by these chemicals. Ellagic acid antimutagenic activity was proposed as the mechanism through which it inhibits the direct binding of these carcinogens to the DNA (Teel et al., 1986; Zhang et al., 1993; Vattem and Shetty, 2005). Studies have shown that ellagic acid is a potent inhibitor of DNA topoisomerases, which are other types of enzymes involved in carcinogenesis (Vattem and Shetty, 2005).

Studies reporting in vitro and in vivo antitumor and antiproliferative activity of cranberry have implicated flavonoid-rich extracts as contributing to these activities (Neto, 2007; 2008). Seeram et al. (2004) reported that total cranberry extract (200 μg/mL) could inhibit the proliferation of human oral, colon, and prostate tumor cells in vitro. Similar effects were also observed with some purified cranberry fractions, including the total phenolic fraction (200 μg/mL), the anthocyanin fraction (7.1 μg/mL), and the proanthocyanidin fraction (6.5 μg/mL). Quercetin (40.90 $\pm$ 1.12 μM) and

quercertin glucoside 3,5,7,3',4'- pentahydroxyflavonol-3-O-β-D-glucopyranoside (49.22 $\pm$ 4.90 μM) isolated from cranberry extract were also reported to inhibit the proliferation of HepG2 human liver cancer cells. These same compounds, quercetin (137.46 $\pm$ 2.5 μM) and 3,5,7,3',4'- pentahydroxyflavonol-3-O-β-D-glucopyranoside (23.90 $\pm$ 3.86 μM) also inhibited the proliferation of MCF-7 human breast cancer cells (He and Liu, 2006). Cranberry anthocyanins could play a role in the fight against cancer through their displayed anti-angiogenic properties (Roy et al., 2002; Bagchi et al., 2004). An anthocyanin-rich extract (55.96 $\pm$ 5.66 pg/mL) obtained from a mixture of berries, including cranberries, inhibited the tumor necrosis factor alpha-induced expression of vascular endothelial growth factor, and decreased hemangioma formation and tumor growth (Bagchi et al., 2004). Proanthocyanidin oligomers from whole cranberry fruit, in a size up to 12 degrees of polymerization and with as many as four A-type linkages, also showed tumor antiproliferative activity (Neto, 2007). A proanthocyanidin fraction from whole cranberry fruit was observed to selectively inhibit the growth of H460 human large cell lung carcinoma, HT-29 colon adenocarcinoma, ME-180 cervical carcinoma cells and K562 chronic myelogenous leukemia cells with GI_{50} in the range of 20–110 μg/L, while a proanthocyanidin subfraction containing oligomers composed primarily of 4–7 epicatechin units with at least 1 or 2 A-type linkages could inhibit the growth of H460 lung tumors, HT-29 colon, and K562 leukemia cells at GI_{50} values ranging from 20 to 80 μg/ml (Neto et al., 2006).

Other in vitro evidence suggests that cranberry phenolics could prevent cancer by decreasing cell invasion and metastasis by inhibiting matrix metalloproteinase activity, and inhibiting the inflammatory processes including cyclooxygenase activity (Neto, 2007). It seems that the presence of A-type linkages can influence the tumor inhibitory and selectivity properties of proanthocyanidins. Indeed, when screening a variety of smaller polyflavan-3-ols from different plants against GLC4 lung and COLO 320 colon carcinomas, Kolodziej et al. (1995) found that a trimer with an A-type linkage was more cytotoxic than dimers with A-type linkages and trimers with only B-type linkages. Overall, the tumor inhibition by cranberry is likely to involve synergistic activities between the cranberry major phytochemicals—the anthocyanins, the flavonols, and the proanthocyanidins.

CONCLUSIONS AND FUTURE PROSPECTS

The data available strongly suggest that cranberry consumption could help protect against cardiovascular diseases, various cancers, and infections involving the urinary tract disorders, dental health, and Helicobacter pylori-induced stomach ulcers and cancers. Through the study of the biological properties of cranberry phytochemical, considerable progress has been made in the understanding of these potential health benefits. The cranberry phytochemical components, the flavonoids, flavonols, and flavan-3-ols, the anthocyanins, the tannins (mainly proanthocyanidins), and phenolic acid derivatives, working in synergy, appear to be responsible for the antioxidant, antibacterial, and antimutagen properties observed mainly in vitro, but also in animal experimental studies and clinical studies. Confirmation of these hypotheses, through mechanistic and clinical studies, may lead to further development of cranberry-based nutraceutical and pharmaceutical preventive and therapeutic treatments.

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