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Review

Flavan-3-ols: Nature, occurrence and biological activity

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Representing the most common flavonoid consumed in the American diet, the flavan-3-ols and their polymeric condensation products, the proanthocyanidins, are regarded as functional ingredients in various beverages, whole and processed foods, herbal remedies and supplements. Their presence in food affects food quality parameters such as astringency, bitterness, sourness, sweetness, salivary viscosity, aroma, and color formation. The ability of flavan-3-ols to aid food functionality has also been established in terms of microbial stability, foamability, oxidative stability, and heat stability. While some foods only contain monomeric flavan-3-ols [(−)-epicatechin predominates] and dimeric proanthocyanidins, most foods contain oligomers of degree of polymerization values ranging from 1 – 10 or greater than 10. Flavan-3-ols have been reported to exhibit several health beneficial effects by acting as antioxidant, anticarcinogen, cardiopreventive, antimicrobial, anti-viral, and neuro-protective agents. This review summarizes the distribution and health effects of these compounds.

Keywords: Catechins / Condensed tannins / Flavan-3-ols / Health effects / Proanthocyanidins

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1 Introduction

The most common group of flavonoids in the diet, flavan-3-ols are considered functional ingredients of beverages, fruits and vegetables, food grains, herbal remedies, dietary supplements, and dairy products. Flavan-3-ols have been reported to exhibit several health beneficial effects by acting as antioxidant, anticarcinogen, cardiopreventive, antimicrobial, anti-viral, and neuro-protective agents. Despite the plethora of data demonstrating positive effects of flavan-3-ols consumption, reports also associate flavan-3-ol consumption with dietary burden and health deleterious effects. This review presents information on flavan-3-ol rich food sources, consumption, bioavailability and metabolism, as well as flavan-3-ol function in prevention, amelioration, and possible induction of human disease according to chemical structure.

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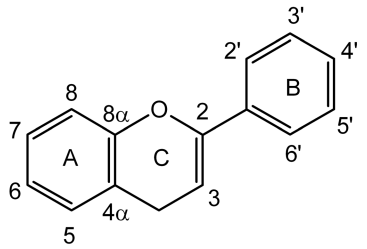
Abbreviation: **DP**, degree of polymerization

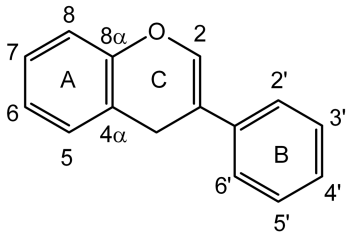
2 Flavonoids

Largely investigated for their health beneficial activity, the flavonoids comprise a group of phenolic secondary plant metabolites characterized by a phenylbenzopyran chemical structure [1]. The general chemical structure (Fig. 1) includes a C_{15} (C_6-C_3-C_6) skeleton joined to a chroman ring (benzopyran moiety) that in turn bears an aromatic ring at C-2, C-3 or C-4. The heterocyclic benzopyran ring is known as the 'C' ring, the fused aromatic ring as the 'A' ring, and the phenyl constituent as the 'B' ring. The A ring can be of two types: a phloroglucinol type that is *meta*-trihydroxylated or a resorcinol type that is *meta*-dihydroxylated [2, 3]. The B ring can be monohydroxylated, ortho-dihydroxylated or vicinal-trihydroxylated. The center heterocycle most commonly exists in one of three forms: pyran, pyrilium, or γ-pyrone [2]. The position of the chroman-aromatic linkage determines benzopyran class (Fig. 1): 2-phenylbenzopyrans comprise the flavonoids, 3-phenylbenzopyrans the isoflavonoids and 4-phenylbenzopyrans the neoflavonoids. All three groups share a chalcone precursor (Fig. 2) that exists in an open chain isomeric form. Flavonoids with a five-member heterocycle ring are referred to as aurones. Other flavonoid modifications include methoxylations, hydroxyl group O-glycosylation, C-glycosylation, presence of prenyl or alkyl groups covalently linked to flavonoid moieties and additional rings condensed to the flavonoid core [1].

2-phenylbenzopyran: Flavonoid



3-phenylbenzopyran: Isoflavonoid



4-phenylbenzopyran: Neoflavonoid

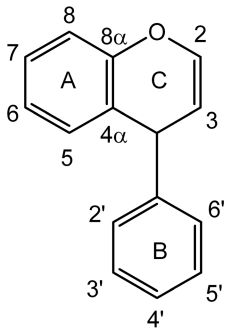


Figure 1. Flavonoid classes.

Based on C-ring saturation and oxidation status, the 2-phenylbenzopyrans further divide into eight groups: flavan, flavanone, flavone, flavonol, dihydroflavonol, flavan-3-ol, flavan-4-ol and flavan-3,4-diol (Fig. 2). Representing the largest and most ubiquitous class of monomeric flavonoids, the flavan-3-ols comprise the major constitutive units of condensed proanthocyanidins [1, 4].

2.1 The condensed tannins: proanthocyanidins

Hemingway and Karchesy [5] divide the proanthocyanidins into two categories based on their A ring classification: phloroglucinol and resorcinol (Fig 3). The proapigeninidins, propelargonidins, proluteolinidins, procyanidins, prodelphinidins, and protricetinidin all possess a phloroglucinol type A ring. Proguibourtinidin, proteracacidin, profisetinidin, promelacacidin and prorobinetinidin all possess a resorcinol type A ring. Proapigeninidins, lacking a hydroxyl group at C-3, and proluteolinidins are considered rare. Proteracacidin has not been found in plants while profisetinidin is generally restricted to leguminosae and anacardiaceae. Procyanidins and prodelphinidins (Fig. 4) represent the most common phloroglucinol type proanthocyanidins found in nature, with 3′,4′-dihydroxy and 3′,4′,5′-trihydroxy substitution patterns of the B ring [6].

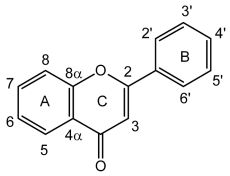
2.2 Proanthocyanidin nomenclature

Various terms have been used to describe polymeric flavan-3-ols in literature. As such, nomenclature used to describe these compounds can be confusing and is often erroneously employed [6]. Of the various terms used in reference to proanthocyanidins (condensed tannins, vegetable tannins, flavans, flavolans, polyflavans, catechins, macromolecular phenolic substances, leucoanthocyanidins, condensed proanthocyanidins, polymeric proanthocyanidins, oligomeric proanthocyanidins, procyanidins, procyanidolic oligomers, plant polyphenols, and pycnogenols) [3, 4, 6, 7], the terms ‚proanthocyanidins' and 'condensed tannins' are used most frequently in literature. However, the use of the term 'tannin' must be carefully considered as its definition lends itself to be used ambiguously in reference to other classes of plant polyphenols such as the hydrolysable and complex tannins [6, 7]. The IUPAC system also works in naming proanthocyanidins and, although its use becomes troublesome in naming larger molecules, it becomes useful in cases that necessitate defining absolute stereochemistry at all chiral centers. Location and stereochemistry about the interflavonoid bond can be denoted with bracketed symbols α and β and because most common flavan-3-ols exist as 2*R* isomers, less common 2*S* isomers are labeled with the prefix '*ent*'. Proanthocyanidins possessing a glycone are given a qualifying ending of '-in', while those lacking a glycone are qualified with an ending of 'idin' [2].

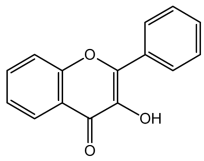
2.3 Proanthocyanidin stereochemistry (Fig. 5)

The hydroxylation on the A-ring strongly influences stereochemistry at C-4. Procyanidins and prodelphinidins with 2,3-*cis* stereochemistry frequently adopt 3,4-*trans* stereochemistry during interflavanoid bond formation with C-4→C-8 or C-4→C-6 interflavanoid linkages occurring in relative proportion of 3:1 [5]. Dimers with these types of linkages are termed 'B'-type and include the following: Procyanidin B-1 ((−)-epicatechin-(4β→8)-(+)-catechin), B-2 ((-)-epicatechin-(4β→8)-(−)-epicatechin), B-3((+)-catechin-(4α→8)-(+)-catechin), B-4 ((+)-catechin-

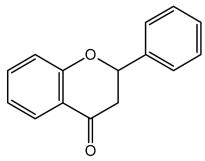
Flavone



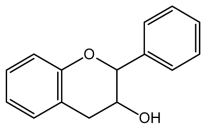
Flavonol



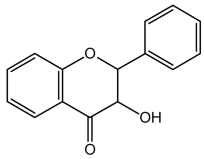
Flavanone



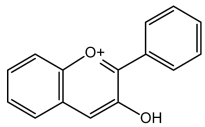
Flavan-3-ol



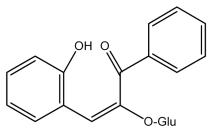
Flavanonol



Anthocyanidin



Chalcone



Aurone

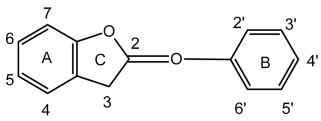
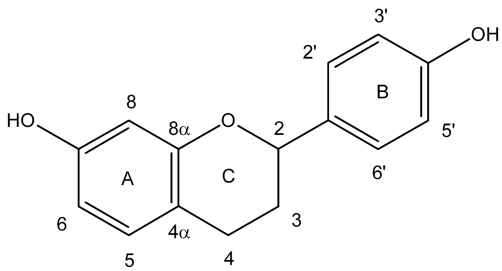
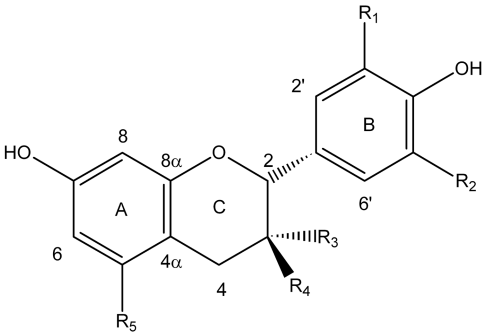


Figure 2. Flavonoid subclasses (the 2-phenylbenzopyrans).



Proanthocyanidin type	3	5	8	3′	5′
Procassinidin	H	H	H	H	H
Probutinidin	H	H	H	OH	H
Proapigeninidin	H	OH	H	H	H
Proluteolinidin	H	OH	H	OH	H
Protricetinidin	H	OH	H	OH	OH
Propelargonidin	OH	OH	H	H	H
Procyanidin	OH	OH	H	OH	H
Prodelphinidin	OH	OH	H	OH	OH
Proguibourtinidin	OH	H	H	H	H
Profisetinidin	OH	H	H	OH	H
Prorobinetinidin	OH	H	H	OH	OH
Proteracacinidin	OH	H	OH	H	H
Promelacacinidin	OH	H	OH	OH	H

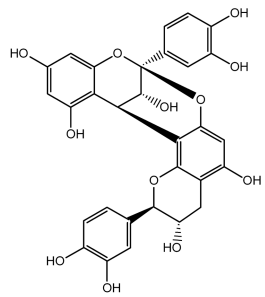
Figure 3. Proanthocyanidin types.



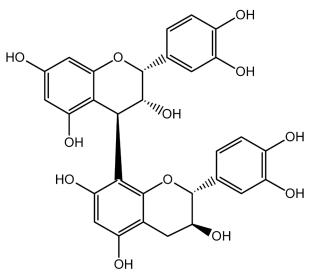
2*R* Flavan-3-ol Monomers	R_1	R_2	R_3	R_4	R_5
(+)-afzelechin	H	H	H	OH	OH
(-)-epiafzelechin	H	H	OH	H	OH
(+)-catechin	H	OH	H	OH	OH
(-)-epicatechin	H	OH	OH	H	OH
(+)-gallocatechin	OH	OH	H	OH	OH
(-)-epigallocatechin	OH	OH	OH	H	OH
(+)-fisetinidol	H	OH	H	OH	H
(-)-epifisetinidol	H	OH	OH	H	H
(+)-robinetinidol	OH	OH	H	OH	H

Figure 4. Flavan-3-ol monomer examples. Most natural flavan-3-ols are 2*R* isomers; the less common 2*S* isomers carry the prefix '*ent*'.

A-type Dimer (A1)



B-type Dimer (B-1)



C-type Proanthocyanidin

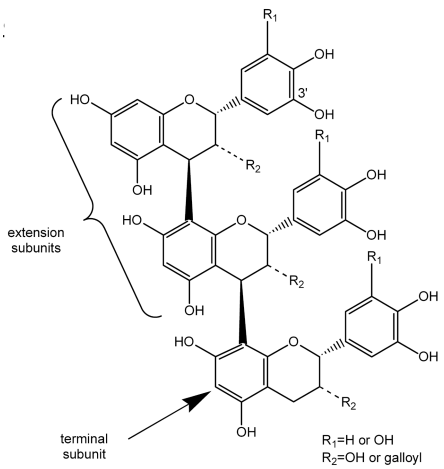


Figure 5. Procyanidin examples.

(4α→8)-(−)-epicatechin) and B-5, a regioisomer of the B-2 dimer that possesses a C-4→C-6 interflavan linkage. Trimers, or 'C' type proanthocyanidins, such as C-1 consist of three flavan-3-ol units linked by two C-4→C-8 interflavan bonds [8]. Another group of proanthocyanidin dimers, the 'A' type, are more rigid in conformation than the B class due to possession of two interflavan linkages: one C-C and the other C-O. Examples of 'A' type proanthocyanidins are: A-1 ((−)-epicatechin (2β→O-7; 4β→8)-(+)-catechin) and A-2 ((−)-epicatechin (2β→O-7;4β→8)-(−)-epicatechin) [9, 10].

Typically 2,3-*trans* stereochemistry dominates the chain extender units although a minority exist in the 2,3-*cis* formation. Proanthocyanidin oligomers most commonly terminate with (+)-catechin subunits [5]. Soluble proanthocyanidin polymers typically possess MW averages of 1000–6000, but sometimes possess MW as high as 20 000 [3].

2.4 Flavan-3-ol function in plants

During the 1960's, researchers feverishly investigated tannins to determine their biological role in plants. In 1973, Bate-Smith proposed that 'vegetable tannins' possess an astringent property that renders plant material unpalatable to both animals and microbes. The precipitation of proteins is what renders tannins astringent, a characteristic vital to plant defense against pathogens. Flavan-3-ol ability to chelate metals such as iron and other essential minerals also reportedly limits growth of invasive microorganisms by causing severe essential mineral-depletion, as has been documented in bacteria [11]. The presence of (−)-epicatechin in plant tissue may also provide a similar resistance against fungal attack. In the case of an invasive species of spotted knapweed, some plant species utilize flavan-3-ols such as *ent*-catechin [(-)-catechin] to prevent proliferation of neighboring plant species. The *ent*-catechin is said to trigger active oxygen production as well as calcium signaling cascades that ultimately promote cell death of neighboring plants' root cells [12, 13]. Flavan-3-ols have also been implicated in a plant's ability to tolerate high lead levels, as demonstrated by the fern *Athyrium yokoscense*. The fern can tolerate over 1000 μg of lead per gram of dry matter by storing proanthocyanidin-lead complexes in its rhizoids [14]. Today, the generally accepted biological role of flavan-3-ols in plants relates to their protection against harmful intruders such as microbes, fungi, insects and herbivorous animals [6, 15].

3 Flavan-3-ols, food, consumption, and bioavailibility

3.1 Effect on food quality

According to Santos-Buelga and Scalbert [16], proanthocyanidins are the most common group of tannins in our diet. Their presence in foods greatly affects food quality. Astringency, defined as a drying or puckering sensation in the mouth results from the interaction of tannins with salivary proline-rich proteins [17]. It has been suggested that the ability of proline rich proteins to bind tannin may be an evolutionary adaptation to deal with dietary tannin burden. Peptide models of salivary proline-rich proteins indicate that tannins of both low and high molecular mass elicit an astringent response [18]. Moreover, astringent substances may also interact with bitter receptors, contribute to sourness, sweetness, or modulate saliva viscosity [19]. Proanthocyanidins present in dairy products have been noted to contribute to both desirable and undesirable taste, odor and color [20]. The ability of flavan-3-ols to aid food functionality has also been established in terms of microbial stability, foamability, oxidative stability, and heat stability [20].

3.2 Food sources

Flavan-3-ols can be found in common foodstuffs as well as in herbal remedies, and dairy products. Quantitative data on the proanthocyanidin content of dietary supplements, botanicals, and herbals is not as abundant as that concerning food sources. Beecher's review on proanthocyanidin biological activity associated with human health provides a good summary of botanical related research conducted prior to 2004. The 1990 CRC release, Dietary Tannins Consequences and Remedies [21] provides a chapter on the occurrence, nature and composition of proanthocyanidins in cereals, legumes, fruits, vegetables, forages and beverages such as wine and tea. Due to limitations in phenolic extraction methodology, most concentrations are reported as total-phenols or catechin equivalents and as a result are not particularly helpful in evaluating consequences of dietary intake.

Not surprisingly, much attention has historically been given to red wine, tea, chocolate, grapes and apples due to their common consumption in the United States [22]. However, few investigations sought to quantify food proanthocyanidins on a broader scale until more recently. De Pascual-Teresa *et al*. [23] quantified flavan-3-ol concentrations in 56 different kinds of Spanish Food products including fruit, vegetables, legumes, beverages, and chocolate. This work provides data on the content and distribution of 15 different flavanols (monomers, dimers and trimers) as well as the detection of 6 unquantified flavanols. Of the foods analyzed, the highest flavanol contents were found in broad beans (154.5 mg/100 g fresh weight), while fruits possessed 10 to 50 mg/100 g fresh weight, and vegetables ranged from 0 to 154 mg/100 g fresh weight. (−)-Epicatechin appeared most prevalent in analyzed samples, followed by (+)-catechin and procyanidin B-2. Catechins were present in the majority of samples, while gallocatechins were detected only in some, and galloyled flavan-3-ols in even fewer samples.

Gu *et al*. [22] report concentrations of flavan-3-ols in common American foods along with estimations of normal consumption. The 41 foods studied include sauces, juices, cereal, wine, beer, nuts and infant foods. Detected flavan-3-ols were quantified and categorized according to degree of polymerization (DP): monomers, dimers, trimers, 4 – 6 mers, 7 – 10 mers and >10 mers. While some foods appear to only contain monomers and dimers, most foods were found to contain flavan-3-ols of DP values ranging from 1 – 10 with DP greater than 10 found in 21 of the tested food sources. Additionally, homogenous B-type proanthocyanidins were found exclusively in 20 of the food types, while procyanidins were found exclusively in 7 of the food types (a small fraction being A-type or gallic acid esters). The propelargonidins and prodelphinidins were only found as minor components of the tested foods. Conclusive evidence of this study indicates that up to 25% of infant cereals, 90% of infant juices, and 85% of fruit based infant foods contain flavan-3-ols, with epicatechin as the most predominant flavan-3-ol.

O'Connel and Fox [20] have investigated and reviewed the occurrence of phenolic compounds in milk and dairy products. Their 2001 publication discusses the implication of both indigenous and exogenous polyphenols on dairy product quality. Although research in this area over the last half century appears scattered and incomplete, evidence suggests that indigenous polyphenols of dairy products play a role in the formation of distinctive flavors and aromas of specialty cheeses. Indigenous dairy polyphenols appear to be simple in nature, with specific profiles dependent on grazing patterns of the milk-producing animals [24]. Exogenous polyphenols, namely tea and wine catechins such as epigallocatechin, epicatechin-gallate, epigallocatechin-gallate, and epicatechin, have also been used in dairy product processing. Such incorporation of catechins has proven to inhibit oxidative rancidity in milk products and been useful in the isolation of whey and cream. Ironically, whey isolated in this manner can be re-incorporated into fermenting musts during wine production [20, 25].

In 2005, Prior and Gu [26] published a review regarding the flavan-3-ol content and biological significance in the American diet. Data from their research helped to establish the flavan-3-ol database posted on the USDA Nutrition Data Laboratory website: http://www.nal.usda.gov/fnic/foodcomp. Their evaluation of varying degrees of flavan-3-ol polymerization from monomer to decamer, as well as polymer, allowed comprehensive determination of both occurrence and structural diversity of flavan-3-ols in foods. Foods in this study were analyzed by normal phase HPLC according to the method of Gu *et al*. [22]. Of 102 food types sampled, most foods were found to exclusively contain homogeneous B-type proanthocyanidins. Foods containing heterogeneous proanthocyanidins were classified into three groups: the propelargonidin, the prodelphinidin, and the A-type proanthocyanidin groups. MS/MS was implemented in order to determine connective sequence of heterogeneous proanthocyanidin oligomers. Only cranberry, peanut, plum, avocado and curry were found to contain A-type proanthocyanidins, with some novel structures identified in cinnamon.

3.3 Factors affecting flavan-3-ol content of food

Flavan-3-ol content in food can be affected by many factors such as environmental, food processing, and food storage conditions. Higher flavan-3-ol levels generally appear in fresh fruits than in either dried or cooked fruits, and in some cases environmental variation can explain large flavan-3-ol content disparity within species, as has been noted in fresh berry fruits. Epimerization, degradation and de-polymerization of oligomers and polymers have been known to occur during food processing and storage as well [26 – 28]. For example, epimerization of (–)-epicatechin to (–)-catechin has been observed to occur in cocoa product processing and transformation of B-type to A-type procyanidin dimers has been linked to oxidation of processed blueberry fruit [27].

Moreover, flavanol content variations, as seen in teas, wine and cocoa, have been attributed to several influences including sample origin, variety, degree of ripeness, refrigeration practice, processing, as well as fat, protein and carbohydrate content [20, 23, 24, 28]. Cooking and industrial food-processing practices such as fruit peeling, dehulling of seeds, decortication and bolting of cereals, grinding, juice filtration and berry maceration can also decrease or chemically modify total flavan-3-ol content [29]. The addition of exogenous polyphenols to foods during production, namely tea and wine catechins such as epigallocatechin, epicatechin-gallate, epigallocatechin-gallate, and epicatechin, can additionally alter food flavan-3-ol content [20, 25].

3.4 Food consumption

Several reviews have been written on the topic of flavan-3-ol consumption and bioavailability. Daily proanthocyanidin intake can vary from 10 mg to 0.5 g/day, with dimers B-1 and B-2 most likely consumed [30, 31]. An estimated 74% of ingested proanthocyanidins possess DP >3 [22]. The estimated mean daily proanthocyanidin intake in the United States is 53.6 mg/person/day excluding monomers and 57.7 mg/person/day including monomers. Proanthocyanidin intake also varies with age and gender [22, 26].

3.5 Flavan-3-ol bioavailability

Upon ingestion, flavan-3-ols first react with proline-rich proteins in the mouth to elicit an astringent response. Those that cross the intestinal barrier travel to the liver via the portal vein, where they further degrade into metabolites and can possibly reach all tissues within hours following con-

sumption as seen in radiolabeled experiments with live rats. Confirmation of the presence of flavan-3-ol low-molecular weight metabolites in the urine and feces of these rats, as well as in chickens and sheep, indicates that polymeric proanthocyanidins may not absorb through the intestinal barrier without first being degraded into low-molecular weight metabolites, most probably by gut microflora [11, 32 – 34]. Although it has been suggested that metabolism of proanthocyanidin polymers (specifically dimer B-3 and trimer C-2) by intestinal microflora is limited [35], human fecal microflora, grown under anaerobic conditions *in vitro,* have shown the ability to degrade proanthocyanidins (mDP = 6) to low-molecular weight metabolites within 48 h [30]. A variety of metabolites such as phenolic acids and lactones reportedly form through fission of the heterocyclic and A-rings, however, the absence of a free hydroxyl at C-5, 7 or 4′ may prohibit cleavage [29, 36 – 38]. A study investigating metabolite excretion in the urine of healthy human subjects revealed that consumption of chocolate [439 mg proanthocyanidins and 147 mg (+)-catechin] results in increased excretion of six flavanol-derived acids [34]. Urine analysis of 69 human subjects after consumption of grape seed extract supplement over 6 weeks (1000 mg/day total polyphenols) corroborated these results, indicating three phenolic acids as breakdown products of proanthocyanidin metabolism: 3-hydroxyphenylpropionic acid, 4-O-methylgallic acid and 3-hydroxyphenylacetic acid [38]. These phenolic acid metabolites are then said to be absorbed or conjugated with glycine, glucuronic acid or sulfate.

Of all the classes of flavonoids, proanthocyanidins appear to be the least well absorbed, 10- to 100-fold less than their monomeric constituents [39]. Reportedly (+)-catechin, dimer B-3 and trimer C-2 are absorbed similarly, while polymers (mDP = 7) are not as well absorbed by the human intestine due to their lower permeability through paracellular absorption (movement of ions through intercellular spaces between epithelial cells), and their likely complexation with luminal and mucosa proteins [16, 31, 36].

Flavan-3-ols, like most other flavonoids, exist in plasma predominantly in their conjugated forms [36], as has been confirmed by multiple investigations. Recent exploration on flavan-3-ol concentrations in the liver, kidney, brain, gastrointestinal tract, plasma, urine and feces of rats indicates that while parent flavan-3-ols and procyanidins may be found in the gastrointestinal tract and sulfated metabolites, as well as procyanidin dimers (B-1, B-2, B-3 and B-4) and trimer (C-2) may be detected in urine, only catechin glucuronides and methylated glucuronide metabolites appear to reach the plasma, liver and kidneys. Although, this investigation did not provide conclusive evidence that flavan-3-ol metabolites reach brain tissue [39], published evidence suggests that cocoa-derived flavanols may interact favorably at the blood/brain barrier [40]. Another recent investigation synthesized oligomeric proanthocyanidin-like compounds linked with ethylidine bridges via acetaldehyde

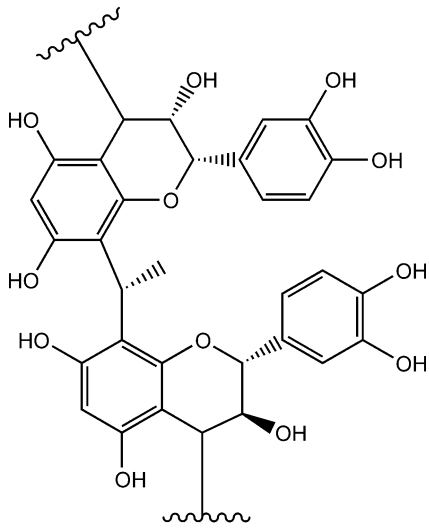


Figure 6. Ethylidene-bridged flavan-3-ol.

induced polymerization of (–)-epicatechin (Fig. 6) in order to investigate the effect of proanthocyanidin structure-related *in vivo* health benefits. The synthetic proanthocyanidin-like material consisted of dimers, trimers, tetramers, and nanomers. Following consumption of 200 mg/kg body weight of the synthetic mixture, male Wistar rats were subject to plasma and liver analysis. Within 1 h of consumption tetramethylated dimeric procyanidin was found in the rats' plasma. Within 2 h of consumption the methylated dimer could be detected in the rats' plasma (14 mg/L) and the liver (15 μg) [41]. Similarly, an investigation on the absorption of orally administered apple procyanidins in rat plasma revealed that procyanidins of various DP (dimer to pentamer) reach a maximum concentration in the plasma within 2 h [42].

4 Flavan-3-ols and health

4.1 Antioxidant character of flavan-3-ols

Flavan-3-ols have been shown to behave as antioxidants via several mechanisms including the scavenging of free radicals, chelation of transition metals, as well as the mediation and inhibition of enzymes [43].

The generation of free radicals by living systems can potentially cause oxidative damage to DNA, lipids, carbohydrates and proteins, to eventually result in impairment of cellular function that in turn causes aging and initiates onset of disease [4, 15, 44, 45]. Reactive-oxygen species can be oxygen or nitrogen radicals, or non-radicals that either oxidize or easily convert to oxidizing radicals. Common radicals include superoxide anion, hydroxyl, alkoxyl, peroxyl and nitric oxide. Examples of non-radicals include hydrogen peroxide, hypochlorous acid, ozone, singlet oxygen, and peroxynitrite.

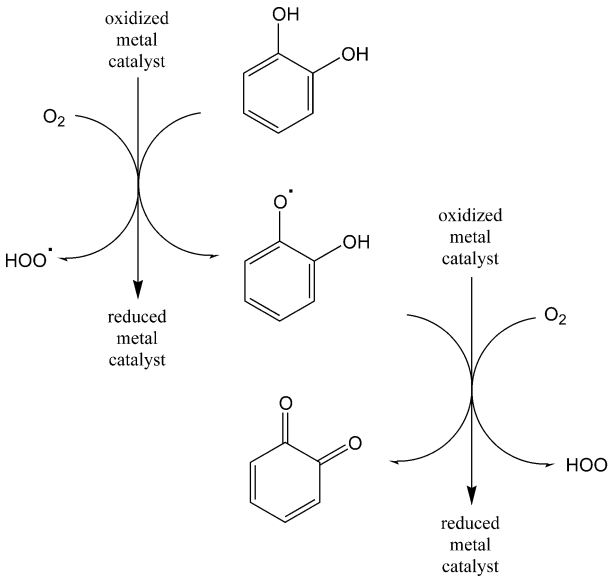


Figure 7. Phenolic oxidation and radical formation.

The electronic configuration of flavan-3-ols allows for easy release of electrons to free radical species ($R^{\bullet}$) (Fig. 7). By release of electrons the radical character of the reactive-oxygen species is transferred to the flavan-3-ol ($F^{\bullet}$). Often considered better than vitamins C and E, flavan-3-ols and their microbial phenolic acid metabolites are particularly good antioxidants in that the derived radical is generally more stable and less harmful than the initial radical species [46, 47]. Moreover, the preferable oxidation of flavan-3-ols has been shown to protect ascorbic acid under both neutral and alkaline conditions [46, 47]. Flavan-3-ols have been suggested to be superior to flavonols in their antioxidant capacity because oxidation of flavan-3-ols predominantly produces semiquinone radicals that couple to produce oligomeric compounds through nucleophilic addition [48]. Coupling in this manner retains the number of reactive catechol/pyrogallol structures and effectively preserves scavenging ability [48, 49]. Flavonols on the other hand form quinones more prone to redox-cycle with potential to behave as pro-oxidants [48]. Common free radicals used to test antioxidant activity include 1,1-diphenyl-2-picrylhydrazyl ($DPPH^{\bullet}$) and 2,2′-azinobis-3-ethylbenzthiazoline-6-sulfonic acid ($ABTS^{\bullet+}$). $ABTS^{\bullet+}$ is used in the trolox-equivalent antioxidant capacity (TEAC) assay. Electron spin resonance (ESR) is also commonly used to measure free radical activity [15, 34, 43, 49].

Flavan-3-ol structure determines relative ease of oxidation and free radical scavenging activity. Although the presence of galloyl groups, number and position of hydroxyl groups (based on redox potential) are said to enhance activity, methoxylation and glycosylation of position 3 apparently inhibit activity [44, 50–52]. While (–)-epicatechin is more easily oxidized than (+)-catechin, type of interflavanoid bond determines relative ease of oligomer degradation, with C-4→C-8 linked dimers such as B-3 and B-4 oxidizing more readily than their C-4→C-6 linked counterparts. However, dimers B-6 and B-7 oxidize less readily than B-1 and B-2 due to the nature of their lower structural monomeric unit [53]. Although, proanthocyanidin B-2 has been shown to scavenge hydroxyl radical and superoxide anion better than proanthocyanidin B-4 or (–)-epicatechin [54]. Additionally, flavan-3-ol antioxidant activity is said to increase from monomer to trimer and then decrease from trimer to tetramer [55, 56]. A recent study utilized full geometry optimizations to investigate the structure-property effect on antioxidant activity of (+)-catechin [57]. Full geometry optimizations considered the orientation of hydroxyl groups relative to the ring as well as the relative position of the aromatic systems of (+)-catechin. From the obtained structural data, the authors propose a deprotonation sequence for the most stable conformer of (+)-catechin: ring A (7-OH), ring B (4′-OH, 3′-OH), ring A (5-OH), ring C (3-OH). The authors note, however, that this proposal disagrees with previously proposed deprotonation sequences for catechin. Selected references related to the antioxidant activity of flavan-3-ols are listed in Table 1.

4.2 Metal chelating activity of flavan-3-ols

The presence of free state iron and copper in biological systems catalyzes free radical reactions such as Fenton and Haber-Weiss reactions [58]. In the Fenton reaction, iron catalyzes the generation of hydroxyl radicals. The ability of flavan-3-ols to bind such divalent transition metals effectively reduces the concentration of these cations and thus the extent of their oxidative activity [15, 59]. Work by Facino *et al*. [60] indicates that procyanidins strongly complex iron and copper cations with stability constants from 9 to 9.35 (log K) in preferred stoichiometric binding ratios of Fe^{+2}/procyanidin (2:1) and Cu^{+2}/procyanidin (4:1). Results of an investigation involving effect of flavan-3-ol hydroxylation patterns and degree of polymerization on aluminum chelating capacity reveal that hydroxyl groups are essential sites for metal chelation, *o*-dihydroxylphenyl groups of the B ring in particular, and that increasing the degree of polymerization leads to higher stability of tannin-metal complexes [61]. The resultant stability of catechin-metal chelate complexes has recently been tested using spectrophotometric analysis. Formation constants for catechin-copper chelate complexes from this study agree with previously reported data: (+)-catechin (KCu/CuL = 14.45). The work also allowed for construction of a Cu^{+2}/(+)-catechin equilibrium model for the formation of $[CuLH_2]$, $[CuLH]^{-}$, and $[CuL]^{-2}$ [57]. Metal-flavonoid complexes are also thought to play a cytoprotective role in superoxide radical scavenging because flavonoid-metal complexes are more effective than the parent flavonoid in scavenging superoxide radicals [62].

Table 1. List of references regarding proanthocyanidins as antioxidants

Proanthocyanidin type or source	Comment	Year	Authors
Red wine and green and black tea	Peroxy nitrite scavenger, effect of structure on antioxidant activity	1996	Rice-Evans *et al*. [51]
Vaccinium vitis-idaea (six tannins):	Anti-lipid peroxidation and periodontal disease	1999	Ho *et al*. [212]
Crataegus sinaica (Rosaceae) procyanidin C-1	Procyanidin C-1 had highest antioxidant activity in microsomal lipid peroxidation and the hydroxyl radical scavenging assay.	2002	Shahat *et al*.[229]
Cacao liquor: C-glycosidic flavan, O-glycosidic dimer and O-glycosidic A-linked trimers	DPPH scavengers	2002	Hatano *et al*. [230]
Carob pods: Condensed tannin as a functional food?	DPPH free radical scavengers, lipid peroxidation inhibitors	2002	Kumazawa *et al*. [231]
Grape Seed Proanthocyanidin	Ameliorates the toxic effects associated with chemo-therapeutic agents.	2000	Joshi *et al*. [139]
Grape Seed Proanthocyanidins	Provides protection against oxidative stress and free radical mediated tissue injury	2000	Bagchi *et al*. [140]
Phaseolus vulgaris L. (10 genotypes of colored dry beans)	Important source of dietary antioxidants	2003	Beninger and Hosfield [232]
Wine of red and white grapes and blueberry	Blueberry wine > red wine> white wine in antioxidants	2003	Sanchez-Moreno *et al*. [233]
Grape Seed proanthocyanidin	*In vitro*: GSPE scavenges H2O2, hydroxyl radical and superoxide and may chelate iron	2003	Shao *et al*. [82]
Grape Seed procyanidins	Protect endothelial cells from peroxynitrite damage	2003	Aldini *et al*. [99]
Red, white and sherry wines	Phenolic content relates to antioxidant activity, flavanol fractions more	2004	Fernandez-Pachon *et al*. [234]
Grape seed extracts	Attenuation of A beta-induced cytotoxicity, intracellular ROS accumulation and lipid peroxidation-fight Alzheimer's disease	2004	Li *et al*. [235]
Green and Black Tea extracts	Antiradical activity: Green tea extracts were 4x less effective. Ethanol extracts were 50% more effect.	2005	Gramza *et al*. [236]
Catechins	Catechins quench singlet oxygen	2005	Mukai *et al*. [237]
Geranium niveum A-type proanthocyanidins	Powerful radical scavengers *in vitro*	2005	Maldonado *et al*. [238]
Commercial grape juice, wine and vinegar	Red wine exhibits higher antioxidant activity than either grape juice or vinegar.	2005	Davalos *et al*. [239]
Grape seed extract: proanthocyanidin	Anti-aging drug prevents oxidative stress	2005	Sangeetha *et al*. [45]
Red wine extract: proanthocyanidin	Attenuate the degree of lipid peroxidation	2005	Fantinelli *et al*. [101]
Catechins	Structure-property studies on antioxidant activity.	2005	Teixeira *et al*. [57]
Stryphnodendron obovatum Benth: procyandin dimer	Antioxidant activity	2005	Sanches *et al*. [240]
Aronia melanocarpa: polymeric proanthocyanins	Antioxidant activity	2005	Oszmianski and Wojdylo [241]
Apple (8 cultivars): epicatechin and procyanidin B-2	Major contributors to antioxidant	2005	Tsao *et al*. [242]
Grape seed extract: proanthocyanidins	GSE stabilizes ascorbic acid under neutral and alkaline conditions	2006	Kitao *et al*. [47]
Carallia brachiata bark A-type trimer	Anti-radical against DPPH, superoxide radical and xanthine oxidase	2006	Phuwapraisirisan *et al*. [243]
Grape seed proanthocyanidin	Effective in attenuating lipid peroxidation and protein oxidation	2006	Devi *et al*. [244]
Lychee fruit proanthocyanidins: B4, B-2 and epicatechin	Hydroxyl radical and superoxide anion activities were good, B-2>B-4 and epicatechin	2006	Zhao *et al*. [54]
Vitis vinifera and *Vitis rotundifolia*: grape seed powder	Aqueous solvents work best for extraction	2006	Yilmaz and Toledo [245]
Vitis vinifera: grape seed powder	Flavans and procyanidins protect against superoxide radicals, hydroxyl radicals and singlet oxygen, low-density lipoprotein and copper induced oxidation.	2006	Janisch *et al*. [59]
Dietary Supplements: *Vitis vinifera* leaves and grape skins	40-43% decline in antioxidant capacity of grape skins over 60 days of storage	2006	Monagas *et al*. [246]
Mammea longifolia buds: spice used in India	Extracts possess antioxidant activity due to proantho-cyanidin content	2006	Rathee *et al*. [247]

4.3 Flavan-3-ols and cardiovascular disease

The U.S. Department of Health and Human Services Center for disease control and prevention estimates that more than 910 000 Americans die of cardiovascular disease each year, representing 1 death every 35 s. In fact, heart disease is the number one leading cause of death for Americans. An estimated 70 million Americans currently suffer from and live with cardiovascular disease, costing Americans a projected $403 billion dollars annually [63]. However, as little as a 10% decrease in total blood cholesterol level potentially lowers an individual's risk of heart disease by 30% [63].

Free radicals and oxidative stress are key contributors to cardiovascular diseases such as congestive heart failure, valvular heart disease, cardiomyopathy, hypertrophy, atherosclerosis and ischemic heart disease [64]. Flavan-3-ols may interfere in the pathogenesis of cardiovascular disease via several mechanisms: antioxidative, antithrombogenic, and anti-inflammatory. In particular, proanthocyanidins and flavan-3-ol monomers aid in lowering plasma cholesterol levels, inhibit the oxidation of LDL, and activate endothelial nitric oxide synthase to prevent platelet adhesion and aggregation that contribute to blood clot formation [46, 64 – 73]. Flavan-3-ols also influence oxidative stress via enzyme modification and modulation of cell signaling pathways; the extent of the effect relies greatly on flavan-3-ol structure-related protein reactivity [74].

High blood cholesterol, high levels of LDL in particular and high ratios of saturated to polyunsaturated fatty acids predisposes individuals to atherosclerosis and coronary artery disease [46, 66, 75, 76]. Atherosclerosis, a type of arteriosclerosis, occurs due to the deposition and build-up of fatty substances such as fats, cholesterol, smooth muscle cells, platelets, cellular waste, and calcium in arterial endothelium. The resultant build up, deemed plaque, hardens and thickens the arteries causing high blood pressure. Plaque build up can cause hemorrhage, thrombosis, or embolism that lead to gangrene, heart attack, or stroke. Atherogenesis may be caused by endothelial damage due to one or several factors including: high blood triglycerides, high cholesterol, high blood pressure, and/or oxidative stress. Damage to the endothelial wall eventually causes platelet adherence that contributes to blood clot formation and loss of arterial elasticity. Hardening of the arteries over time affects blood pressure, resistance, and blood flow that force the heart to work harder, enlarge, and eventually fail [77]. Normally, circulating blood platelets do not adhere to healthy endothelium. The release of nitric oxide (NO) prevents platelet adhesion to healthy endothelial tissue [71]. Evidence suggests that flavan-3-ols increase nitric oxide production and thus possess great potential for use in treatment and prevention of cardiovascular disease [78 – 82].

Reports blame both initiation and propagation steps of atherosclerosis on lipid oxidation [67, 83]. An excess of lipoprotein, especially LDL, in the blood may accumulate within the arterial wall, then become modified via oxidation. A common mechanism in oxidative stress pathological conditions, lipid peroxidation occurs via reactive-oxygen species-initiated auto-oxidation of polyunsaturated fatty acids. Smooth muscle cells uptake modified lipoproteins, resulting in foam cell formation that causes connective and cellular tissue deposition. The modified LDL remains soluble in blood plasma and thus easily traverses the endothelium to contribute to plaque, or atheroma, formation [71, 77]. Consequently, lipoprotein metabolism, principally occurring in the liver, receives much attention from investigators of atherogenesis [84]. Factors including the presence of copper ions, cell antioxidant capacity, and composition and/or location of polyunsaturated fatty acids affect lipid peroxidation.

Polyunsaturated fatty acids such as the Omega 3's are especially effective in protecting against cardiovascular disease. The distribution of double bonds in these compounds allows for high resistance against lipid peroxidation and thus reduced susceptibility for induction of clot formation [46]. Antioxidants such as those found in common fruits, vegetables, herbs and beverages also effectively prevent the oxidation of LDL [67, 83, 85]. Consumption of flavan-3-ol-rich foods such as grapes, wine, berries, apples, chocolate, and teas act to reduce cholesterol, restore lipid balance, and prevent conversion of LDL to harmful oxidized chemical states [85].

While flavan-3-ols' strong antioxidant and free radical scavenging characters make them ideal candidates for treatment of cardiovascular disease, as of 2003 few published studies indicate structure-cardioprotective function relationships. This is most likely due to the fact that experiments employ different mechanisms to incur as well as measure oxidation and endothelial damage [58]. Work by Steinberg *et al*. [86] reveals that cocoa-derived flavan-3-ol and oligomeric procyanidins decrease LDL oxidative susceptibility with increasing chain length; however, equivalent concentrations of monomers equally inhibit LDL, suggesting that antioxidant power of procyanidins on biologic substrate depends on ring structure and number of catechol groups, not chain length alone. Recent work by Ottaviani *et al*. [74] highlights possible mechanisms for flavan-3-ol-structure mediation of blood pressure. Flavan-3-ols influence angiotension I (Ang-I) converting enzyme (ACE), also known as peptidyl-dipeptidase A. ACE is a glycoprotein peptidyldipeptide hydrolase that catalyzes angiotension I hydrolysis to angiotension II (a potent vasoconstrictor). ACE also facilitates the cleavage of bradykinin, a vasodilator mediated by NO release. Flavan-3-ols inhibit ACE-mediated angiotension II formation in cultured HUVEC cells. Tetramers show greatest inhibition potential, while dimers and trimers prove ineffective, most likely due to their relatively low or reduced affinity for cellular constituents. Ottaviani *et al*. [74] suggest that despite the number of (–)-epicatechin units (high hydroxyl moiety count capable

of binding protein), other factors determine flavan-3-ol-enzyme interactions and that ACE activity and Ang-I hydrolysis are dependent on Cl- binding. Actis-Goretta *et al*. [87] recently demonstrated that procyanidins (dimer and hexamer) as well as epigallocatechin all inhibit ACE activity, while flavan-3-ol monomers such as (+)-catechin and (−)-epicatechin do not. ACE activity inhibition thus depends on both phenolic and flavan-3-ol content of food.

The following studies are summarized and presented in chronological order according to flavan-3-ol source: herbal remedy, grape or wine, cocoa or chocolate, green tea, and cranberry.

4.3.1 Herbal remedies

A flavan-3-ol rich Ginkgo biloba extract may abolish arteriolar constriction due to an 'NO-like' action [88]. Pycnogenol™, a French maritime pine extract, provides cardiovascular benefits including vasorelaxant activity, angio-tension-converting enzyme inhibiting activity, and enhancement of microcirculation and increased capillary permeability. Pycnogenol's cardioprotective activity is attributed to the strong free radical scavenging activity of its oligomeric procyanidin components. Accordingly, Pycnogenol modulates NO metabolism in activated macrophages by quenching the NO radical and modulating redox-sensitive signal transduction pathways and altering gene expression [79]. Hawthorn proanthocyanidin extract, most notably B-2 dimer, inhibits Cu^{+2}-induced LDL oxidation [89]. Hawthorn berry extracts act equally as grape seed extracts to inhibit endothelin-1 (ET-1) synthesis. ET-1 synthesis results in reduced endothelium-dependent vasodilation characteristic of endothelial dysfunction and heart failure. These flavan-3-ols are thought to stimulate a pseudo laminar sheer stress response in endothelial cells that aids to repair endothelial function [90]. High degree of polymerization pine bark extract fed to rats at 500 mg/kg body weight provides greater total antioxidant activity than lower degree of polymerization pine bark extract [91].

4.3.2 Grape and wine

As early as 1994, a diet rich in polymeric grape tannins proved to lower plasma total cholesterol, triacylglycerol, and low density lipoprotein cholesterol concentrations in rats more than a diet rich in monomeric catechins [92, 93]. A polymeric rich diet also helps to increase plasma HDL cholesterol level and excretion of cholesterol, while reducing platelet aggregation in rats [94] with red wine polyphenols inhibits the proliferation and DNA synthesis of cultured rat aortic smooth muscle cells, suggesting the down-regulation of cyclin A gene expression via inhibition of transcription factor expression as a possible mechanism [75]. Red wine flavan-3-ols cause endothelium-dependent vasorelaxation (EDR) associated with marked formation of NO. The same red wine extract also causes extracellular calcium ion increases in endothelial cells, but not in smooth muscle cells [78]. Procyanidin-rich grape seed extract (74.3% procyanidins) decreases oxidized LDL-derived atherosclerotic foam cell lesions in rabbit aorta via reactive oxygen scavenging in the plasma and interstitial fluid [95]. A dealcoholized red wine extract added to whole blood *in vitro* increases PAC-1 binding and P-selectin expression and inhibits platelet activation in response to epinephrine [96]. Grape proanthocyanidin trimers, tetramers, pentamers and polymers and their gallates as well as a dimer gallate provide EDR of blood vessels *in vitro* by increasing nitric oxide production. Phenolic acids and flavan-3-ol monomers did not instigate the same EDR result [81]. Treatment with 100 mg grape seed proanthocyanidin (GSP) extract/kg/day reduces the number of apoptotic cells in rats with ischemic/reperfused hearts. In this case, the GSP cardioprotective effect was thought due to reduced expression of JNK-1 (proapoptotic transcription) factor and c-Jun gene- both apoptotic factors in the ischemic/reperfused myocardium [97]. Supplementation of grape seed proanthocyanidin extract IH636 (50 mg/kg and 100 mg/kg) reduces atherosl-cerosis (% of aorta covered in foam cells) by 50 and 63% in hamsters. In this model, grape seed proanthocyanidin extract also causes formation of thibarbituric acid reactive substances, demonstrative of its effect on cholesterol and triglyceride as well as oxidative LDL damage [98]. During investigation of vascular smooth muscle cell EDR activity *in vitro*, administration of grape seed extract procyanidins was found to increase endothelial NO release and subsequent increase in cyclic GMP levels. Crushed Concord grape seed activity also showed that EDR activity increases with degree of polymerization, epicatechin content, and extent of galloylation [80]. The cardioprotective effects of GSP do not always require PKC, mito K-ATP channel or NO synthase, instead GSP may dose dependently attenuate oxidant formation by scavenging H_2O_2, hydroxyl radical, superoxide and possibly by chelating iron [82]. Grape seed procyanidins dose dependently inhibit AAPH induced lipid oxidation and reverse cell loss of viability, but are ineffective for the mediation of intracellular Cu-driven oxidation. The protective effect of these procyandins is thought due to specific binding and quenching of exogenous harmful radicals. Procyanidins also dose dependently relax human internal mammary aortic rings (max effect at 50 μM). The vasodilating effect is said to involve a Cox-dependent mechanism, verified by dose-dependent stimulation of prostacyclin [PGI(2)]. Protection against peroxynitrite attack on vascular cells occurs via procyanidin layering on the surface of coronary endothelial cells and is enhanced by endothelial NO-synthase-mediated relaxation [99]. GSP application and supplementation improves cardiac post-ischemic left ventricular function, reduces myocardial infarction size, ventricular fibrillation, and tachycardia, as well as decreases the amount of reactive oxygen species and malondialdehyde formation in heart perfusate. Cardioprotective properties of GSP may be due to its ability to inhibit

anti-death signaling by JNK-2 and c-fos proteins that mediate apoptosis. Treatments with GSP also inhibit doxorubicin-induced cardiotoxicity, serum creatine kinase activity, DNA damage as well as alter histopathology. A human clinical trial indicates that GSP supplementation significantly reduces oxidized LDL in hypercholesterolemic subjects. GSP also inhibits induction of endothelial CD36 expression, a known cardioregulatory gene [64]. The 4-week treatments of 2 g/day grape seed extract improves flow mediated dilatation by 1.1% in men and women at risk for cardiovascular disease. This is thought due to influence on endothelial NO production [100]. The cardioprotective effects of a non-alcoholic extract of red wine on ischemia-reperfusion injury can be correlated to attenuation of lipid peroxidation. Polymeric proanthocyanidin rich de-alcoholized wine extract improves post-ischemic recovery of left ventricular pressure development better than a monomeric flavan-3-ol fraction [101]. Incubation of platelets with red wine and purple grape juice extracts leads to a decrease in platelet aggregation from 68.8 to 45% (seeds) and 27% (skin). Incubation also leads to a decrease in superoxide release from 73 to 2% (seed) and 0.33% (skin). A significant increase in radical scavenging activity, decrease in release of reactive oxygen species, attenuation of CD40 ligand (inflammatory mediator) release and enhancement of platelet NO also result from incubation in purple grape juice extract [68]. Red wine and extracts of grape skin inhibit phosphodiesterase activity on human recombinant PDE5A1 isoform, while seed extracts do not. It is proposed that the induced vasorelaxation may be sustained by phosphodiesterase inhibition by anthocyanins also present in the wine and grapes [102]. Red wine proanthocyanidins improve atherosclerotic risk index in the prostprandial state by altering liver mRNA levels and cholesterol biosynthetic enzyme levels (SHP and CYP7A1). An induction of overexpression of CYP7A1 increases cholesterol elimination via bile acids and SHP (a nuclear receptor key to regulation of lipid transcriptional homeostasis) [84].

Red wine extract inhibits blood thrombotic reactivity rather than reducing the development of atherosclerotic lesions [103]. *Vitis vinifera*-derived oligomeric procyanidins reduce incidence of ventricular fibrillation from 86.6 to 55.6% and shorten the duration of episodes from 76.1 to 36.6% (expressed as percentage relative to the total duration) [104].

4.3.3 Cocoa

Cocoa flavonoids protect against cardiovascular disease due to antioxidant, antiplatelet, anti-inflammatory effects as well as the ability to increase serum HDL while lowering serum LDL cholesterols [69]. Cocoa procyanidin trimers, pentamers added to whole blood *in vitro* increase PAC-1 binding and P-selectin expression as well as inhibit platelet activation in response to epinephrine [105]. Ingestion of procyanidin-rich chocolate (5.3 mg total procyandin/g) increases plasma antioxidant capacity and decreases plasma lipid oxidation [106]. Oligomeric chocolate and cocoa derived procyanidins also inhibit LDL oxidation *in vitro* [107]. Chocolate and cocoa catechins and their C-4→C-8-linked oligomers lower human LDL oxidation *in vitro*; however, the antioxidant strength of such monomers and polymers depends on the system used to measure activity. Catechin proves more powerful at delaying liquid oxidation than other monomers, dimers (B-2 and A-2), or trimer C-1 in systems that employ ions for radical generation, However, when replacing 2,2′-azobis as the radical generator epicatechin proves most powerful, followed by dimer B-2, trimer C-1, and lastly catechin [108]. Work by Pearson *et al*. [109] exhibits that flavanol-rich cocoa inhibits epinephrine-stimulated human platelet activation similar to the effect of aspirin. Cocoa also decreases platelet P-selectin expression- a marker of platelet activation- as well as diphosphate- a collagen- induced platelet aggregator [71]. Platelet effects of cocoa may be due to secondary changes in eicosanoid metabolism; procyanidin decreases plasma leukotriene-prostacyclin ratios (protective alteration of proinflammatory/anti-inflammatory eicosanoid balance) both *in vitro* and *in vivo* [110]. Chocolate flavanol and procyandin oligomer consumption improve nitric oxide-dependent vasorelaxation in the presence of pre-existing endothelial dysfunction [111]. Cocoa-derived flavanol-rich beverages lower the plasma level of F-2 isoprostanes, indicators of *in vivo* lipid peroxidation, relative to low-flavanol cocoa drink (187 *vs*. 14 mg/100 mL) [73]. Dark chocolate consumption increases flow-mediated dilation in both healthy and hypertensive human subjects [112, 113]. Cocoa-induced vasodilation may depend on epicatechin concentration; oral administration of pure epicatechin improves both nitric oxide production as well as vasodilation in healthy human volunteers [114]. Administration of a flavanol-rich cocoa beverage increases brachial artery hyperemic blood flow by up to 76% (446 mg flavanol) and decreases soluble vascular cell adhesion molecule-1 in plasma by 11% [115]. A series of experiments by Heptinstall *et al*. [116] validate the effects of cocoa flavanols on both platelet and leukocyte function *in vitro*. Effects on platelet aggregation, platelet-monocyte conjugate formation, platelet-neutrophil conjugate formation and platelet activation are similar to those of aspirin; however, no additive effects of aspirin + proanthocyanidin have been observed. Consumption of a flavan-3-ol-rich cocoa beverage significantly inhibits platelet aggregation, platelet-monocyte conjugate formation, platelet-neutrophil conjugate formation and platelet activation induced by collagen *ex vivo* [116].

4.3.4 Tea

Green tea flavan-3-ols are antithrombotic due to antiplatelet rather than anticoagulation effects. The antiplatelet activity is thought due to inhibition of an intracellular pathway preceding GPIIb/IIIa complex exposure. Green tea flavan-3-

ols also inhibit rise in intracellular calcium ion concentration induced by thrombin treatment [117]. Green tea flavan-3-ols effectively lower plasma LDL levels [66]. Green tea consumption improves endothelial dysfunction in chronic smokers by increasing circulating endothelial progenitor cells (EPC). Consumption also improves flow-mediated endothelium dependent vasodilation of the brachial artery [118]. EPC act to improve neovascularization by improving blood flow, cardiac function and reducing scarring. Green tea consumption provides cardioprotective effects including: antioxidant, antithrombogenic, anti-inflammatory as well as improvement of coronary flow velocity [70, 119].

4.3.5 Cranberries

A diet rich in cranberry juice powder significantly reduces both total and LDL cholesterol *in vivo*. More information on cranberry effects on atherosclerosis may be found in the works of Reed [120] and Leahy *et al*. [121].

4.4 Flavan-3-ols and cancer

A recent scientific focus dedicates research to understanding the mechanisms behind fruit and vegetable protective effects against multiple chronic and degenerative diseases including various types of cancers. Most studies link polyphenols to their chemopreventative effects, some to cancer-inducing effects, while other studies show no effects [44, 122].

Animal studies and cell models suggest that flavonoids act as anticarcinogens through influencing molecular events in the initiation, promotion and progression stages of cancer [123]. Several proposed mechanisms explain flavonoid effects on cellular function. Flavonoids can act as anticarcinogens via antioxidant scavenging of free radicals, regulation of signal transduction pathways of cell growth and proliferation, suppression of oncogenes and tumor formation, induction of apoptosis, modulation of enzyme activity related to detoxification, oxidation and reduction, stimulation of the immune system and DNA repair, and regulation of hormone metabolism [124 – 130]. Of particular interest are polyphenol roles in programmed cell death, also known as apoptosis. Apoptosis differs from cell necrosis, the accidental form of cell death, in that it minimizes leakage of potentially toxic cellular constituents from dying cells. During apoptosis cell shrinkage, chromatin condensation and inter-nucleosomal DNA fragmentation lead to formation of apoptopic bodies, which then become phagocytosed. Oxidative stress, cancer pathogenesis, viral infections and other degenerative diseases all correlate to improper regulation of apoptosis. A damaged or blocked apoptotic pathway results in uncontrolled cell division that eventually leads to tumor formation. Several genes, both promoters and inhibitors, regulate apoptotic death. Promoter genes related to apoptosis include: Bax, Bcl-xs, c-myc, p53, and c-fos. Bcl-2 and Bcl-xL represent apoptosis inhibitor associated genes [58]. Also essential to apoptosis are the caspases, a group of cysteine protease enzymes capable of cleaving proteins following aspartic acid residues. Of the eleven caspases identified in humans, two types are known: initiator caspases and effector caspases. Initiator caspases such as caspase-9 activate pro-forms of effector caspases such as caspase-3 to eventually initiate apoptosis. Nuclear factor-kappa B (NF-κB), a ubiquitous transcription factor involved in several cancer-related signal transduction pathways protects cells from apoptotic stimuli via regulation of gene transcription [66]. Activation of NF-κB occurs through exposure of cells to inflammatory cytokines such as tumor necrosis factor (TNF) or IL1, viral infection, UV irradiation, B or T cell activation, and other stimuli. NF-κB is primarily controlled via interaction with the inhibitor protein I kappa B. I kappa B proteins inhibit DNA binding, prevent nuclear uptake of NF-κB complexes and terminate NF-κB nuclear activity. Phosphorylation and degradation of I kappa B initiates movement of NF-κB into the nucleus. In the nucleus, NF-κB dimers bind to target DNA elements to activate transcription of genes that encode immune and cell growth regulatory proteins. Because the activation of NF-κB depends on kinase activity as well as cell redox status the potential intervention of NF-κB by antioxidants such as flavan-3-ols remains a strong focus of current chemopreventative research [131, 132]. TNF proteins such as TNF alpha aid in apoptosis of cells in which no NF kappa B is present, as is the case of normal cells. The presence of free NF-κB, as seen in cancerous cells, allows for cell proliferation and tumor formation. However, once bound to I kappa B proteins, NF-κB loses its anti-apoptotic activity. Presence of TNF in these types of cells directly affects tumor cells, promoting shrinkage of tumors by apoptosis. Since the goal in cancer therapy is to kill tumor cells, recent research focuses on TNF protein chemotherapeutic potential [133 – 135]. Other genes linked to NF-κB regulation include the cytokines IL2, IL6, IL8, CMCSF [136]. Flavan-3-ols may also be effective hormone metabolism regulators. Some tumors such as breast cancer tumors are 'estrogen sensitive' in that the presence of estrogen helps their growth. Aromatase, a cytochrome P450 enzyme, catalyzes C-19 androgen conversion to C-18 estrogen. Research attributes cancers of several tissue types to aromatase overexpression: ovary, placenta, adipose and bone tissues. Human aromatase genes contain at least nine translated exons (II-X) and eight untranslated exons (I.1-I.7 and PII). Current research targets both aromatase and estrogen receptors as potential treatments for breast cancer. Evidence suggests that flavan-3-ols function as aromatase inhibitors. Aromatase inhibitors block tumor growth by reducing circulating estrogen levels *in vivo*. A few selective aromatase inhibitors are currently available in the United States that reduce circulating estrogen to 1 – 10% of pretreatment levels. However, since treatments such as these still cause undesirable side effects, researchers continue to seek out

natural product alternatives. Flavan-3-ols may prove valuable as alternative treatments for hormone metabolism-related cancers [137, 138]. Very few past investigations report research relating flavan-3-ol structure to anti-carcinogenesis mechanisms. Initially, scientists investigated green tea flavan-3-ols, later as a result of excitement over the phenomenon known as the French paradox, a majority of studies focused on grape, grape seed or wine flavan-3-ol effect on cancer. More recently, flavan-3-ol extracts of pine, cocoa, apple, blueberry and other plant sources receive more attention.

4.4.1 Grape-seed and grape flavan-3-ols

Grape seed proanthocyanidin (GSP) extract regulates the expression of cell cycle-related genes such as p53, Bcl-2 and c-myc [139]. GSP treatment decreases the expression of p53 and c-myc in Chang liver cells, indicating that proanthocyanidins ameliorate toxic effects associated with cancer therapy treatments. GSP extract cytotoxicity to human breast, lung and gastric adenocarcinoma cells may coincide with up-regulation of Bc1-2 and down-regulation of the oncogene c-myc [140, 141]. GSP extract also provides better protective effects than vitamins C and E to tobacco-induced oxidative stressed human oral keratinocytes, most likely due to modified expression of cell regulatory genes p53 and Bcl-2 [140]. 12-O-Tetradecanoylphorbol-13-acetate (TPA) promotes anti-apoptotic mechanisms associated with high levels of Bcl-2 by modulating DNA synthesis and differentiation of human lymphoid cells [142, 143]. Bomser *et al*. [144] investigated the effect of time and dose of grape polyphenolic extract on TPA-induced tumor promotion, ornithine decarboxylase (ODC) activity and protein expression, and on protein kinase C activity in mouse skin epidermis. The findings of the study show that grape polyphenolic extract effectively inhibits TPA induced ornithine decarboxylase activity.

Activator protein-1 (AP-1) activity may be involved in biological apoptosis, cell proliferation, transformation, and differentiation related to cancer development. AP-1 binds to palindromic DNA sequences present in the regulatory region of many genes and has been linked to mitogen-activated protein kinase (MAPK) pathways. Jeong *et al*. [145] report that procyanidins B-1 and B-2 have no effect on AP-1 reporter gene activity in HT-29 cells. To the contrary, Agarwal *et al*. [146, 147] found that procyanidin rich grape seed extract inhibits the growth and induction of apoptotic cell death. Agarwal *et al*. applied GSP to both human prostate carcinoma (DU145) and rodent skin cells. Results suggest that GSP modulates mitogenic signaling and cell-cycle regulators, and induces arrest of the crucial cell growth phase G1 to instigate apoptosis. Furthermore, GSP may cause mitochondrial damage responsible for releasing cytochrome C into the cytosol and signaling capsases-3 via capsase-9 activation to eventually cause DU145 cell apoptosis [146]. GSP, consisting primarily of 5 – 10 mers, increases caspase-3 activity and eventual apoptosis in rat colon cancer cell line RCN-9 *in vitro* [148]. Procyanidins are more effective than constituent flavan-3-ol monomers at preventing DNA hepatocytic lesion [149]. Polyphenolic grape extracts tested by Matito *et al*. [150] exert potent anti-proliferative effects in mouse hepatoma Hepa-1c1c7 cells. Fractions pertaining to higher degree of polymerization and galloylation influenced cell cycle and apoptosis induction more greatly. According to Faria *et al*. [151] 30 μg/mL procyanidin GSE provides protection of human breast cancer cell membranes against peroxyl radical up to 800 molecular weight units but decreases with the increase of structural complexity.

A recent report by Kaur *et al*. [152] provides mechanistic understanding concerning the effects of GSE on LNCaP human prostate cancer cells. The mechanism associated with cell death appears to involve both caspase-dependent and caspase-independent apoptosis. Treatment of cells with GSE triggers anoikis (apoptosis of cells that have become detached from extracellular matrix) as well as release of cytochrome c and apoptosis-inducing factor into the cytosol within 12 h. The study also suggests that GSE initiates activation of ataxia telangectasia mutated kinase and Chk2, as well as p53 Ser15 phosphorylation and translocation to mitochondria of DNA damage-induced cells. GSE-induced apoptosis, cell growth inhibition and cell death appear to involve reactive-oxygen species.

Bagchi *et al*. [153] investigated the mechanistic cytoprotective pathways of GSPE on acetaminophen-induced hepatoxicity and nephrotoxicity, amiodarone-induced pulmonary toxicity, doxorubicin-induced cardiotoxicity, DMN-induced immunotoxicity and MOCAP-induced neurotoxicity in mice. Results relate anti-toxic potential of GSPE to inhibiting or modulating the drug metabolizing enzyme cytochrome P450 2E1. GSPE treatment also decreases TNF alpha-induced adherence of T cells to HUVEC by inhibiting VCAM-1 expression. At doses greater than or equal to 50 μg/mL GSE inhibits constitutive and TNF alpha-induced NF-κB DNA binding activity in advanced human prostate carcinoma DU145 cells. Inhibition of Ik B-alpha phosphorylation and kinase activity accompany binding activity inhibition and result in apoptosis [154].

Work by Cheng *et al*. [155] demonstrates that GSE induces a marked dose-dependent decrease in nicotine-DNA adduct formation *in vivo*. Other work from 2003 by Eng *et al*. [156] reveals structure- activity relationships for procyanidin B dimers of red wine and grape seeds. *In vivo* testing of procyanidin B dimers on an aromatase-transfected MCF-7 breast cancer xenograft model indicates that procyanidin B dimers suppress *in situ* estrogen formation and reduce androgen-dependent tumor growth. The inhibition mechanism on aromatase activity is due to competitive binding of androgen substrate at active site residues: Asp-309, Ser-378, and His -480. Work by Kijima *et al*. [138] suggests that GSE proanthocyanidin B dimers act as a potent aromatase inhibitor by down-regulating two transcription factors:

cyclic AMP-responsive element binding protein-1 (CREB-1) and glucocorticoid receptor (GR). Both CREB-1 and GR are known aromatase up-regulators. Treatments of 60 mg/mL of GSE suppress levels of exon I.3-, exon PII- and exon I.6-containing aromataste mRNA in MCF-7 and SK-BR-3 cells. Aromatase up-regulation in breast cancer appears to rely on promoters I.3, II, and I.4. Procyandin B-2 also provides potent catalytic inhibition of topoisomerase II [157, 158]. Topoisomerase II enzymes are essential for DNA replication and thus are considered critical targets for anti-tumor drugs. GSP strongly enhances the anti-tumor activity of the chemotherapeutic drug doxorubicin. The mechanism may be partially related to enhancement of lymphocyte proliferation, NK cell cytotoxicity, $CD4^+/CD8^+$ ratio, IL2 and IFN-gamma production [159, 160]. Sharma and Katiyar [161] found that GSP reduce UVB-induced increase in immunosuppressive cytokine IL10 in skin and draining lymph nodes in mice.

4.4.2 Green tea

With green tea comprised of significant amounts of flavan-3-ols, tea and tea extracts have been used experimentally as inhibitors against carcinogenesis. The effects of tea polyphenolic extracts on several cancer types (lung, skin, esophagus, liver and stomach) have been demonstrated in many animal models. Green tea polyphenolics have effectively inhibited multiple stages of carcinogenesis: initiation, promotion, and progression stages. However, as of 1998 the mechanisms of growth inhibition and apoptosis were not well understood or easy to identify. The majority of studies investigating green tea extract effect on cancer focus on monomeric flavan-3-ols.

Lin and Lin [162] report that (–)-epigallocatechin-3-*O*-gallate (EGCG) inhibits NF-κB activation via prevention of its binding to DNA. Follow up investigations of EGCG effect on various cell lines confirm this activity through multiple mechanisms [13, 163 – 165]. Yang *et al*. [166] suggest that green tea polyphenols-induced production of H_2O_2 plays a role in apoptosis mediation. Investigation of the effect of green tea flavan-3-ols on the growth and apoptosis of human prostrate cancer DU145 cells indicates that apoptosis induction occurs through increased reactive-oxygen species formation and mitochondrial depolarization. In this study, apoptosis induction appeared unrelated to members of the Bcl-2 family of cell cycle regulating genes [167]. EGCG also increases AP-1 factor associated responses via MAPK signaling [145]. Chen *et al*. [168, 169] report EGCG potently induces MAPK induction in human cancer cell lines: HepG1, Ht-29 and HeLa (hepatoma, colon and cervical squamous).

4.4.3 Cocoa

Recently, cocoa-derived procyanidins have been evaluated for their potential chemotherapeutic roles. Kenny *et al*. [170] isolated a cocoa procyanidin pentamer of 1442 DA to evaluate its effect on tyrosine kinase ErbB-2 expression. ErbB-2 is an important receptor in angiogenesis regulation. Angiogenesis, the growth of new blood vessels, is necessary for cancerous tumor growth (www.cancer.gov). Kenny's procyanidin pentamer proved to be a potent inhibitor of tyrosine kinase expression and thus an inhibitor of angiogenesis. Ramljak *et al*. [171] report that cocoa derived pentameric procyanidin selectively inhibits proliferation of human breast cancer cells by causing depolarization of mitochondrial membranes of MDA MB-231 cells and down-regulation of G1 modulatory proteins CdC-2 and p53 of MDA MB-468 cells. Normal epithelial cells appear unaffected by Ramljak's procyanidin pentamer. Another study published in 2005 [172] applied B and C type cocoa and A-type peanut procyanidins to phosphatidyal choline liposomes. Both dimers and trimers act to inhibit lipid oxidation in a concentration dependent manner and protect the lipid bylayer from Triton X-100 disruption via interaction with membrane phospholipids. Such interactions suggest that procyanidins could provide antioxidant protection to cell membranes. A 2006 *in vitro* study [173] testifies that cocoa polyphenol extracts inhibit growth of both metastatic and nonmetastatic human prostate cancer cell lines (DU145 and 22Rv1), yet do not exert any effect on normal prostate cells (RWEP-1).

4.4.4 Apple

Apples contain flavan-3-ol monomers as well as oligomeric procyanidins. Hibasami *et al*. [174] induced DNA fragmentation and eventual apoptosis on human stomach cancer KATO III cell DNA by administration of apple (*Rosaceae-Malus pumila*) derived B and C-type procyanidins. Active oxygen is suggested to have induced apoptosis. Gosse *et al*. [175, 176] studied the anti-proliferative mechanisms of apple polyphenols on human metastatic colon carcinoma SW620 cells to evaluate their anti-carcinogenic properties *in vivo*. Apple procyanidin application inhibited protein kinase C activity by 70%, significantly increased presence of extracellular signal regulated kinases, down regulated polyamine biosynthesis, activated caspase-3, and eventually inhibited SW620 cell growth. Gosse *et al*. [176] also tested the effects of apple procyanidins *in vivo* on Wistar rats. Procyanidin treatments reduced the number of preneoplastic lesions compared to controls. A follow up study by Gosse *et al*. [175] further investigated apple procyanidin effect on polyamine metabolism. Apple procyanidins were found to enhance polyamine catabolism and reduce polyamine biosynthesis activity similar to known inducers of Spermidine N (1)-acetyltransferase (SSAT) without sharing their toxicity. Results of these experiments indicate that apple could be used for chemopreventative as well as chemotherapeutic treatments.

4.4.5 Pine

Gali *et al*. [177] report that Douglas fir flavan-3-ols inhibit ornithine decarboxylase activity (ODC) according to

degree of polymerization: trimer (C-1) > dimer (B-1, B-2 and B-3) > monomers. According to Jeong and Kong [66], the biological roles of procyanidins in NF-κB signaling pathways may depend on the degree of procyanidin polymerization, cell models used and occurrence of extracellular stimuli. Park *et al*. [178] report that procyanidin dimers repress interferon-γ-induced NF-κB dependent gene expression while C-2 trimers induce expression. Jeong *et al*. [163] state that procyanidin dimers B-1 and B-2 have little or no effect on LPS-induced NF-κB transcription activation, while pycnogenol, a procyanidin rich extract from pine, inhibits NF-κB dependent gene expression in immortalized human keratinocytes yet has no effect on activation of NF-κB in a murine macrophage RAW 264.7 cell line [79, 179]. Tourino *et al*. [180] suggest that gallate free pine procyanidins may serve as better chemopreventative agents for applications in food and skin because galloylation esters interfere with crucial cell functions. The authors work indicates the pine bark procyanidin mixtures devoid of gallate esters act as free radical scavengers against ABTS(.+), DPPH, and HNTTM and thus galloylation does not appear to play a role in protection against lipid peroxidation.

4.4.6 Blueberry

Other sources of concentrated proanthocyanidins to receive recent attention are edible berries. As early as 2002, Roy *et al*. [181] report that edible berry extracts impair angiogenesis. Six edible-berry extracts proved to inhibit both H_2O_2 and TNF alpha-induced vascular endothelial growth factor (VEGF) expression in human keratinocytes: blueberry, bilberry, cranberry, elderberry, raspberry and strawberry. VEGF levels increase with tumor progression and thus provide clinicians with a useful marker for sarcoma and carcinoma monitoring [182]. Blueberries in particular have received a great deal of attention due to their anti-promotion and anti-proliferative activity against various forms of cancer [127]. According to Schmidt *et al*. [183] blueberry proanthocyanidin (4→8 oligomers) with mDP 3.25 – 5.65 inhibit adhesion of *Escherichia coli* responsible for urinary tract infections, while a fraction of higher mDP (5.65) provide anti-proliferation activity against human prostate and mouse liver cancer cell lines. Proanthocyanidin-rich low-bush blueberry extract also effectively decreased matrix metalloproteinase activity in DU145 human prostate cancer cells *in vitro* [184]. Kraft *et al*. [185] report that more polar wild low-bush blueberry extract fractions act as inhibitors of later stages of carcinogenesis and that proanthocyanidin dimers demonstrate activity against both promotion and proliferation stages of chemogenesis. Another report of Matchett *et al*. [186] provides information related to blueberry flavonoid anti-cancer mechanism. Blueberry proanthocyanidins may use multiple mechanisms in down regulating matrix metalloproteinase activity with possible involvement of protein kinase C and mitogen-activated kinase pathways. Schmidt *et al*. [127] suggests that blueberry proanthocyanidin antiproliferation activity is not simply a function of molecular weight. The authors' investigation on the inhibitory effects of blueberry proanthocyanidin isolates on the proliferation of two prostate cell lines, LNCap (androgen sensitive) and DU145 (androgen insensitive) led to the suggestion of several explanatory hypotheses. The hypotheses include modulation of PSA and androgen receptor gene expression, androgen agonist action, influence on androgen receptor ligand-binding specificity, and modulatory action on growth factor production and receptor expression. Blueberry proanthocyanidin potent antiproliferative effects on androgen-sensitive cell lines suggest their potential for use as novel therapeutic agents for early stage androgen-dependent cancer prevention.

4.4.7 Herbal remedies and others

Other proanthocyanidin-rich plants have been studied for their chemopreventative effects. Toyokuni *et al*. [187] report that 12 Mauritian endemic plants extracts may provide chemopreventative potential due to their ability to modulate the expression of antioxidant enzyme genes on Cu, Zn-SOD promoter activity. Hostanska *et al*. [188] report that St. John's wort-derived procyanidin B-2 inhibits growth of leukemia K562 and U937 cells as well as brain glioblastoma cells LN229. Procyanidin B-2 treatments induced phosphatidylserine and morphological changes in cell size and granulosity, loss of membrane phospholipids and eventual apoptosis in all cell lines. Saito *et al*. [189, 190] synthesized six galloyl-substituted procyanidins: B-1, B-2, 3-*O*-gallate, 3″-*O*-gallate, and 3,3″-di-*O*-gallate. The 3,3″-di-*O*-gallate dimers acted as strong inhibitors against DNA polymerase alpha and beta, whereas the desgalloyl and monogalloyl compounds did not exhibit inhibitory activity against DNA polymerase beta.

4.5 Flavan-3-ols as antimicrobial, anti-viral, anti-parasitic and neuro-protective agents

Flavan-3-ols have also been associated with other health beneficial effects by acting as antimicrobial, anti-viral, anti-parasitic and neuro-protective agents. The anti-microbial effects of several tannin extracts on yeast, filamentous fungi, bacterial, and viral toxicity have been reviewed by Chung *et al*. [4]. Although the review references several types of tannins and sources, it does not offer substantial structural information regarding condensed tannin extracts. However, the organization of information in table format allows for quick and straightforward referencing. Polymeric proanthocyanidins isolated from *Zizyphus* fruits and *Zanthoxylum* fruit peels may be useful as suppressors of antibiotic resistance in *Staphylococcus aureus* and show promise of use as an alternative treatment to antibiotic use against *Staphylococcus aureus* infection [191]. Howell *et al*. [192] report that A-type cranberry and B-type grape proanthocyanidins elicit *in vitro* anti-adhesion activity of *E. coli* from

uroepithelial cells. Min and Hart suggest that proanthocyanidins in forages have the potential to aid in the control of gastrointestinal parasites [193]. Their 2003 publication lists proanthocyanidin contents of various forage species, including molecular weight ranges and subunit composition data. According to the authors, proanthocyanidin-containing forages possess potential to aid in the control of antihelmintic-resistant gastrointestinal parasites, to decrease fecal egg counts in sheep and goats, and to decrease hatch rate as well as larval development in feces.

A 2004 review by Youdim *et al*. [40] provides information concerning proanthocyanidin roles in neuro-physiology. This in depth review covers interactions of flavonoids at the blood/brain barrier as well as their physiological effects on the central nervous system. A 2006 [194] review entitled 'Polyphenols and Cognition' provides information on therapeutic benefits of food-derived flavan-3-ols in neurodegenerative conditions such as Alzheimer's and Parkinson's diseases. Collie proposes, as published evidence suggests, that flavan-3-ols, specifically those of cocoa and cocoa-derived products, may positively affect cognition both acutely and over long-term dosing.

5 Flavan-3-ols and health detriment

5.1 Deleterious effects

Despite the plethora of research conducted relating flavan-3-ol consumption to health beneficial effects, relatively few publications report their deleterious effects on human health. Reported flavan-3-ol-related health detrimental effects include activation of procarcinogens, reactive-oxygen species formation (pro-oxidant activity), hemorrhage formation, initiation of hepatotoxicity, alteration of pharmacokinetics of therapeutic drugs, increased estrogenic tumor formation, mutagenicity [195], modification of plasma biochemistry, instigation of gastroenteritis, antinutritive activity and weight loss.

While a multitude of natural products and commonly consumed foods possess a high degree of antiangiogenic activity, proanthocyanidin-rich products also possess the ability to cause cell toxicity to normal, healthy tissue [125]. Several studies published prior to 1990 link proanthocyanidin consumption to cancer development. Investigations of tea-consuming populations indicate that habitual consumption of high tannin teas correlates to a high incidence of esophageal cancer. Esophageal cancer has also been linked to consumption of proanthocyanidin-rich *Yerba mate* and *Catha edulis* (also known as khat or Abyssian tea) [196]. The procarcinogenic action of proanthocyanidins is most likely due to pro-oxidant activity. Redox cycling of phenolics, catalyzed by metals such as copper and iron, may result in reactive-oxygen species (ROS) as well as phenoxyl radical formation. ROS and phenoxyl radicals alter DNA, lipids, glutathione, proteins, enzymes, ATP, as well as other biological molecules [197, 198]. Ironically, these same pro-oxidant effects of proanthocyanidins have been linked to both chemopreventative and apoptotic characters. In fact, some types of phenolic compounds behave similarly to anti-cancer drugs at the molecular level: both act to bind and cleave DNA and both generate ROS upon exposure to transition metals [198]. Of the major flavan-3-ols found in green tea, those containing a gallic acid moiety appear more cytotoxic than those without. Gallic acid moieties bind with Fe^{+3} to form a complex capable of scavenging ROS. However, in oxidizing phenolics Cu^{+2} rapidly reduces to Cu^{+1} that further auto-oxidizes to form more ROS. Thus copper increases cellular toxicity of proanthocyanidins and ROS formation while iron prevents both. However, if Fe^{+3} concentration is more than twice the gallic acid concentration, the complex shows pro-oxidant activity by forming H_2O_2-derived hydroxyl radicals via a Fenton-type reaction. Pro-oxidant phenoxyl radicals may also cause cytotoxicity and encourage apoptosis in healthy cells by disrupting mitochondrial cell membrane potential [198, 199].

The cytotoxic effects of proanthocyanidins on the alimentary canals of animals have been documented in rodents and ruminants. At low doses (1.41 – 2.15 g extract/kg animal weight) novel IH636 proanthocyanidins, grape seed, and skin extracts fed to rats produced no toxicologically significant effects [95, 200, 201]. However, in a dermal irritation study, low levels of grape seed proanthocyanidin extract were described as 'moderately irritating' [201]. Although generally considered safe, ingestion of higher concentrations of proanthocyanidins instigates destruction of mucosal lining of the digestive tract, gastroenteritis, and congestion of the intestinal walls in rats, hemorrhagic gastroenteritis in rabbits [21], and striking lesions in the digestive tract of sheep [202]. Hervas' work indicates that 5 days of supplementation of quebracho proanthocyanidin extract (3.0 g/kg live weight) causes weakness and depression in sheep. Sheep cease to take food and suffer significant weight loss at 6 days, become recumbent at day 8, and deathly ill at 10 days. Supplementation for 10 days invoked formation of 'well demarcated ulcers filled with necrotic material in the mucosa of the rumen and reticulum, distension of abomasums and small intestine and dense mucous material in the caecum'. An equivalent dose of quebracho tannin extract proved toxic to sheep rumen microbial populations [202, 203]. However, sheep fed 1.5 g/kg body weight did not show any signs of toxicity. Changes in biochemistry also resulted, as seen in significant alterations in glutathione and cytochrome (CYP) P450 enzyme concentrations. CYP P450 enzymes aid in the metabolism of therapeutic drugs, xenobiotics, and foreign compounds. CYP enzymes induce activation of phase I or II enzymes that are responsible for the inactivation of reactive metabolites and promote carcinogen detoxification. However, CYP enzymes also metabolize carcinogens to chemically reactive electrophilic metabolites capable of covalently binding

to DNA to induce carcinogenicity [204 – 206]. Capable of procarcinogen activation, the CYP1 family of enzymes contributes to several types of carcinogenesis: pulmonary, colorectal, tobacco, and estrogen-related cancers.

Although proanthocyanidin roles as procarcinogens are not well understood, it is known that flavonoids induce CYP production through a variety of mechanisms: direct stimulation of gene expression through receptor specific processes, binding to ligand-activated transcription factor (AhR) and possibly other mechanisms yet to be determined [204]. Binding to AhR is believed to occur due to the similarity of flavonoid structure to typical AhR substrates characterized by planar aromatic compounds with few bulky substituents [207]. Work done by Ciolino *et al*. [208] showed that despite having similar skeletal structures, quercetin and kaempferol behave differently. Quercetin acts as a CYP1A1 inducer while kaempferol appears to have no effect on CYP expression. Their differential effects are thought due to quercetin's extra B-ring hydroxyl group. The lack of an additional B-ring hydroxyl group prevents kaempferol from binding AhR to activate transcription. Tsyrlov *et al*. [209] tested the importance of the ortho-orientation of B-ring hydroxyl groups by comparing quercetin (3′, 4′-OH) to morin (2′, 4′-OH). However, CYP induction appears to vary with CYP type, as quercetin has been shown to inhibit CYP1A-2 and various tea extracts have been shown to both suppress and induce differing types of CYP in animal models [129, 210, 211]. According to Ho *et al*. [212], flavonoid-CYP activity depends both on the number and on position of hydroxyl groups: flavonoids with hydroxyl groups inhibit CYP activity; those that lack hydroxyl groups may induce CYP activity [198].

Structurally similar to endogenous steroid hormones, flavonoids may also promote estrogenic activity by initiating increased expression of aromatase (CYP19). Increased expression of CYP19 correlates with tumor initiation, promotion and progression [213, 214]. Computer modeling estimated that flavonoid-aromatase binding occurs because flavonoid A and C rings mimic androgen D and C rings [215]. Evidence also suggests that 3′, 4′ B ring hydroxylation allows flavonoids to optimally bind aromatase [216].

Phase II enzymes are responsible for metabolizing and detoxifying carcinogens. Members of the phase II enzyme class include: glutathione S-transferase (GST), quinone oxidoreductase (NQO) and UDP-glucuronyltransferase (UGT). Phase II enzyme induction appears to correlate with Michael reaction acceptor functionality, increased by presence of *o*-hydroxy substituents [204]. Both green and black tea extracts induce phase II enzymes due to their concentrations of catechin and epicatechin. However, other flavonoids such as kaempferol, quercetin, myricetin and apigenin induce phase II enzyme NQP [198].

The inhibition of phase II enzymes could increase toxicity of xenobiotics and result in drug toxicity or overdose. Of therapeutic anti-cancer drugs, 50% are metabolized by human CYP3A4. Thus, flavonoid consumption may affect pharmacokinetics of therapeutic drugs by either increasing the drugs toxicity or lowering its effectiveness [217]. The actual effect on drug metabolism depends on flavonoid structure. One such example is the effect of grapefruit juice consumption on calcium channel blockers [218]. Naringenin, the most abundant flavonoid of grapefruit juice, inhibits intestinal CYP3A4 within 30 min after consumption, competitively inhibits glucoronidation, and effectively reduces the metabolization of prescription medications [219]. Naringenin (200 mg/kg) has also shown to increase *in vivo* phenolic anesthetic duration and hepatotoxicity in mice [198].

Toxicity studies indicate pyrogallol moieties cause hepatic damage in rats at 100 mg/kg ip dosing. Several major tea catechins contain pyrogallol and/or gallate moieties. EGCG, characterized by both pyrogallol and gallic acid moieties, and proanthocyanidin significantly increase plasma alanine amino transferase (ALT) levels at 120 mg/kg dosing (24 hours ip administration) in male CD-1 mice, signifying liver damage. EGCG has also caused death to mice less than 24 h following a 150 mg/kg body weight administration. Serum enzyme level increases of ALT are thought due to free radical formation and pro-oxidant activity [198, 199]. Tea flavonoids (50 mg/kg, ip for 7 days) have shown to cause hepatic necrosis *in vivo* in rodent models. Extensive hepatic necrosis and elevated ALT activities resulted, with a 20% mortality rate in male Swiss Webster mice, mild hepatoxicity in male BALB/c mice, and 20% mortality rate in BALB/c female mice [220].

Metabolism studies reveal that glucuronidation does not detoxify gallic acid or EGCG while methylation does. EGCG forms glutathione (GSH) conjugates when oxidized by peroxidase/H_2O_2 in the presence of GSH due to the possession of a catechol B-ring and low redox potential. The formation of GSH conjugates in this manner consumes hepatocyte GSH and leads to hepatoxicity [199].

Tannin toxicity of fodder can be detrimental to livestock, especially in countries where resource limitations require animals to consume tannin-rich fodder. The feeding of *Acacia angustissima* fodder without adaptation causes toxicity to sheep. Rats fed diets containing 20% *Acacia angustissima* died within 2 – 5 days while intake and average daily gain were both significantly reduced in rats fed a diet of 70% extract. Investigation indicates that condensed tannins contribute to the negative effects of *Acacia angustissima*. However, the gradual increase in dietary levels over time appears to prevent toxicity, most likely due to changes at the microbial level [221].

5.2 Antinutritive effects

Proanthocyanidins can also lower the nutritional value of food and fodder due to their ability to form complexes with proteins, starch, essential amino acids, carbohydrates, and

digestive enzymes [3, 4, 222]. Proanthocyanidin antinutritive effects have been associated with weight loss, decreased protein and amino acid utilization, decreased vitamin and mineral facilitation, digestibility, feed utilization, feed intake, and even fatality when used in high quantity. Proanthocyanidin-protein precipitation interactions depend on both chain length and presence of galloyl groups [223]. The proanthocyanidin content of grains also varies; in barley, rye and common beans it is generally low, while in rapeseed, sorghum and millet it is high. It is thought that tannin-bound proteins form hydrolysis resistant, less digestible complexes. There is also evidence, as in the case of faba bean hull, that such complexes impair sugar transport at the intestinal brush border [4, 21, 224]. Studies on experimental animals have used whole seed or grain meal, extracts or mixtures of commercially available tannins in a multitude of animal models: chicks, rats, pigs, swine and hamsters. Cattle and deer, goats and other ungulates appear to select their feed on the basis of tannin content, rejecting plants with over 5% tannin. Mountain gorillas of the African Congo and Colobus monkeys of Western Uganda also select plants on the basis of tannin levels, generally avoiding those that contain significant concentrations of tannins in their leaves [225]. The parotid-derived proline-rich proteins (PRP) display high affinity for tannin. Consumption of a tannin-rich diet initially leads to body mass loss until a quick adaption produces up to 45% PRP to restore body mass in mice and rats [222]. Hamsters lack the ability to induce synthesis of PRP and consequently die due to toxicity if kept on a proanthocyanidin-rich diet [226].

Flavan-3-ols also affect utilization of vitamins and minerals. Tannnins have been implicated in a reduction of vitamins A, and B-12, and mineral iron utilization. In the case of divalent iron, tannins chelate the metal into a less absorbable complex [4]. Insoluble iron-phenol complexes may form in the gastrointestinal tract, rendering iron nutritionally unavailable from high tannin food sources [224]. Work done by Brune *et al*. [227] suggests a correlation between phenolic degree of galloylation and iron adsorption inhibition. Proanthocyanidin content of rapeseed/canola plays an important role in iron-binding properties of the meal. However, removal of proanthocyanidins could improve the nutritional value of canola/rapeseed protein products [224]. Consumption of a proanthocyanidin-rich grape seed extract produces similar effects. Rats fed grape seed extract (2.0%) experienced decreased serum iron levels and decreased serum iron/total iron binding capacity ratios *in vivo* [228].

6 Conclusion

Substantial evidence suggests that diets rich in flavan-3-ol containing foodstuffs may provide one or more of several health beneficial effects. Flavan-3-ols of varying structure possibly act as antioxidants, free radical scavengers, anticarcinogens, cardiopreventatives, antimicrobials, anti-virals, and may play a significant role in maintaining neurological health. However, evidence on the contrary must equally be considered as flavan-3-ols may also behave as anti-nutrients, procarcinogens, pro-oxidants, hemorrhage inducers, mutagens, or hepatotoxins depending on the source, type, quantity and existence of other dietary burdening factors.

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