NPC Natural Product Communications

2011
Vol. 6
No. 1
149 - 156

Anthocyanins as Antimicrobial Agents of Natural Plant Origin

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Received: July 23rd, 2010; Accepted: December 9th, 2010

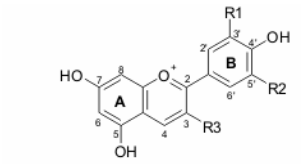
Anthocyanins are particularly abundant in different fruits, especially in berries. The beneficial effects of these compounds for human health have been known from at least the 16th century. Despite the great number of papers devoted to the different biological effects exerted by anthocyanins only a limited number of studies is focused on the antimicrobial activity of these compounds. Anthocyanin content of berry fruits varies from 7.5 mg/100 mg fresh fruit in redcurrant (*Ribes rubum*) up to 460 mg/100 g fresh fruit in chokeberry (*Aronia melanocarpa*). After consumption, anthocyanins are intensively metabolized, mainly in the intestines and liver. Glucorination, methylation and sulfation are the most typical metabolic reactions. Antimicrobial activity of crude extracts of plant phenolic compounds against human pathogens has been intensively studied to characterize and develop new healthy food ingredients as well as medical and pharmaceutical products. However, there is very little information available about the antimicrobial activity of the pure anthocyanins. In the last part of this review we present the collection of papers describing the anthocyanin profiles of different fruits (mainly berries) and the antimicrobial properties of the identified compounds. Generally, anthocyanins are active against different microbes, however Gram-positive bacteria usually are more susceptible to the anthocyanin action than Gram-negative ones. Mechanisms underlying anthocyanin activity include both membrane and intracellular interactions of these compounds. Antimicrobial activity of berries and other anthocyanin-containing fruits is likely to be caused by multiple mechanisms and synergies because they contain various compounds including anthocyanins, weak organic acids, phenolic acids, and their mixtures of different chemical forms. Therefore, the antimicrobial effect of chemically complex compounds has to be critically analyzed.

Keywords: flavonoids, anthocyanins, berries, antimicrobial activity.

Anthocyanins (Greek: *antos*, flower and *kyanos*, blue) are a class of plant constituents sharing the same diphenylpropane skeleton ($C_6C_3C_6$) and collectively known as flavonoids. Anthocyanins are water-soluble and their spectral properties usually are responsible for blue, purple and red coloring of different plant parts (flowers, fruits and other plant tissues). They are particularly abundant in different fruits, especially in almost all types of berries. Owing to their common occurrence in fruits the daily intake of anthocyanins is rather high – in the USA it was estimated to be at the level of ca. 200 mg/day [1]. The exact profile of the composition of the consumed anthocyanins depends, however, on factors like the age of the consumer and type of diet [2,3].

The beneficial effects of anthocyanins on human health were known at least from the 16th century, when blackberry juice was used in the treatment of mouth and eye infections [4]. Thanks to numerous studies we now know that anthocyanins display a variety of biological activities: they act as strong antioxidants [5,6], anti-inflammatory [4], antiproliferative [7] and anti-carcinogenic [8] agents. They could also exert positive effects on the cardiovascular system [1]. The above mentioned anthocyanin properties were the main reason for the substantially increased interest in their study in the last decade. Despite the great number of papers devoted to the different biological effects exerted by anthocyanins, only a limited number of studies has focused on the antimicrobial activity of these compounds. In the current paper we review the recent advances in this field together with some information concerning the metabolic transformation occurring after consumption of anthocyanins. This last issue seems to be of some importance for antimicrobial flavonoid activity since anthocyanins usually are intensively metabolized in the gastrointestinal tract prior to reaching their target. It is worth emphasizing also that anthocyanidins could be easily transformed into various products during food processing [9].

Chemically anthocyanins are mono- or diglycosides of anthocyanidins, which are polyhydroxyl and polymethoxyl derivates of 2-phenylbenzopyrylium (flavylium) salts (see Figure 1). In anthocyanins, sugar moieties (usually glucose, rhamnose, xylose, galactose, arabinose or fructose) can be attached at positions 3, 5, 7, 3', and 5'; the 3- and 5-positions being dominant. Depending on pH anthocyanins may exist in many different protonated, deprotonated, hydrated, and isomeric forms. Due to



	R1	R2	R3
delphinidin	OH	OH	OH
cyanidin	OH	H	OH
petunidin	OMe	OH	OH
peonidin	OMe	H	OH
malvinidin	OMe	OMe	OH
pelargonidin	H	H	OH

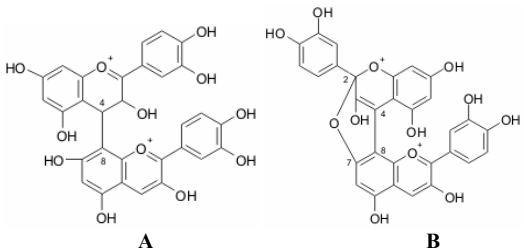
Figure 1: Chemical structure of six most common anthocyanidins.

changes in protonation and hydration at acidic pH (pH 1-3) anthocyanins are red, at pH 4-5 they are colorless or yellow, and at pH values between 6 and 7 they became blue-purple [6].

Anthocyanidin synthesis (like other flavonoids) occurs in plants on the cytoplasmic leaflet of the endoplasmic reticulum and then they are accumulated in the large central vacuole [10]. In many plants anthocyanidins might occur in oligomeric form – in this case they are called proanthocyanidins. Depending on the type of bond between the oligomer-forming anthocyanidin molecules two general types of proanthocyanidins are distinguished. In less common A-type proanthocyanidins, two bonds are formed between 2β-7 and 4β-8 carbons of oligomer-forming molecules (see Figure 2A). In B-type proanthocyanidins, only one 4β-8 bond is formed (Figure 2B). Apart from proanthocyanidins anthocyanidin-flavonol adducts can also appear, mostly during the wine production process [9].

Two main chemical structural features providing anthocyanins with antioxidant properties are shared with other flavonoids. The first is the presence of a catechol moiety in the B-ring (R2 = OH in Figure 1), and the second is the double bond between C-2 and C-3 [11]. These common features are, however, strongly supplemented by the hydroxylation and/or glycosylation pattern of individual compounds. At this point it seems to be important to stress that, as can be found in the literature, the role of glycosylation is rather controversial. According to Rice-Evans [5], Satué-Gracia *et al.* [12] and Wang *et al*. [13a] glycosylation of anthocyanidins decreases their antioxidant potency, while Kähkönen *et al*. [6] reported that the antioxidant efficiency of petunidin and malvidin increased along with glycosylation.

Anthocyanins are present in many plant families, but are almost absent in flavanone-rich plants [5]. Among different plant sources berry fruits are particularly rich in different polyphenols, including anthocyanins. The anthocyanin content of berry fruits varies from 7.5 mg/100 mg fresh fruit in redcurrant (*Ribes rubum*) up to 460 mg /100 g fresh fruit in chokeberry (*Aronia melanocarpa*) [13b]. The chemical structure profiles of anthocyanins differ between individual berry species; typical are mono-,



A **B**

Figure 2: Two general types of proanthocyanidins: A-type (**A**) and B-type (**B**).

di- and triglycosides. Among different berries cyanidin-3-glycosides are most abundant, but delphinidin-3-glycosides can also be quite often found. It is characteristic that different berry species belonging to the same genus (e.g. *Vaccinium*) might significantly differ in the number of anthocyanidin types [13b]. Anthocyanin profiles of individual berries might also be profoundly affected by factors like berry maturity at harvest [13c]. Therefore, the commercially available extract mixtures are composed for optimal beneficial properties, like antioxidant potential [14].

Apart from berries that are common in European diets, some tropical fruits can also be a valuable source of anthocyanins. These compounds have been found, for example, in acerola (*Malphigia emarginata*; 23-48 mg/100 g fresh fruit), guajiru (*Chrysobalanus icaco*; 104 mg/100 g f.f.), jambolão (*Syzygium cumini*; 79 mg/100 g f.f.) and jussara *(Euterpe edulis*; 290 mg/100 g f.f.) [15].

Not only fresh fruits supply consumers with anthocyanins as their processed forms also contain these compounds. Similar to berries, red grapes and in consequence red wine also contain anthocyanins, which provide wine their red color. In many countries, red wine is an important source of different beneficial polyphenols, including resveratrol and anthocyanins. The total anthocyanin content depends on age: in young red wine it reaches 400 mg/L, but in aged wines (at least two years) this amount decreases to 90 mg/L [16]. Red wines generally contain glycosides of five major anthocyanidin types: delphinidin, cyanidin, petunidin, peonidin and malvidin. The exact amounts of these components should, however, strongly depend on such factors as geographical origin and cultivar. These differences could be so significant that they have been proposed as a scientific base for wine differentiation and quality control [17].

The beneficial health effects of anthocyanins depend on their absorption, metabolism, distribution in the tissues and excretion. The general scheme of anthocyanin fate in humans is presented in Figure 3. It was reported that anthocyanins are poorly bioavailable. The proportion of total anthocyanins, in both native glycosylated form, as well as metabolites, absorbed and excreted in the urine was often very low compared with the ingested doses [18,19]. The concentrations of these flavonoids in the urine and

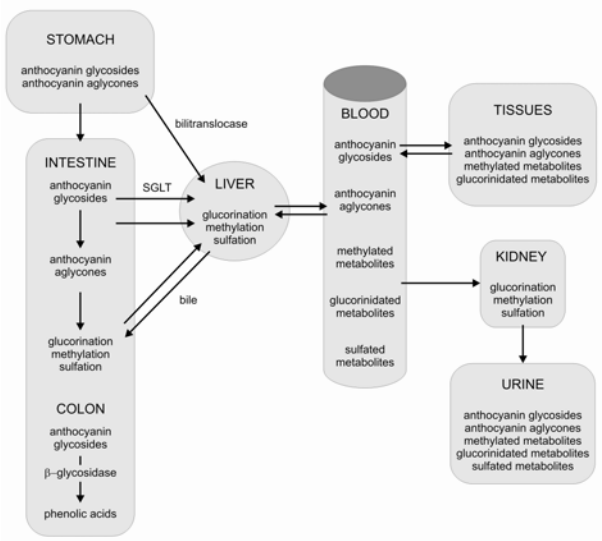


Figure 3: Potential routes for anthocyanin absorption, metabolism and elimination in humans.

plasma reached only low nanomolar levels [20,21]. The majority of dietary anthocyanins undergo deglycosylation before absorption and are subsequently modified during first metabolic transformation. The main metabolites of anthocyanins detected in the urine are glucuronide conjugates and methylated compounds. Sulfonated derivatives are also present in very low concentrations [21]. Despite the occurrence of the above described transformations, many ingested anthocyanins appear in plasma and urine unaltered [20b]. Several pharmacokinetic animal studies have documented that anthocyanins are absorbed mainly in their intact form and moved into the blood within 15-120 minutes after ingestion [20-24]. Many investigators suggest that anthocyanins are not metabolised before release into the systemic circulation [25].

Talavera *et al.* [26,27] have shown that, after consumption, anthocyanins are efficiently absorbed from both stomach and small intestine. Direct evidence of the absorption from the stomach has been demonstrated using *in situ* gastric exposure to anthocyanins [26,28a]. Transport of anthocyanins across gastric mucosa is carried by a specific enzyme, bilitranslocase, which is found in stomach and liver plasma membranes [28b,29]. Passamonti *et al.* [28b] demonstrated also that anthocyanins can serve as ligands for bilitranslocase.

There is also direct evidence that anthocyanins are absorbed from the rat small intestine. Using *in situ* intestinal perfusion, it was shown that anthocyanins were efficiently absorbed and excreted into the bile and urine [27]. Matuschek *et al.* [30a] measured the absorption of cyanidin-3-glucosides from various tissues of the mouse digestive tract using an *in vitro* method. Cyanidin-3-glucoside was significantly absorbed by tissue from the jejunum and slightly absorbed by duodenal tissue. No absorption was measured for tissue from the ileum and colon. The results of clinical trials and experiments performed on animal models indicate that anthocyanins are absorbed in a very short time [30b,31]. Anthocyanins appear in blood a few minutes after consumption, and their maximum blood levels were observed after approximately 60 minutes [32,33a]. The rapid appearance of anthocyanins in the bloodstream indicates that the absorption may take place in the stomach and small intestine.

Anthocyanins are present in foods as glycosides and aglycones. Aglycones are primarily hydrophobic and can passively diffuse across the mucosal epithelium. On the other hand, anthocyanin glycosides are deglycosylated in the small intestine by the action of β-glucosidase, β-glucuronidase and α-rhamnosidase. The linkage with sugars increases anthocyanin water solubility and limits its passive diffusion [33b]. Therefore, the absorption of an anthocyanin glycoside requires either a specific active transport mechanism or hydrolysis of the β-glycoside before absorption [34]. A sodium-glucose co-transporter (SGLT) enables the transport of the intact glycosides across membranes of enterocytes [35].

Anthocyanins can be modified by pH and microbial populations of the digestive tract. *In vitro* digestion studies confirm that anthocyanins occur as the flavylium cation (most stable form) in the stomach as a result of low pH [36,37a]. In contrast to the stomach, the environment in the small and large intestines is alkaline, where multiple molecular forms of anthocyanin can be present. In these circumstances anthocyanins are much less stable and can be modified to glucuronidated, methylated and sulfated forms [37b]. Glucuronide conjugation is regarded as the major conjugation reaction involved in flavonoid metabolism. This reaction occurs predominantly in the liver, intestine and kidneys. Methylation appears to be the second most significant conjugation reaction involving flavonoids.

Keppler and Humpf [38a] using an *in vitro* model have shown that microflora isolated from pig caecum can modify the structure of anthocyanins. They investigated six different anthocyanins and showed that exposure to gut microflora resulted in rapid deglycosylation and demethylation to the corresponding aglycones. The aglycones were unstable at alkaline pH and rapidly degraded to the corresponding phenolic acids and aldehydes. Similar results were obtained in studies in which anthocyanidins and anthocyanins were incubated with human fecal microbiota [38b,39]. It is well known that anthocyanins can be metabolized and transformed to phenolic acids by intestinal microflora, mainly in the colon [19]. It was also hypothesized that those phenolic acids can be partially responsible for their protective and/or antioxidant effects, catalysed by UDP-glucuronosyl-tranferases, which are the effects exerted by anthocyanins [40,41]. Intestinal microflora exhibit significant hydrolytic activities and can cleave glycosidic bonds and generate degradation products such as aglycones and phenolic acids that are metabolized by the liver [42]. Absorption and bio-availability of anthocyanins significantly depends on their

chemical structure. McGhie *et al.* [43] reported that the relative concentrations of delphinidin-based anthocyanins were lower than those of malvidin-based anthocyanins in the urine of rats and humans. The authors suggested that this might be caused by the hydrophobicity difference between these compounds. Malvidin, possessing less hydroxyl groups and thus more hydrophobic, can be more easily transported into cells and tissues. A similar result was obtained by Yi *et al.* [44]. The comparison between delphinidin-3-glucoside and malvidin-3-glucoside showed that the latter anthocyanin had higher transport efficiency.

Sugar moieties may influence the bioavailability of anthocyanins. Variations in absorption between different phenolic aglycones and glycosidic forms were observed [20a,20b,30b]. The comparison between galactosides and glucosides with the same aglycone showed that glucosides had higher transport efficiencies [45].

Considering urinary excretion as an indication of absorption, in rats relatively more delphinidin glycoside was absorbed than malvidin glycoside and relatively more galactoside was absorbed than arabinoside [43]. This finding indicates that absorption of anthocyanins depends on the presence as well as on the type of sugar moiety attached to the phenolic core. Such a conclusion was supported by another study devoted to the pharmacokinetic properties of 15 anthocyanins derived from bilberry [20b]. It was reported that plasma concentrations were higher for galactosides and lower for arabinosides, and among the aglycones, plasma concentrations were higher for the delphinidins and cyanidins than for the malvidin anthocyanins. It is worth emphasising that anthocyanins containing more than one sugar in their structure are much more resistant to any transformation by intestinal microflora [30b].

Measurement of urinary excretion has often been used to assess bioavailability. Most studies reported very low relative anthocyanin urinary excretions, ranging from 0.018% to 3% [33b]. Talavera *et al*. [26] reported intact as well as methylated and glucuronidated metabolites of anthocyanins in the kidneys of rats. Similar results were obtained by Felgines [46] and Matsumoto [47]. The former reported that after 8 days consumption of lyophilized blackberry powder anthocyanins were detected in the urine in intact and methylated forms. Matsumoto *et al.* [47] indicated that anthocyanin 3-glycosides can be excreted in urine as intact forms in rats within 4 h of ingestion. The excretion of anthocyanins after ingestion of cranberry juice was also investigated in humans. Anthocyanin urinary levels reached a maximum between 3 and 6 h after consumption [48]. In another human study, urinary levels of anthocyanins reached a maximum concentration 4-8 h after consumption of black raspberry *(Rubus occidentalis)* and decreased during the following 8-12 h [49]. The relatively low permeability of anthocyanins across the epithelium of the gastrointestinal tract causes them to be moved to the colon and eliminated from the organism in the feces [50]. The results presented by He *et al.* [51] show that feces are a major excretion route of ingested anthocyanins.

Antimicrobial activity of crude extracts of plant phenolic compounds against human pathogens has been intensively studied to characterize and develop new healthy food ingredients as well as medical and pharmaceutical products. However, there is very little information available about the antimicrobial activity of the pure anthocyanins. Burdulis *et al.* [52] determined the total anthocyanin contents in bilberry (*V. myrtillus*) and blueberry (*V. corymbosum*) fruits and their skins and characterized the antimicrobial properties of their extracts. The anthocyanin composition of bilberry is more complicated than that of most other anthocyanin-containing berries, as 15 different anthocyanins have been identified [2]. In bilberries, anthocyanins comprise 90% of the phenolic compounds, including delphinidin 3-arabinoside (15.3%), delphinidin 3-galactoside (14.9%), delphinidin 3-glucoside (13.7%), cyanidin 3-arabinoside (13.6%), cyanidin 3-galactoside (9.0%), cyanidin 3-glucoside (8.5%), malvidin 3-glucoside (8.4%), petunidin 3-glucoside (6%) and other monoglycosides of malvidin, petunidin and peonidin (from 3.1 to 0.1%). Burdulis *et al.* [52] observed the highest amount of anthocyanins in fruit skins of blueberry cultivars. On the other hand, in fruit samples the highest content of anthocyanins was found in bilberry compared with blueberry fruits. In addition, the variation in individual anthocyanidin composition in bilberry and blueberry cultivar fruits and fruit skins was evaluated and it was found that the biggest amounts of delphinidin, cyanidin, petunidin and peonidin are accumulated in bilberry fruits, while malvidin was the most abundant in blueberry cultivar fruits. The highest contents of delphinidin, petunidin and malvidin were found in blueberry fruit skins, whereas cyanidin and peonidin prevailed in bilberry skin samples. Investigation of the antimicrobial properties of extracts of berry and berry skin demonstrated no significant differences. These extracts showed inhibitory effects on the growth of Gram-positive (*Listeria monocytogenes*, *Staphylococcus aureus*, *Bacillus subtilis* and *Enterococcus faecalis*) and Gram-negative strains (*Citrobacter freundii, Escherichia coli, Pseudomonas aeruginosa,* and *Salmonella enterica* ser. Typhimurium). *C. freundii* and *E. faecalis* strains were the most sensitive, while *E. coli* showed the largest resistance among the tested bacteria. The yeast strains *Debaryomyces hansenii*, *Trichosporon cutaneum*, *Kluyveromyces marxianus* var. *lactis*, *Saccharomyces cerevisiae*, *Candida parapsilosis*, *Torulaspora delbrueckii*, *Pichia kluyveri*, and *Rhodotorula rubra* showed complete resistance to these berry extracts.

Anthocyanin profile in berries of European cranberry (*Vaccinium oxycoccos*) species was also evaluated [53]. Authors have reported that fruits of different clones of this berry accumulated from 224.1 mg/100 g to 498.2 mg/100 g of phenolic compounds and anthocyanins comprised

18.3-42.7% of the total phenolic content. The dominant anthocyanin was peonidin 3-galactoside (30% of total anthocyanin content). The average composition of other anthocyanins was the following: cyanidin 3-arabinoside (21.7%), cyanidin 3-galactoside (19.8%), peonidin 3-arabinoside (17.4%), peonidin 3-glucoside (7.4%), and cyanidin 3-glucoside (3.4%). Investigation of the antimicrobial properties showed that European cranberry extracts inhibited the growth of a wide range of human pathogenic bacteria, both Gram-negative and Gram-positive. *L. monocytogenes* and *E. faecalis* strains were the most sensitive, *S. enterica* ser. Typhimurium, and *S. aureus* were found to be of moderate resistance and *E. coli* rods were the least sensitive. Evaluation of the closely related American cranberry species (*Vaccinium macrocarpon*) has revealed the same antibacterial properties of its concentrate on foodborne pathogens [54,55]. Furthermore, these authors found that exposure to cranberry concentrate (5 μL/ml) resulted in morphological damage of bacterial cells such as loss of the structural integrity of the wall, membrane and intracellular matrix. Cell deformation, breakage of cell wall and membrane, condensation of cellular material, and presence of significant amounts of cytoplasmic material and membrane debris outside of the cells were observed in all concentrate-treated bacteria. Lacombe *et al*. [55] demonstrated that anthocyanin fractions from American cranberry produced *E. coli* O157:H7 growth reduction below detectable limits after treatment with 14.8 mg/L anthocyanin, at native pH. However, at neutral Ph, anthocyanins showed reduced antimicrobial activity (growth inhibition below detectable limits in treatments with 29.15 mg/L), possibly due to their instability under these conditions. *E. coli* rods exposed to anthocyanins displayed localized disintegration and irregularity in the outer membrane and leaking of cytoplasm. The composition of individual anthocyanins in whole fruits of American cranberry was the following: peonidin 3-galactoside (32.7%), cyanidin 3-galactoside (20,5%), cyanidin 3-arabinoside (19%), peonidin 3-arabinoside (6.7%), peonidin 3-glucoside (3.5%), and cyanidin 3-glucoside (2.3%) [56]. Anthocyanins extracted from berries and their press cakes revealed antimicrobial properties against Gram-positive and Gram-negative strains. *Bacillus cereus* and *Micrococcus luteus* strains were the most sensitive, *L. monocytogenes* displayed average resistance, while *E. coli* was the least sensitive. Furthermore, cranberry extracts did not have any effect on the growth of any of the 8 yeast species [52].

Pomegranate fruit (*Punica granatum*) also contains a high proportion of phenolic compounds, such as delphinidin, cyanidin, and pelargonidin [57a]. The crude extract, fractions, and pure isolated compounds (pelargonidin 3-galactose, cyanidin 3-glucose) from the juice of *P. granatum* were found to be active against Gram-positive (*B. subtilis*, *Corynebacterium diphtheriae*, *C. diphtheriticum*, *Micrococcus lysodiecticus*, *S. aureus*, *Staphylococcus epidermidis, S. saprophyticus*, *E. faecalis*, *Enterococcus faecium*, *Streptococcus pneumoniae*, and *S. pyogenes*), and Gram-negative (*E. coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *P. aeruginosa*, *Salmonella typhi*, *S. paratyphi* A and B, *Shigella dysenteriae*, *S. sonneie*, and *S. flexneriae*) bacterial species. The activity against Gram-positive organisms was higher compared with Gram-negative species due to the outer membrane of Gram-negative bacteria acting as a permeability barrier, so that uptake of the compounds in the cell was reduced. The mechanisms thought to be responsible for the toxicity of pure phenolic compounds to microorganisms include enzyme inhibition by the oxidized compounds, possibly through reaction with sulfhydryl groups or through more non-specific interactions with proteins leading to their inactivation and loss of function. Probable targets in the microbial cell are surface-exposed adhesions, cell wall polypeptides, and membrane-bound enzymes. Phenols may also render substrates unavailable to microorganisms [57a].

Moreover, the influence of elderberry *(Sambucus nigra)*, blackcurrant concentrates, and a purified anthocyanin mix from blackcurrant concentrate (48% cyanidin 3-rutinoside, 21% delphinidin 3-rutinoside, 6% cyanidin 3-glucoside, and 4% delphinidin 3-glucoside) was tested on the growth of *E. coli*, *S. aureus*, *E. faecium*, and *S. cerevisiae* strains [57b]. Blackcurrant concentrates inhibited the growth of *S. aureus* and *E. faecium* strains. Only mild effects were observed in the case of *E. coli* rods, whereas the *S. cerevisiae* strain was slightly stimulated by the fruit concentrates. The purified anthocyanin mix did not influence the growth of the tested microorganisms.

The component profile of fresh blue honeysuckle fruits (*Lonicera caerulea* var. *kamtschatica*) was analyzed and the phenolic fraction (0.4% of fresh fruits) has been found to contain anthocyanins (77%), flavonoids, and phenolic acids [58]. Among the anthocyanins, cyanidin 3-glucoside predominated (83.3%), followed by peonidin 3-glucoside (5.9%), cyanidin 3-rutinoside (3.3%), delphinidin 3-glucoside (2.1%), and delphinidin 3-rutinoside (1.2%). As minor anthocyanins (0.1-0.6%), some glycosylated derivatives of delphinidin, peonidin, pelargonidin, and petunidin were also found. The impact of freeze-dried fruits and their phenolic fractions on the antiadherence activity and the inhibition of biofilm formation by *E. coli*, *E. faecalis*, *S. epidermidis*, *S. mutans*, and *C. parapsilosis* were studied. Suppression of biofilm formation by the freeze-dried fruits was statistically significant only in the case of *C. parapsilosis*, *S. epidermidis*, *E. faecalis*, and *S. mutans*. Freeze-dried fruits were found also to exert no effect on biofilm formation by *E. coli* strains. Unfortunately, the authors were unable to evaluate the results of the tested phenolic fraction due to coagulation of the sample with the culture medium and adhesion to the polystyrene wells. Additionally both the freeze-dried fruits and its phenolic fraction significantly reduced adhesion to the artificial surface for *S. epidermidis*, *E. coli*, *E. faecalis*, and *S. mutans*. A statistically significant effect of the phenolic fraction was found only for the *C. parapsilosis* strain.

Nohynek *et al*. [59] studied antimicrobial activity of phenolic extracts of bilberry, lingonberry (*Vaccinium vitis-idaea*), cranberry (*Vaccinium oxycoccus*), raspberry (*Rubus idaeus*, var. Ottawa), cloudberry (*Rubus chamaemorus*), strawberry (*Fragaria ananassa* Senga Sengana), blackcurrant (*Ribes nigrum*, var. Öjeby), sea buckthorn berry (*Hippophae rhamnoides*), chokeberry (*Aronia mitschurinii*), highbush bilberry (*V. myrtillus*), rowanberry (*Sorbus aucuparia*), and crowberry (*Empetrum nigrum*) against selected human pathogenic microbes (*B. cereus*, *Campylobacter jejuni*, *Candida albicans*, *Clostridium perfringens*, *E. coli*, *Helicobacter pylori*, *L. rhamnosus*, S. *enterica* sv. Typhimurium, *S. enterica* sv. Infantis, S. *epidermidis*, and *S. aureus*). They reported that microbial strains had different susceptibilities against berry extracts, and that the antimicrobial effects of the tested berries were variable. The phenolic extract of cloudberry possessed the strongest antimicrobial activity, followed by raspberry and strawberry. The weakest effects were detected for chokeberry, rowanberry, crowberry, and buckthorn berry. Antimicrobial effects of phenolic extracts were stable during 1 year of frozen storage of berries. Although the content of anthocyanins in the berries decreased to 57-68% of the original amount, antimicrobial activity of the berry extracts against S. *enterica* sv. Typhimurium and *S. enterica* sv. Infantis strains remained constant. They also found also that *H. pylori* and *B. cereus* strains were the most sensitive against the berry extracts. Cloudberry, raspberry and strawberry were potent inhibitors of microbial growth of all microorganisms, including *C. jejuni* and *C. albicans*, which were not inhibited by the other berry extracts. In addition, phenolic extracts of cloudberry and raspberry, but not anthocyanin fractions of raspberry, disintegrated the outer membrane of S. *enterica* sv. Typhimurium, and *S. enterica* sv. Infantis strains. This membrane perturbation was demonstrated by the increased uptake of an hydrophobic fluorochrome (NPN) and liberation of [^{14}C]Gal-LPS. On the other hand, phenolic extracts of blackcurrant, cranberry and bilberry did not increase the NPN uptake, but efficiently released LPS from the examined *Salmonella* strains [59,60].

The antimicrobial activity of pure pelargonidin, delphinidin, cyanidin chloride, and cyanidin 3-glucoside, as well as phenolic extracts from blueberry, raspberry, lingonberry, blackcurrant, cloudberry, cranberry, sea buckthorn berry, and strawberry were analyzed for antimicrobial properties against probiotic bacteria from the genus *Lactobacillus* (*L. crispatus*, *L. johnsonii*, *L. paracasei*, *L. reuteri*, and *L. rhamnosus*) and other intestinal bacteria (*E. coli*, *S. enterica* ser. Typhimurium, *E. faecalis*) [61]. Sensitivity to the pure compounds was found to differ significantly among the tested organisms. Lactic acid bacteria, in general, were more resistant than the other microorganisms. The anthocyanidins and cyanidin 3-glucoside inhibited only the growth of *E. coli* and had no effect on the other bacterial strains. The berry extracts mainly inhibited the growth of Gram-negative bacteria, but were not active against the Gram-positive species. The antimicrobial effects of berry extracts decreased in the following order: cloudberry > raspberry > strawberry > lingonberry > blueberry > cranberry > sea buckthorn berry > blackcurrant. Moreover, Puupponen-Pimiä *et al*. [62] compared the antimicrobial effects of all mentioned above lyophilized berries and their phenolic extracts against *S. enterica* sv. Typhimurium, *S. enterica* sv. Infantis, *S. aureus*, *S. epidermidis*, *L. monocytogenes*, and *Listeria innocua* strains. They observed that pathogenic bacterial strains were selectively inhibited by bioactive berry compounds. Cloudberry and raspberry were the best inhibitors, and *Staphylococcus* and *Salmonella* the most sensitive bacteria. *Listeria* strains were not affected by the phenolic extracts. According to Puupponen-Pimiä *et al.* [60,63], the mechanisms of action in the growth inhibition of bacteria of purified berry-derived compounds included destabilisation 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. Antimicrobial activity of berries may also be related to antiadherence of bacteria to epithelial cells, which is a prerequisite for colonization and infection for many pathogens.

As reviewed, anthocyanin rich products may have a protective effect on human health. However, more studies are needed in order to establish the real implications of anthocyanins in these health promoting properties, since most studies have used fruit extract and thus other substances may be totally or partially responsible for the mentioned biological activities [64]. Antimicrobial activity of berries and other anthocyanin-containing fruits is likely to be caused by multiple mechanisms and synergies because they contain various compounds including anthocyanins, weak organic acids, phenolic acids, and mixtures of their different chemical forms [59]. Therefore, the antimicrobial effect of chemically complex compounds has to be critically analyzed.

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