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Review

Antioxidant activity and antimicrobial effect of berry phenolics – a Finnish perspective

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In Finland, berries are part of the traditional diet significantly contributing to the intake of flavonoids and other phenolic compounds. Compositional data on phenolic compounds in berries has been rapidly accumulating and included in the national food composition database. Among the different bioactive substances in berries, phenolic compounds including flavonoids, tannins, and phenolic acids have received considerable interest due to their effects in food and health. A great amount of *in vitro* evidence exists showing that berry phenolics are powerful antioxidants. However, the antioxidant effect of berry phenolics is strongly dependent on the choice of berry raw material, as the antioxidant activity differs between the different phenolic constituents, including anthocyanins, ellagitannins, and proanthocyanidins. In foods, the antioxidant effect is also influenced by the structure of food. Tannin-containing berries exhibit antimicrobial properties against pathogenic bacteria, thus offering many new applications for food industry. Much of the interest in berry phenolics has focused on cranberries and both cultivated and wild blueberries, although also other berries including black currants, cloudberries, lingonberries, and red raspberries posses promising bioactivities that may have relevance to human health. Antioxidant activity of berry phenolics, in addition to other mechanisms, may contribute to human health, but the possible relationship remains yet to be scientifically substantiated.

Keywords: Antioxidants / Antimicrobial effect / Berries / Bioactivities / Phenolics

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1 Berries and berry phenolics in the Finnish diet

Berries are part of a traditional diet in Finland and other Scandinavian countries. In recent years, the average consumption of berries in Finland has been 15 kg/year [1]. The most widely cultivated berries are strawberries (*Fragaria ananassa*) and black currants (*Ribes nigrum*) while among the commercially most valuable wild berries are lingonberries (*Vaccinium vitis-idea*), bilberries (*Vaccinium myrtillus*), and cloudberries (*Rubus chamaemorus*). The estimated total amount of wild berries growing in Finland is about 50 of which 37 species are edible. Most of the berries, ca. 80%, are consumed as various berry products [1] such as juices, jams, and purées. Among the different bioactive substances in berries, phenolic compounds including flavonoids, tannins, and phenolic acids have received considerable interest in bearing possible relations to human health. An estimated value of flavonoid intake in Finland is 150 mg/day based on national food consumption data (Fig. 1) with berries and berry products being an important source especially due to their significant contribution to the anthocyanin intake [2]. This intake value is in accordance with other estimated dietary intakes of flavonoids ranging from below 100 to 210 mg/day [3–5], but much higher regarding the anthocyanin intake compared to a recent estimation of 12.5 mg/day in the USA [6].

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2 Phenolic compounds in berries

Compositional data on phenolic compounds in berries grown in Finland have been rapidly accumulating since late 1990's [7–19] and have been included in the national food composition database in much similar way as in other countries including Scotland [5] and the USA [20]. In addition to genetic differences and several environmental factors

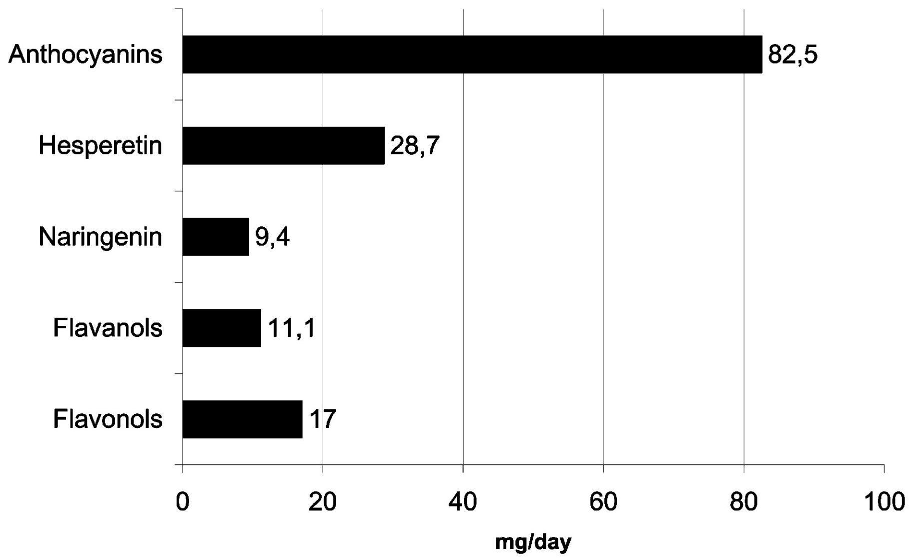

Figure 1. Estimated intake of different flavonoids in the average Finnish daily diet [2].

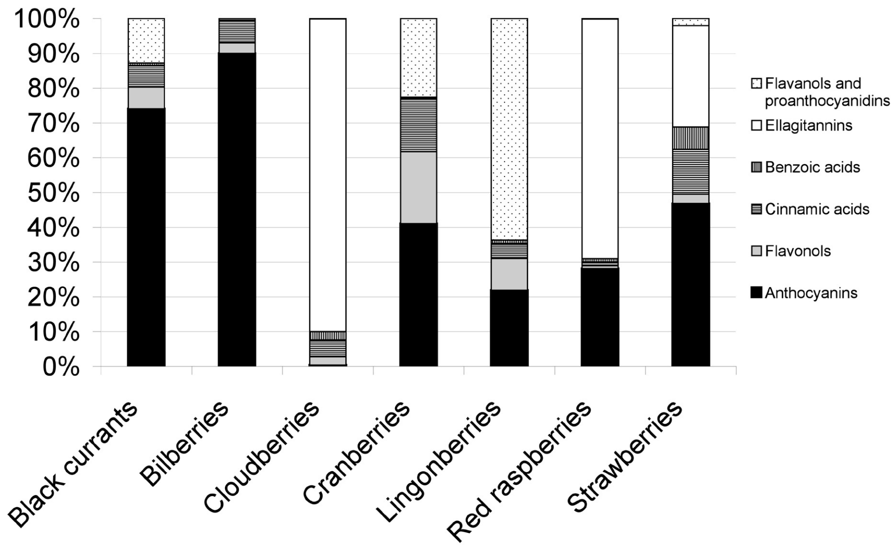

Figure 2. Phenolic profiles of black currants (*Ribes nigrum)*, bilberries (*Vaccinium myrtillus)*, cloudberries (*Rubus chamaemorus*), cranberries (*Vaccinium oxycoccus*), lingonberries (*Vaccinium vitis-idaea*), red raspberries (*Rubus idaeus*), and strawberries (*Fragaria ananassa*) obtained with aqueous acetone extraction [13, 43].

such as light, temperature, and humidity, the extraction method affects remarkably the phenolic composition. Use of aqueous acetone results in higher yields anthocyanins, ellagitannins and hydroxycinnamates compared to extraction with aqueous methanol [13]. Ethylacetate has been used for extraction of berry proanthocyanidins [18, 21], although for efficient extraction of oligomeric and polymeric proanthocyanidins 80% methanol as well as acetone are recommended [21]. Hydrolysis of the native glycosylated forms of phenolics is often used to simplify analytical procedure, however, thereby loosing essential information on the relationship between bioactivity and phenolic composition. In general, the phenolic profile of black currants, bilberries, cloudberries, cranberries (*Vaccinium oxycoccus*), lingonberries, red raspberries (*Rubus idaeus*), and strawberries appear as shown in Fig. 2.

Anthocyanins are especially abundant in bilberries, the wild lowbush blueberry species. The anthocyanin composition of bilberry is more complicated than that of most other anthocyanin-containing berries, as a total amount of 15 different anthocyanins have been identified, including monoglycosides of delphinidin, cyanidin, petunidin, peonidin, and malvidin (Table 1) [22]. In bilberries, anthocyanins comprise ca. 90% of phenolic compounds while black currants contain on the average 75% anthocyanins with lower percentage found in strawberries, cranberries, lingonberries and red raspberries [22, 23]. The most common anthocyanins in red berries are cyanidin glycosides followed by delphinidin glycosides. Berries belonging to *Rubus* and *Fragaria* in family Rosaceae including cloudberries, red raspberries, and strawberries are rich in hydrolysable ellagitannins comprising 90, 85, and 30%, respectively, of their phenolic

Table 1. Anthocyanin composition (%) of black currants, bilberries, lingonberries, and red raspberries [22, 44]

Anthocyanins	Black currant	Bilberry	Lingonberry	Red raspberry
Cyanidin-3-glucoside	6.9	8.5	1.9	15.6
Cyanidin-3-rutinoside	38.7			
Cyanidin-3-galactoside		9.0	74.2	
Cyanidin-3-sophoroside				5.3
Cyanidin-3-arabinoside		13.6	23.9	59.4
Cyanidin-3-glucosylrutinoside			15.6	
Delphinidin-3-glucoside	15.3	13.7		
Delphinidin-3-rutinoside	39.1			
Delphinidin-3-galactoside		14.9		
Delphinidin-3-arabinoside		15.3		
Malvidin-3-glucoside		8.4		
Malvidin-3-galactoside		3.1		
Malvidin-3-arabinoside		2.4		
Pelargonidin-3-glucoside				0.9
Pelargonidin-3-rutinoside				0.7
Pelargonidin-3-sophoroside				2.5
Petunidin-3-glucoside		6.0		
Petunidin-3-galactoside		2.1		
Petunidin-3-arabinoside		1.3		
Peonidin-3-glucoside		0.1		
Peonidin-3-galactoside		0.6		
Peonidin-3-arabinoside		1.0		

composition [13]. These berries are the major sources of ellagitannins in Western diets [11, 24]. The main ellagitannins in both cloudberries and red raspberries have been identified as dimeric sanquiin H-6 and trimeric lambertianin C (submitted for publication) supported by Scottish research on red raspberries [25]. Ellagitannins are also well represented among phenolic compounds of arctic bramble (*Rubus arcticus*) [18]. Other types of polymeric tannins exist in berries of the *Vaccinium* species with rare A-type low-molecular weight proanthocyanidins found in wild lingonberries, cranberries, bilberries and bog-whortleberries (*Vaccinium uligonosum*) [26]. Other berries containing proanthocyanidins include Saskatoon berries (*Amelanchier alnifolia*) [21], elderberries (*Sambucus nigra*), aronia (*Aronia melanocarpa*) [27], and rowanberries (*Sorbus aucuparia*), (Mattila, P., personal communication 2006). Nordic berries contain to a lesser extent flavonols [8, 10, 13, 16, 19, 28] and phenolic acids [7, 13, 14, 17, 19, 28], which are also contributing to the antioxidant, antimicrobial, anti-inflammatory and other types of health-related bioactivities of berry phenolics.

3 Bioactivities of berry phenolics

3.1 Antioxidant activity of berry phenolics

Considerable *in vitro* evidence exists, showing that berry phenolics are powerful antioxidants. A great diversity of methods have been applied to study both the radical-scavenging activity as well as the antioxidant activity of berry phenolics, resulting in great differences in the outcome [29–31]. Currently, as a lot of antioxidant evaluation is conducted merely by means of radical-scavenging tests, it must be emphasized that the hydrogen-donating ability of antioxidants in a simple test model does not necessarily indicate their activity in the presence of oxidizable lipid (or protein) containing environment. Preferably, in evaluating for *in vitro* antioxidant activity of plant phenolics, an initial screening procedure using simple methodology such as total phenolic content and radical-scavenging assays should be followed by structure-activity investigations including compositional analyses and antioxidant activity testing in the presence of oxidizable substrates. Using various radical-scavenging assays, aronia, black currants, different blueberries *(Vaccinium corymbosum*), bilberries, cranberries, elderberries (*Sambucus nigra*), lingonberries, red raspberries, and rowanberries have been reported to exhibit significant radical-scavenging activities [28, 32–38]. In screening for antioxidant activity of different plant phenolics, berries scored among the most promising among 92 plant materials examined [39]. Berry phenolics were also potent antioxidants toward oxidation of bulk lipids. The formation of methyl linoleate hydroperoxides was inhibited over 90% by crowberries (*Empetrum nigrum*), rowanberries, cloudberries, cranberries, bog-whortleberries, aronia, gooseberry (*Ribes grossularia*), bilberry, and lingonberry phenolics at concentrations of 500 μg/mL. Red raspberries and black currants were somewhat less active, whereas red currants (*Ribes rubrum*) and strawberries were the least active in inhibiting lipid oxidation. In more complex environments such as phosphatidylcholine liposomes with or without incorporated protein (lactalbumin), bilberry, lin-

Table 2. Radical scavenging (DPPH-test) and antioxidant activities of berry phenolics [a] investigated in different environments containing various oxidizable lipids

Berry phenolics	DPPH[b] 17 μg/mL	Bulk oil[c] 500 μg/mL	Emulsion[d] 100 μg/mL	Liposomes[e] 4.2 μg/mL	LDL[f] 4.2 μg/mL	Reference
Bilberry phenolics		96 ± 2		57 ± 3	97 ± 0[g]	[13, 43]
Bilberry anthocyanins	52 ± 1		70 ± 5	87 ± 5	95 ± 1	[22, 43]
Bilberry proanthocyanidins	47 ± 2[h]		85 ± 1		92 ± 1	[26]
Black currant phenolics		83		41 ± 3	97 ± 0[g]	[43]
Black currant anthocyanins	58 ± 1		60 ± 0	75 ± 3	96 ± 0	[13, 22, 39, 43]
Cloudberry phenolics	36 ± 1	94 ± 0			96 ± 1	(submitted for publication)
Cloudberry ellagitannins	85 ± 0	56 ± 1			94 ± 3	(submitted for publication)
Cranberry phenolics			95		96 ± 0	[39]
Cranberry proanthocyanidins	58 ± 2[h]		87 ± 1		86 ± 2	[26]
Lingonbery phenolics		97 ± 1		57 ± 3	34 ± 5[g]	[13, 43]
Lingonberry anthocyanins	36 ± 0		61 ± 3	68 ± 1	97 ± 0	[22]
Lingonberry proanthocyanidins	74 ± 0[h]		84 ± 0	88 ± 1	96 ± 1	[26, 43]
Red raspberry phenolics	56 ± 2	93 ± 1		54 ± 2[i]	97 ± 1	[13, 35]
Red raspberry anthocyanins	48 ± 2			82 ± 5[i]	96 ± 1[g]	[35]
Red raspberry ellagitannins	88 ± 4	67 ± 3		57 ± 4[i]	97 ± 0	[35, submitted for publication]
Strawberry phenolics		56 ± 4			53 ± 2[g]	[39]

a) Extracted with aqueous acetone and further fractionated using column chromatography. Interfering sugars removed by solid phase extraction prior to radical scavenging or antioxidant activity analysis.
b) Expressed as radicals scavenged (%) after 4 min reaction time.
c) Inhibition (%) of hydroperoxide formation of bulk methyl linoleate after 72 h of oxidation.
d) Inhibition (%) of hydroperoxide formation of emulsified methyl linoleate after 72 h of oxidation.
e) Inhibition (%) of hexanal formation in phosphatidylcholine-lactalbumin liposomes after 72 h of oxidation.
f) Inhibition (%) of hexanal formation in LDL lipids after 2 h of oxidation.
g) Previously unpublished data.
h) Concentration of 8.3 μg/mL.
i) No lactalbumin present.

gonberry, red raspberry, and black currant phenolics were only moderate inhibitors toward oxidation [23, 35]. On the other hand, berry phenolics, as well as other plant phenolics, respond favorably in assays measuring inhibition of oxidation of LDL (Table 2). In most antioxidant assays, however, the antioxidant activities measured reflect the effect of the principal phenolic compounds of the various berries with different phenolic profiles.

In red or purple colored berries such as aronia, black currants, blueberries, bilberries, cranberries, elderberries, lingonberries, and red raspberries, anthocyanins are among the principal antioxidant berry constituents [13, 22, 32, 35, 40, 41]. Despite of their lack of effect in bulk lipids, berry anthocyanins exhibit good free radical-scavenging activities [22, 27, 34, 38, 42] and are powerful antioxidants in lipid-containing hydrophilic environments such as toward oxidation of emulsified lipids, liposomes, and LDL [22, 23, 35, 40, 42, 43]. However, anthocyanins and antioxidant effect can significantly vary across species and cultivars of berries [22, 27, 34, 38, 42–44]. Anthocyanins isolated from bilberries prevent the formation of emulsified methyl linoleate hydroperoxides efficiently but in LDL black currant, bilberry, and lingonberry anthocyanins all exhibit high antioxidant activity [22]. In all of these berries, the monoglycosides of cyanidin and delphinidin are dominant (Table 1). Compared to the effect of a natural mixture of berry phenolics, the isolated anthocyanins of black currants, bilberries, lingonberries, and red raspberries provide significantly better antioxidant protection in liposomes (Table 2). In bilberries and black currants, anthocyanins contribute the most to the antioxidant effect while in red raspberries the coexistence of anthocyanins and ellagitannins may not be the best combination for inhibition of liposome oxidation, as raspberry phenolics were among the least potent antioxidants [43]. In liposomes, the protective antioxidant effect of bilberry anthocyanins have been shown to be further improved by co-pigmentation reactions [42]. Black currant anthocyanins followed by red raspberry and lingonberry anthocyanins as well as red raspberry and blackberry (*Rubus laciniatus*) berry juice anthocyanins inhibit effectively also oxidation of dairy emulsions [23, 44]. In these whey protein emulsions, berry anthocyanins partition into the aqueous phase of the emulsion, thus being located favorably for antioxidant protection.

Tannins are known to interact with proteins, resulting in complex formation responsible for the astringent character of foods and beverages rich in tannins [45]. Hydrolysable tannins, ellagitannins, in raspberries and cloudberries, and the proanthocyanidins (condensed tannins) in bilberries, cranberries, and lingonberries are also effective antioxidants in various food environments such as bulk oil, emulsions, liposomes as well as toward oxidation of LDL (Table 2). Compared to proanthocyanidins, ellagitannins exhibit better radical-scavenging activities. As ellagitannins have not been commercially available, their bioactive properties such as antioxidant activity are less studied than those of ellagic acid or many other phenolic compounds. Isolated red raspberry ellagitannins are equally active or more potent than anthocyanins in protecting both dairy proteins and liposome phosphatidylcholine from oxidation [35, 43]. Sanquiin H-6 is the major ellagitannin contributing to the antioxidant capacity of red raspberries [25]. Both red raspberry and cloudberry ellagitannins (80–90% of the phenolics) are excellent antioxidants toward oxidation of LDL and bulk methyl linoleate (submitted for publication). Dimeric and trimeric proanthocyanidins isolated from bilberries, bog-whortleberries, cranberries, and lingonberries inhibit effectively the oxidation of methyl linoleate emulsion, lactalbumin-containing liposomes, and LDL [26, 43]. Especially lingonberry proanthocyanidins were efficient in inhibiting oxidation of lactalbumin measured by loss of natural fluorescence of trypthophan [43]. In addition, catechins, the monomers, exhibited comparable activity to the fractions containing dimers and trimers in inhibiting oxidation [26]. Especially bog-whortleberry catechins were excellent antioxidants toward the oxidation of human LDL even in lower concentrations.

Contribution of other flavonoids such as flavonols as well as phenolic acids to the antioxidant effect of berries is generally much less significant compared to the activity of anthocyanins and tannins. In berries where phenolic acids are the main phenolic constituents, such as in rowanberries [7, 14], they are most likely to contribute to the radical-scavenging and antioxidant activity. In addition, berries, including cultivated and wild blueberries, with comparatively high amounts of hydroxycinnamates such as chlorogenic acid [13, 14, 19] benefit from their antioxidant effect. Flavonols are well represented among other phenolics in for example sea buckthorn (*Hippophaë rhamnoides*) and black and green currants (*Ribes nigrum*) [19] where they might add to the antioxidant protection.

3.2 Antimicrobial effects of tannins in berries

Certain berries rich in tannins have been identified as berries with properties beneficial toward bacterial infections. Two different types of polymeric tannins in these berries protect against pathogenic bacteria. Presence of the A-type linkage of cranberry (*Vaccinium macrocarpon*) proanthocyanidins may enhance both *in vitro* and urinary bacterial anti-adhesion activities [46]. In addition, other tannin-containing berries may contribute to this effect, as berry juices of a mixture of cranberries (*Vaccinium oxycoccus*) and lingonberries as well as cloudberry juice protect from urinary tract infection [47, 48]. Among different berries, cranberries, cloudberries, red raspberries, strawberries, and bilberries possess clear antimicrobial effects against human pathogens [49]. Berry ellagitannins are strong antimicrobial agents acting as possible anti-adherence compounds in preventing the colonization and infection of many pathogens. The phenolic extract of cloudberry comprising of mostly ellagitannins possessed the strongest antimicrobial effect, followed by red raspberry and strawberry. *Salmonella, Staphylococcus, Helicobacter,* and *Bacillus* are the most sensitive bacteria for the berry phenolics. In addition, the growth of *Escherichia, Clostridium* and *Campylobacter* species but not *Lactobacillus* and *Listeria* species is inhibited by berry phenolics [49–53]. Red raspberry phenolics and its ellagitannin fraction also have powerful antimicrobial properties against the growth of human colonial pathogens, *Klebsiella oxytoca* and *Proteus mirabilis* [53]. Several mechanisms of action in the growth inhibition of bacteria are involved, such as destabilization of cytoplasmic membrane, permeabilization of plasma membrane, inhibition of extracellular microbial enzymes, direct actions on microbial metabolism and deprivation of the substrates required for microbial growth [49].

4 Impact of berry phenolics on foods and health

The antioxidant effect of berry phenolics toward inhibiting lipid and protein oxidation is a well-recognized factor in improving the quality of food. The antioxidant effect of berry phenolics is, however, strongly dependent on the choice of berry raw material, as the antioxidant activity differs between the different phenolic constituents, including anthocyanins, ellagitannins, and proanthocyanidins [43, 44]. In addition, the antioxidant effect depends on the environment, *i.e.* the structure of food, whether it is bulk oil, emulsion, or containing liposomal particles. In preventing oxidation of food, berries are used as food ingredients or as concentrates, *e.g.* for color improvement (E163). Utilization of antimicrobial activity of berry phenolics may offer many new applications in food industry. For example, natural food preservatives targeted to foods that are easily contaminated by bacteria, such as *Salmonella* and *Staphylococcus*, are highly desired [49].

There is strong evidence supporting the role of polyphenols in human health [54]. In the past few years, there have been major advances regarding the knowledge of polyphenol absorption, metabolism and bioefficacy [55–58]. Significant mechanisms of action of berry phenolics may include antioxidant action as well as interaction reactions with proteins such as those in bacterial cell walls and fimbria, as well as participation in enzyme reactions including metalloproteinases. Antioxidant activity, *i.e.* inhibition of oxidation of lipids and proteins as well as radical-scavenging activity of berry phenolics have been related to the onset of many diseases, including cardiovascular diseases and cancer. According to a review of 93 intervention studies concerning relevance of polyphenols to humans [55], ingestion of cranberry juice, black currants, as well as red wine have been reported to increase plasma antioxidant activity or to decrease formation of lipid-oxidation products while ingestion of blueberries did not significantly affect plasma antioxidant activity. As oxidation of LDL is one of the biomarkers associated with cardiovascular diseases, berries inhibiting *in vivo* oxidation of LDL are postulated to have potential benefits in disease prevention. Berry anthocyanins and their possible protective role toward cardiovascular health were investigated also in an EU-funded collaboration (QLK1-1999-00124). For 3 weeks in a strictly controlled study, 58 healthy young subjects consumed black currant juice or anthocyanin soft drink, amounting to an intake of 285–450 mg of anthocyanins/day The only differences between treatment effects were found in plasma vitamin C, red blood cell glutathione peroxidase activity, and bleeding time (to be published). Antioxidant activity of berry phenolics may thus be relevant to human health, but the possible relationship remains yet to be scientifically substantiated.

Another type of health beneficial effect has been proven regarding berry phenolics and pathogenic bacteria. Certain types of cranberries have been shown to aid in maintaining urinary tract health [46, 55] with other tannin-containing berries contributing to this effect as well [47, 48]. In a Cochrane review [59], the conclusion based on seven human intervention studies was that cranberry juice might reduce symptoms of urinary tract infections. The first ever health claim on berry phenolics was issued by French Food Safety Authority in April 2004. According to this claim, cranberry proanthocyanidins, in a dose of 36 mg, "help reducing the adhesion of certain *E. coli* bacteria to the urinary tract".

Bioactive berry phenolics are exploited in marketing of food supplements. For example, anthocyanin extracts of wild and cultivated blueberries sold as food supplements are claimed to enhance eye health. Generally, the daily dose recommended is 25 mg anthocyanins, which is equivalent to less than a handful of blueberries. However, according to a review by Canter and Ernst [60], the claim regarding improvement of night vision cannot be substantiated by human studies.

Berry phenolics have also been investigated mainly *in vitro* and in animal experiments for many other bioactivities related to various disorders and diseases [25, 35, 55, 61–69]. Much of the research on berry phenolics has focused on cranberries and both cultivated and wild blueberries, although also other berries including black currants, cloudberries, lingonberries, and red raspberries posses promising bioactivities that may have relevance to human health.

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