Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2007, 151(2):163–174.

BERRY FRUITS AS A SOURCE OF BIOLOGICALLY ACTIVE COMPOUNDS: THE CASE OF *LONICERA CAERULEA*

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Received: September 7, 2007; Accepted: October 12, 2007

Key words: Lonicera caerulea/Berries/Polyphenols/Biological activity

Background: *Lonicera caerulea* L. (blueberry honeysuckle, Caprifoliaceae) is a traditional crop in northern Russia, China, and Japan. Its fruits are little known as edible berries in North America and Europe. This review deals with the botany and chemical composition of *L. caerulea* and the biological activity of its main constituents, focusing on the potential health benefits of the berries.

Methods and Results: PubMed, Science Direct and ISI Web of Knowledge[SM] databases were used for this paper. Literature sources include the period 1935–2007.

L. caerulea berries a are rich source of phenolic compounds such as phenolic acids as well as anthocyanins, proanthocyanidins and other flavonoids, which display potential health promoting effects. Chemopreventive, antimicrobial, anti-adherence and antioxidant benefits, among others are described for these compounds.

Conclusions: The potential of *L. caerulea* berries to prevent chronic diseases such as diabetes mellitus, cardiovascular diseases and cancer seems to be related above all to their phenolic content.

INTRODUCTION

Lonicera caerulea L. (blue berry honeysuckle, Caprifoliaceae) is a traditional crop used in folk medicine in northern Russia, China, and Japan but its fruits are little known as edible berries in North America and Europe[1]. In recent years a large number of studies have investigated the therapeutic effects of various fruits and vegetables in the prevention of a range of diseases and there is increasing interest in herbal medicine products. Berries constitute one of the most important sources of potential health supporting phytochemicals in the human diet[2]. They are a rich source of ascorbic acid and phenolic compounds, particularly phenolic acids, anthocyanins, proanthocyanidins and other flavonoids. These compounds provide the pigmentation of fruits and prove beneficial to human health[3, 4]. Their biological activities include: protection against the incidence and mortality rates of cancer[5], protection against ischemic heart disease mortality[6] and as well as they have antitumorigenic[7], antimicrobial[8], anti-inlammatory-allergic[9] and antimutagenic properties[10]. The aim of this paper was to review the current literature on berry-derived biologically active compounds, with the focus on *L. caerulea*.

1. BOTANICAL DESCRIPTION

The genus *Lonicera*, which includes almost 180 species of deciduous or evergreen shrubs, belongs to the Caprifoliaceae family and is native to the Northern Hemisphere (Fig. 1) (ref.[1]). The nomenclature of honeysuckles is complicated; a lot of species are sometimes classified as varieties and have many synonyms. Large genetic variation is common among the *Lonicera* genus. For example 10 common species of *L. involucrata* are only hybrids of one known origin[11]. Many species of Caprifoliaceae family have non-edible fruits and are cultivated only as decorative shrubs or rambling plants[12], especially for their glorious blooms and ornamental fruits[1]. The fruit colour, which ranges from white or yellow to scarlet or navy blue, is used as an indicator of plant maturity[1]. Honeysuckles are also favoured because of their extreme hardiness to the cold[13]. A few species are used in indigenous medicine as antipyretic, stomachic, diuretic and antidysenteric in India[14].

L. caerulea L., also known as blue honeysuckle, honeyberry, edible honeysuckle or sweet berry honeysuckle[15] is native to the northern temperate zone, especially Russia (Kamchatka Peninsula, Siberia), North Eastern Asia, and Japan[15, 16]. In Europe, it occurs rarely in the Alps and Scandinavia[3]. *L. caerulea* is currently commercially produced in Russia and Japan, but this species was unknown as an edible berry in North America until very recently[17].

Native *L. caerulea* plants grow from 0.8 to 3.0 m tall, but under cultivation the shrubs reach 1.0 m wide to 1.8 m tall[17]. They do not require special soil type; even wet sandy or loamy soil is suitable, pH can range from 5 to 7 and the organic proportion can be higher[12]. Plants have strong

***Lonicera caerulea*:**	
Kingdom:	*Plantae*
Subkingdom:	*Tracheobionta* – Vascular plants
Superdivisio:	*Spermatophyta* – Seed plants
Divisio:	*Magnoliophyta* – Flowering plants
Class:	*Magnoliopsida* – Dicotyledons
Subclass:	*Asteridae*
Order:	*Dipsacales*
Family:	*Caprifoliaceae*
Genus:	*Lonicera* L. – Honeysuckle
Species:	*Lonicera caerulea* L. – Sweetberry honeysuckle

Fig. 1. Botanical subsumption of *Lonicera caerulea* L.

tolerance to severe low-temperature conditions. They can survive a temperature of –46 °C without damage[17, 18]. The freezing tolerance of perennial plants increases in fall and winter to prevent injury under cold conditions. It is known as cold acclimation and seems to be connected with the content and accumulation of specific type of carbohydrates and proteins (see below). The raffinose family of oligosaccharides have been shown to be potential cryoprotectants because of their capacity to modify the freezing behaviour of aqueous solutions[18, 19]. Also the presence of galactose-containing oligosaccharides strongly correlates with increases in freezing, as well as desiccation tolerance[18]. Blue honeysuckle shrubs are long-lived and can survive 25 to 30 years[12].

Blue honeysuckles are not self-pollinating so at least two single plants are required**.** Fruits become ripe very early in May or June in European conditions. The plants can begin fruiting within one year after planting and after three years almost 500 g of fruits can be acquired from one plant[12]. Fruit shapes are oval to long and dark navy blue to purple in color[15] with blue waxy coating according to the genotype[12]. They can grow up to more than 2 cm long and weigh more than 1.5 g[17]. Their flavour is similar to that of bilberries or black currants and it can vary from bitter to sweet[15]**.**

2. CONSTITUENTS AND THEIR BIOLOGICAL ACTIVITY

L. caerulea berries and their juice contain saccharides, lipids, proteins, organic acids and polyphenols as major components and also ascorbic acid (45–93 mg · 100 g^{-1})(ref.[15]), vitamin B, magnesium, phosphorus, calcium and potassium as minor compounds[20].

2.1. Saccharides

L. caerulea **fruit** contain 7.20 % saccharides. Free saccharides include 3.2 % glucose and 2.9 % fructose, bound saccharides are 0.8 % glucose, 0.2 % galactose and 0.1 % arabinose[21]. Five kinds of saccharides have been identified in *L. caerulea* **shoot apices** according to Imanishi et al[18]. The content of fructose, glucose, saccharose, raffinose and stachyose varies depending on climatic conditions and season. The highest level of sugars has been measured in January, and then the saccharide content decreased to May and after that increased again. According to the literature[18] the dominant sugar is saccharose with 50–90 % of total saccharides. The content of stachyose and raffinose changes dramatically during the year. While it is negligible from April to November, it increases rapidly from November to March. Fructose and glucose constitute only small proportion of total sugars and their content does not change over the year. Accumulation of raffinose and/or stachyose has strong relationship to the freezing tolerance and desiccation[18]. *L. caerulea* **leaves** comprise three glucosides, named caerulosides A, B (Fig. 2) and C. These compounds are formed from secologanin attached through acetal bonds to C-4'and C-6' of the saccharide part of loganin and sweroside, respectively. Caerulosides are the first bis-iridoids, that are composed of two units of iridoids bound by acetal linkages[22, 23].

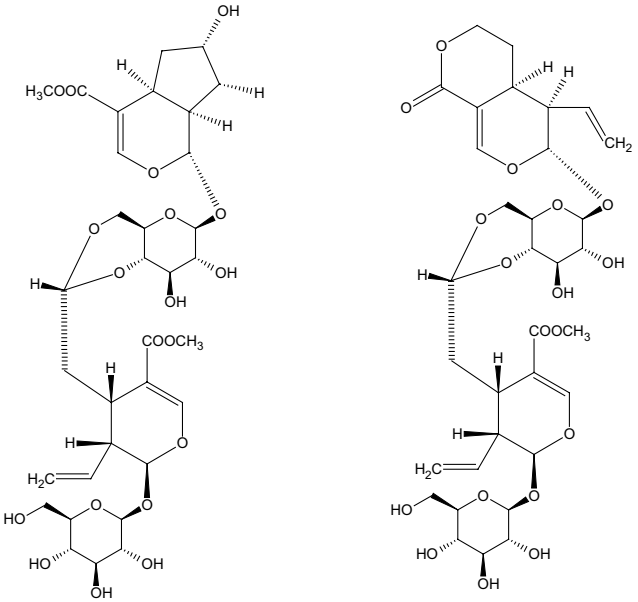


Fig. 2. Caerulosides A and B.

2.2. Lipids

Blueberry honeysuckle fruit contain only 1.52 % lipids. The lipid fraction is mainly hydrocarbons, sterols, triacylglycerols and phosphatidylcholine (Table 1a) (ref.[21]). The weight of fatty acids is 0.89 % total weight of berries. Main acids include palmitic (38.2 %), oleic (27.6 %), stearic (14.7 %), myristic (9.2 %), linolic (5.9 %), palmitoleic (2.8 %) and lauric acid (1.6 %). The entire weight of the unsaponifiable proportion (sterols, alcohols and hydrocarbons) is 0.46 % of the berries, and includes α-amyrin (29.6 %), β-amyrin (24.7 %), stigmasterol (14.8 %) and ursolic acid (11.5 %) (Table 1b) (ref.[24]). Ursolic acid and its 19-hydroxy derivative pomolic acid were reported to inhibit proliferation and DNA synthesis in HL-60 leukemia cell line at micromolar concentrations[25]. Ursolic acid, β-amyrin, and glucoside of β-sitosterol inhibits the growth of HCT 116 human colon cancer cells and PC-12 adrenal pheochromocytoma cells at micromolar concentrations[26].

Table 1. *Lonicera caerulea* berries **a)** lipid fraction **b)** unsaponifiable matter.

a)

Compounds	Relative content (%)
Hydrocarbons+sterols	32.0
Triglycerols	27.0
Phosphatidyl choline	21.0
Free fatty acids	7.0
Phosphatidic acid, phosphatidyl serine + min. phospholipids	6.0
Digalactosyl diglycerol	4.0
Phosphatidyl ethanolamine	3.0
Hydrocarbons+sterols	3.3

b)

Compounds	Relative content (%)
α-Amyrin	29.6
β-Amyrin	24.7
Stigmasterol	14.8
Ursolic acid	11.5
Triterpens	5.3
Sitosterol	4.9
Oleanolic acid	3.3
Δ7-Stigmastenol	3.3
Campesterol	2.0
Brassicasterol	0.6

2.3. Other components

Fruit content includes 14.62 % dry matter[21], of which 14.8 % is soluble fiber[27]. Organic acids (12.2 %) are represented by citric (3.7 %), malic (18.0 %) and others (2.4 %) (ref.[20]).

2.4. Phenolic compounds

Small berries are one of the most important sources of phenolic compounds with potential health promoting effects. *L. caerulea* contain a huge amount of these compounds. The phenolic content is dependent on the degree of maturity, genetic diversity, preharvest climatic, postharvest storage conditions and processing[20]. We prepared a phenolic fraction from *L. caerulea* var. *kamtschatica* (4 % of fresh fruits) containing 33.5 % of phenolics, including anthocyanins (18.5 %), flavonoids and phenolic acids[21].

The polyphenols comprise a range of chemical classes of secondary plant metabolites that they all share the ability to act as chain breaking antioxidants[28]. Phenolic compounds are essential for the growth and reproduction of plants and are produced as a response to plant injury by pathogens. At low concentrations they also protect food from oxidative deterioration. At high concentrations they or their oxidation products may interact with proteins, carbohydrates and minerals[29]. The health benefits of polyphenols are usually linked to two properties: (i) inhibition of certain enzymes such as xanthine oxidase, aldose reductase, and (ii) antioxidant activity[30]. Polyphenols can protect other food components such as carotenoids and vitamin C and also digestive enzymes and gut epithelial cells from oxidation due to free radicals generated in stomach[31, 32].

Little is known about the bioavailability, absorption and metabolism of polyphenols in humans and it is likely that single groups of flavonoids have different pharmacokinetic properties[33]. Phenolics are powerful antioxidants in *in vitro* models, but there is lack of information about whether they can remain a sufficient time in efficient chemical forms in the human body[29]. A large proportion of the ingested polyphenols from berries are not taken up into the circulation and pass through the upper GIT into the large intestine where they may be transformed or broken down by the indigenous microflora[34]. Phenolic compounds are metabolized by deconjugation and reconjugation reactions. They are hydrolyzed to their free aglycones, and then they are conjugated by methylation, sulphation, glucuronidation or their combination. The subsequent metabolic pathway is similar to that of drug metabolism. Since drugs are usually administrated in hundreds of milligrams in one dose while dietary phenolics are presented in much lower concentration, drugs usually saturate these pathways. When food phenolics are administrated at pharmacological doses, they are found in free forms in the blood[35]. Large doses are metabolized primarily in the liver. Small doses may be metabolized in the intestinal mucosa, the liver has a secondary role in their metabolism[35]. Hollman et al.[36] proposed, based on indirect evidence, that flavonoid glycosides actually may be absorbed intact in the small intestine, using sodium-dependent glucose transporter 1 (SGLT1). On the one hand this postulate has been confirmed[37]. On the other hand it has been demonstrated that the efficiency of such absorption is dramatically suppressed by efflux of at least some flavonoid glycosides by the apical transporter multidrug resistance-associated protein 2 (MRP2) (ref.[38]). Some flavonoid glycosides could be hydrolyzed in the small intestine[39]. If the flavonoid glycosides are able to enter the intestinal epithelial cells (enterocytes), which may include shredded cells, they may be hydrolyzed by broad-specific β-glucosidase (BSβG) (ref.[40]). For some flavonoid glycosides, lactase phloridzin hydrolase (LPH), located in the brush border of the mammalian small intestine,

could perform this hydrolysis[41]. It has also been shown, that phenolics, which have rhamnose in their molecule, cannot be absorbed through the small intestine. They are degraded by the action of rhamnosidases produced by the colonic microflora[29].

2.4.1. Phenolic acids

Phenolic acids form approximately one third of the total intake of plant polyphenols in the human diet[42]. Daily consumption of phenolic acids has been estimated as 25 to 1000 mg (ref.[43]). They are present in free and bound forms in plant material. Bound phenolic acids may be linked through ester, ether, acetyl or other bonds[42]. Simple phenolic acids may also be formed by colonic microflora from ingested flavonoids[44].

Total content of phenolic acids in *L. caerulea* berries ranges from 2845.8 ± 141.0 to 5418.2 ± 228.0 mg · kg^{-1}, dry weight (DW) (ref.[45]). Chlorogenic (0.42 %), caffeic (0.14 %) and ferulic acid (0.10 %) were the most abundant in our phenolic fraction of *L. caerulea* var. *kamtschatica* berries; the content of protocatechuic, gentisic, rosmarinic, and vanillic acids was 0.08 % in total[21]. Other hydroxycinnamic acid and hydroxybenzoic acid derivatives are mentioned in the literature, especially *m*-coumaric and *p*-coumaric. The amount of hydroxycinnamic acids derivatives is described as comparable to blueberries or blackcurrant[46]. Also dimethoxycinnamic, hydroxycaffeic, gallic, *o*-pyrocatechuic, protocatechuic, salicylic, *p*-hydroxyphenylacetic and *p*-hydroxyphenyllactic acid are reported[45].

Free phenolic acids stand for only a minor portion of phenolic acids. Their amounts ranged from 1.7 to 4.2 % for all berries. Caffeic, ferulic and *p*-coumaric acids predominate. Free phenolic acids levels do not exceed taste threshold and they do not influence the taste of the berries[45].

Bound phenolic acids are present in ester (69.7 %) form. *L. caerulea* again contains *m*-coumaric acids as the dominant acid. Also caffeic, ferulic, gallic, *p*-hydroxyphenylacetic, *p*-hydroxyphenyllactic and vanillic acid occur in minor amounts. Phenolic acids bound by glycosidic linkages constitute 28.6 %. The majority are represented by hydroxycinnamic acid derivatives (61.1 %), especially *m*-coumaric. The content of ferulic, gentisic, protocatechuic and vanillic acids does not reach 50 mg · kg^{-1}, DW (ref. [45]).

Chlorogenic acid is formed by the esterification of caffeic acid with quinic acid. It can be also degraded to caffeic and quinic acid by esterases produced by the colonic microflora[47]. **Caffeic acid** is known as an antioxidant both *in vitro* and *in vivo*[48]. While both caffeic and chlorogenic acid have been reported to be absorbed in humans[49], caffeic acid absorption is nevertheless hampered when it is esterified with quinic acid. Caffeic acid is still listed under older Hazard Data sheets as a potential carcinogen because of two early experiments on rats and mice. More recent data show that bacteria in the rat's guts may alter the formation of caffeic acid metabolites. There have been no known adverse effect of caffeic acid in humans[50,51]. **Ferulic acid** could be absorbed by passive diffusion or by facilitated transport that appears not be saturated even at a luminal concentration of 50 µmol/l (ref.[52]). The absorption of ferulic acid and also free cinnamic acid are controlled by the Na^+/dependent carrier-mediated transport process in rat jejunal segments[53]. Some food products also contain oxidatively coupled product of the ferulic acid, the diferulic acid[54]. **Coumaric acid** is a hydroxy derivative of cinnamic acid. There are three isomers, *o*-coumaric, *m*-coumaric and *p*-coumaric acid, that differ by the position of the hydroxy substitution of the phenyl group. *p*-Coumaric acid is the most abundant isomer in nature, has antioxidant properties[55] and is believed to reduce the risk of stomach cancer[56].

2.4.2. Flavonoids

Flavonoids are polyphenolic compounds, whose structure is formed by the diphenyl propane skeleton (C_6-C_3-C_6). The differences within each group flow from variation in numbering and order of the hydroxyl groups, as well as the nature and extent of alkylation and glycosylation of these groups. The degree of hydroxylation is a determinant for their tendency to degradation in the colon and products formed by colonic microflora. The absence of hydroxyl group in the molecule prevents the degradation of the ring structure; the degree of hydroxylation magnifies the tendency to degradation. The absence of the methyl group causes a decrease in the tendency to degradation[57]. Food flavonoids are usually glycosylated mostly with glucose or rhamnose, but galactose, arabinose, xylose; glucuronic acid and other sugars can also be found. The number of sugar molecules can be one, two or three in different possible positions of the ring substitution. The glycosylation influences chemical, physical and biological properties of these compounds[35].

The extent of absorption and bioavailability of drugs has long been known to be affected by membrane transporters, mainly efflux transporters, in addition to metabolism. The traditional efflux transporter for drugs, e.g. P-glycoprotein, does not seem to be involved in the transport of flavonoids. Other transporters have been found to play a role, e.g. the absorptive transporter SGLT1 (ref.[38]), the absorptive monocarboxylate transporter (MCT), MRP2, but probably also other MRP isoforms, for glucuronide and sulphate conjugates[38, 58].

The metabolism of flavonoids was initially described to be mediated by cytochrome P450 (CYP) enzymes[59] in liver microsomes from induced rats and from humans, but it has never been shown to be important *in vivo* or in intact cells, where conjugative metabolism may be expected to compete with oxidation[60]. Glucuronic acid conjugates of flavonoids have been well-documented with respect to both the molecular site of glucuronidation and the UD P-glucuronyltransferase (UGT) isoforms involved[61]. One of the tea flavonoids, epicatechin gallate (ECG), showed only sulphate conjugation[62]. Other metabolic pathways include O-methylation by soluble catechol-O-methyltransferase (COMT) (ref.[63]) or by bacterial enzymes. Bacteria

from faecal flora may be responsible for the hydrolysis of flavonoids glycosides as well as flavonoid glucuronides and sulphates. The reaction proceeds via degradation of the flavonoid backbone into numerous phenolic and carboxylic acid products[64], as well as carbon dioxide[65]. Many of these products are absorbed and can be detected in human urine[64]. Other types of metabolites are those resulting from oxidation by reactive oxygen species (ROS)[66]. Covalent binding of oxidized quercetin to DNA and cellular protein has been demonstrated in human cells[67].

2.4.2.1. Flavonols, flavons and flavan-3-ols

Flavonols (e.g. quercetin) have a similar C-ring structure with a double bond in the 2–3 position as flavones (e.g. apigenin). **Flavones** lack a hydroxyl group at the 3-position. **Flavan-3-ols** (e.g. epicatechin) lack a double bond in the 2–3 position. Quercetin (0.1 % of the phenolic fraction), its 3-glycoside (0.06 %) and 3-rutinoside (0.75 %) and minor quantities of epicatechin and apigenin were found in our *L. caerulea* var. *kamtschatica* phenolic fraction[21].

Quercetin is one of the most extensively studied flavonoids apropos its anticancer activity because of its prevalence among fruits and vegetables (Fig. 3) (ref.[68]). Quercetin glucosides are resistant to hydrolysis by HCl in stomach[69] and the absorption occurs in the small intestine into enterocytes probably via active transport[70,71]. They can be methylated into isorhamnetin immediately after absorption in the human body. Quercetin rutinosides are absorbed from the colon following the deglycosylation[71]. After intravenous dose, quercetin can be detected in urine, in particular after hydrolysis with β-glucuronidase/aryl sulphatase[72]. The main route of quercetin excretion is as carbon dioxide, 23 – 81 % of the dose, measured by trapping exhaled air[73]. **Apigenin** is a nontoxic dietary flavonoid that has been shown **to have anti-tumour and anti-inflammatory activities** (Fig. 3)**.** Apigenin can block the formation of uric acid leading to beneficial effects in gout[74]. Catechin and epicatechin are epimers, with (-)-epicatechin and (+)-catechin being the most common optical isomers found in nature (Fig. 3) (ref.[75]). Epicatechin can reduce the risk of four of the major health problems: stroke, heart failure, cancer and diabetes[76]. Acylated flavonoids, such as epicatechin are reported to be absorbed without deconjugation and hydrolysis[77].

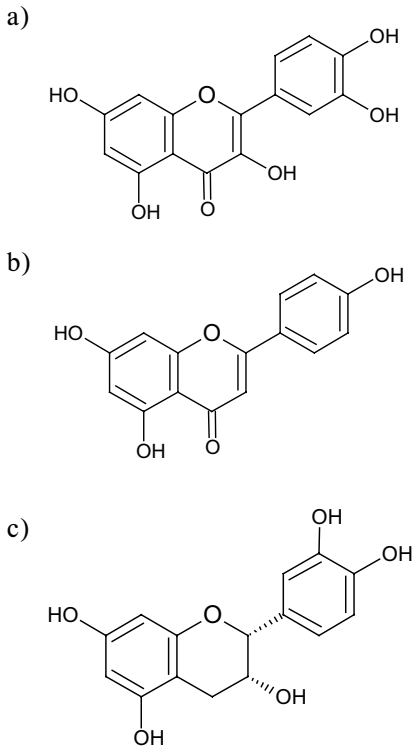


Fig. 3. Chemical structures of a) quercetin; b) apigenin; c) epicatechin.

2.4.2.2. Anthocyanins

Anthocyanins are secondary plant metabolites, derivatives of 2-phenylbenzopyrylium, generally found in glycosidic forms[78–80]. The aglycones (anthocyanidins) are rarely found in fresh plants. They occur as 3-glycosides and 3,5-diglycosides linked with glucose, galactose, rhamnose or arabinose[78]. Anthocyanins represent the most important group of water-soluble pigments, responsible for the blue, purple and red colour of many plant tissues due to their ability to associate into complexes characterized by higher absorbance of light lengths, co-pigmentation and formation of complexes with metals. In aqueous solution, anthocyanins exist in a number of different molecular forms that are in dynamic equilibrium depending mostly on pH (Fig. 4). The red flavylium cation is the most abundant molecular form at pH < 2. As the pH increases, there is rapid loss of a proton to generate the blue quinonoidal structure. At the same time, much slower hydration of the flavylium cation occurs to yield the colourless hemiketal form that further tautomerises to generate the chalcone form[81]. The positively-charged oxonium anthocyanin form may not be affected by MRP2 efflux, which seriously limits the absorption of other flavonoid glycosides[38]. Anthocyanins seem to be able to be absorbed intact as glycosides[82]. However, the proportion of anthocyanins absorbed and excreted in the urine appears to be quite small, perhaps much less than 0.1 % of the intake[83]. The clearance of anthocyanins from the circulation is rapid; very little is generally detected in the plasma 6 h after administration[82]. Some anthocyanins can be metabolized to colourless forms, oxidized, or degraded into other compounds. Researchers have also found that no glycoside hydrolysis takes place during digestion[84]. The absorption of anthocyanins without removal of glycoside has also been demonstrated[85].

Anthocyanins and proanthocyanidins have antibacterial properties as well as the ability to inhibit adhesion of bacteria to mucosal membrane of urinary tract[86]. Anthocyanins also act as anti-inflammatory and anti-mutagenic agents and provide cardioprotection by maintaining vascular permeability[4]. The ability to regulate the perme-

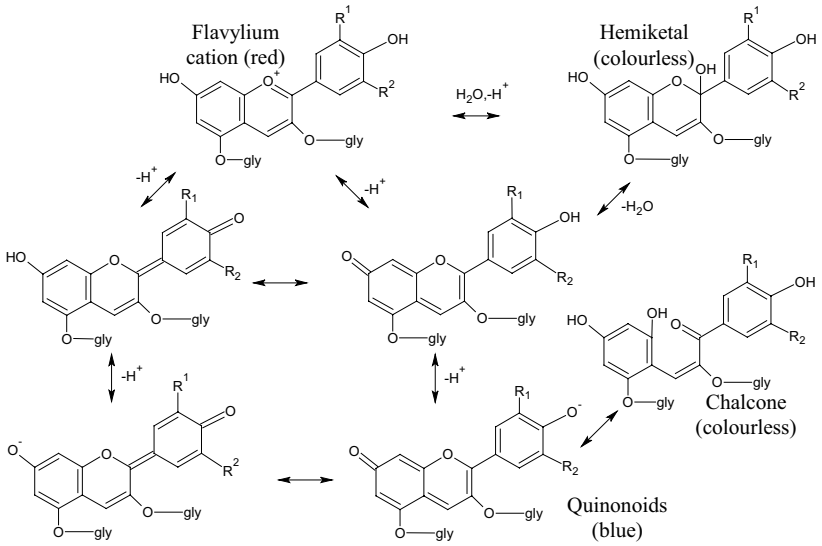


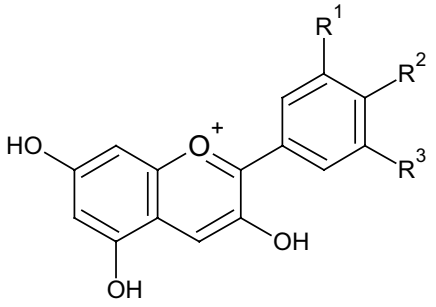
Fig. 4. Various anthocyanin forms existing in aqueous solutions depending on pH (gly = glycoside).

ability of capillary vessels has become the basis for their definition as vitamin P. They show protection against hepatitis A and B and also against paracetamol hepatotoxicity[87]. Berry extracts rich in anthocyanins have been linked to protective effects including the modulation of age-related neurological dysfunctions and improved resistance of red blood cells against oxidative stress *in vitro*[88].

Anthocyanins are very good antioxidants due to the presence of hydroxyl groups in position 3 of the C ring, which can chelate metal ions (Fe, Cu), and 3' and 4' of the B ring. Antioxidant activity is also increased by acylation of sugar residues with aromatic hydroxy acids[89]. These compounds have higher antioxidative activities than vitamin E, ascorbic acid or β-carotene[79].

The major anthocyanins in *L. caerulea* fruit are the glucosides and rutinosides of cyanidin, peonidin, dephinidin and pelargonidin[21] (Fig. 5). Quantities of these compounds vary depending on a number of the factors[46]. Table 2 shows a comparison of *L. caerulea* phenolic fraction anthocyanin content with those of *Vaccinium macrocarpon*[90] and *Vitis vinifera*[91].

Cyanidin is the most common anthocyanidin, with a 3', 4'-dihydroxylation of the B ring, present in 90 % of fruits[92]. Wu et al.[83] detected the methylated form of anthocyanins in human urine after consumption of elderberries and blueberries. The cyanidin glycosides tend to have higher antioxidant capacity than peonidin or malvidin glycosides likely due to the free hydroxyl groups on the 3' and 4' positions of cyanidin[93]. The key difference compared to other flavonoid glucosides, is that **cyanidin-3-glucoside** appears to be absorbed after oral ingestion, although to a limited extent[38]. **Cyanidin-3-rutinoside** from sweet cherry exhibited cyclooxygenase I and II inhibitory activities[94]. It showed potential in the treatment of diabetes, obesity, hyperlipidemia[95] and B and C type viral hepatitis[96]. **Delphinidin** may preserve endothelium integrity and protect against endothelial cell apoptosis[97]. Delphinidin, malvidin and petunidin are metabolized in the liver through the catechol-O-methyltransferase reaction in the 3' position[83]. **Pelargonidin** itself displays an orange red color. Pelargonidin-3-monoglucoside, isolated from frozen strawberries, protected the amino acid tyrosine from the highly reactive oxidant peroxynitrite, inhibited the growth



Name	R^1	R^2	R^3
Cyanidin	OH	OH	H
Delphinidin	OH	OH	OH
Pelargonidin	H	OH	H
Peonidin	OCH_3	OH	H
Petunidin	OCH_3	OH	OH
Malvidin	OCH_3	OH	OCH_3

Fig. 5. Structures of main anthocyanidins.

Table 2. Content of anthocyanins in *Lonicera caerulea*, *Vaccinium macrocarpon* and *Vitis vinifera* extracts.

Compounds	**Relative content (% w/w)**		
	L. caerulea[21]	***V. macrocarpon***[90]	*V. vinifera*[91]
Cyanidin-3-arabinoside	–	5.5	–
Cyanidin-3-galactoside	–	7.1	–
Cyanidin-3-glucoside	**60.0**	12.3	7.0
Cyanidin-3(6'-acetyl)-glucoside	–	–	1.8
Cyanidin-3,5-diglucoside	9.9	–	–
Cyanidin-3-pentoside	–	2.9	–
Cyanidin-3-rutinoside	7.3	–	–
Delphinidin	–	1.8	–
Delphinidin-3-glucoside	1.2	–	3.7
Delphinidin-3-rutinoside	2.0	–	
Pelargonidin-3-glucoside	3.3	–	–
Pelargonidin-3,5-diglucoside	0.6	–	–
Pelargonidin-3-rutinoside	0.1	–	–
Peonidin-3-arabinoside	–	5.7	–
Peonidin-3-galactoside	–	10.2	–
Peonidin-3,5-digalactoside	–	24.1	–
Peonidin-3-glucoside	5.8	**60.7**	18.7
Peonidin-3,5-diglucoside	8.1	–	
Peonidin-3(6'-coumaroyl)-diglucoside	–	–	1.2
Peonidin-3-rutinoside	0.5	–	–
Petunidin-3-glucoside	–	–	10.7
Malvidin-3-glucoside	–	–	**64.6**
Malvidin-3(6'-acetyl)-glucoside	–	–	1.0
Malvidin-3(6'-caffeoyl)-glucoside	–	–	1.8
Malvidin-3(6'-coumaroyl)-glucoside	–	–	2.5
Total anthocyanins	**100**	**100**	**100**

of *Escherichia coli* and *Staphylococcus aureus*, and exerted both a stimulatory and inhibitory effect on *Lactobacillus casei* culture. The stimulation may be due to a decrease in the oxidation-reduction potential of the media affected by the pigment, and/or the ability of the organism to split the β-glycosyl bond and use the glucose moiety[98]. **Peonidin** has been patented for use as food colouring agent[99].

2.4.3. Proanthocyanidins

Proanthocyanidins (condensed tannins) are oligomeric and polymeric end products of the flavonoid biosynthetic pathway and are metabolized to anthocyanidins[79]. They can be considered as the fifth class of plant biopolymers besides polynucleotides, proteins, lignins and polysaccharides. As a class, proanthocyanidins can be complex in structure and composition, featuring various flavan-3-ols (most commonly catechin, epicatechin, and galloylated catechins) linked together in different ways. Berry proanthocyanidins are primarily dimers, trimers and other oligomers[100]. These molecules may contain two types of linkages between epicatechin units. The B-type (4β → 8) is widely found in apples and grapes and A-type (4β → 8 and

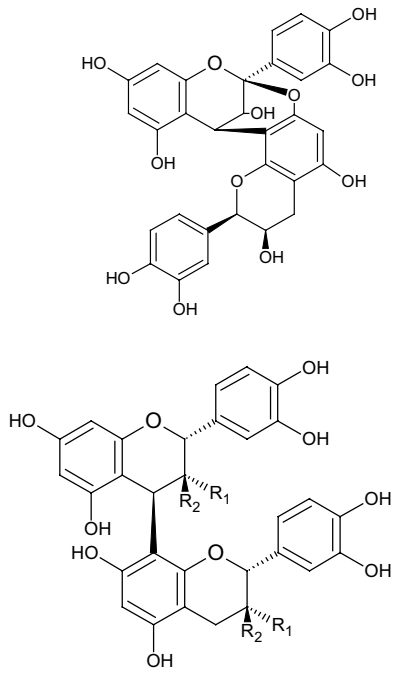


Fig. 6. Chemical structures of proanthocyanidin A and B type.

2β → O →7) found in cranberries can inhibit adherence of uropathogenic P-fimbriated *Escherichia coli* (Fig. 6) (ref.[101, 102]). The stereochemistry of the linkage at C4 may be α or β[103]. The major function of proanthocyanidins in plants is to provide protection against microbial pathogens, insect pests and larger herbivores. Their deposition in the endothelial layer of the seed coat appear to be an example of a pre-formed protective barrier[104]. *Salmonella*, *Staphylococcus*, *Helicobacter* and *Bacillu*s are the most sensitive bacteria to the berry phenolics. In addition, the growth of *Escherichia*, *Clostridium* and *Campylobacter* species but not *Lactobacillus* and *Listeria* species are inhibited[105]. The main mechanisms of action are 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[106].

3. TRADITIONAL USE AND PUTATIVE HEALTH BENEFITS

L. caerulea berries have long been harvested from wild plants in regions of Russia, China and Japan where superior edible forms are native. Recently, research in Russia and Japan has resulted in cultivars being selected for commercial production because of its very early maturity, unique flavour and health benefits that have long been acknowledged in Russia[16]. Recent research has supported some of the folkloric claims for the therapeutic uses of blue honeysuckle berries in atherosclerosis, hypertension, gastrointestinal disorders and bacterial infection. The main beneficial effect is due to the presence of vitamin C and high levels of polyphenolics [16, 107–110].

L. caerulea phenolics, as secondary plant metabolites, have been shown to provide defence against **oxidative stress** from endogenous ROS and free radicals[111]. Tumor inhibition by the berries is likely to involve synergic activities between its phytochemicals, including flavonols (quercetin), proanthocyanidins and others and to be primarily based on reducing oxidation of lipoproteins, improving antioxidant status and lipid levels and mitigating the effect of oxidative stress and inflammation on the vascular system[112]. Phenolics protect cardiomyocytes after ischemic episodes by inhibition of free radical formation in the process of reperfusion[113]. These compounds are able to reduce nitric oxide synthase activity and nitric oxide (NO) level[114]. They inhibit the cyclooxygenase activity, adhesion and reaction of leukocytes with endothelial cells, degranulation of mast cells and decrease in the level of interleukin (IL)-2, interferon (INF)-γ and tumor necrosis factor (TNF)-α (ref.[115]). Polyphenolics have been regarded as a potential novel, safe and nontoxic strategy for the modulation of inflammation dependent on the nuclear factor (NF)-κB pathway[27]. An extract of *L. caerulea* showed significant anti-inflammatory effects on **endotoxin-induced uveitis** in rat. The possible mechanisms for this effect may depend especially on its ability to inhibit activation of NF-κB and the subsequent production of proinflammatory mediators such as TNF-α, prostaglandin (PG)-E_2 and NO (ref.[27]).

Berry anthocyanins act as novel **cardioprotectants** by maintaining vascular permeability, reducing inflammatory responses and platelet aggregation, and offer superior vascular protection compared to other cardioprotective drugs[116, 117]. Bioactive compounds from *L. caerulea* have been also demonstrated in *in vitro* models to inhibit the oxidation of lipoproteins. A recent study of ours showed inhibition of copper-induced lipoprotein oxidation by a phenolic fraction of *L. caerulea* var. *kamtschatica*[118]. Reduction of **atherosclerotic** plaques by polyphenolics has been found in animal models; reduction of carcinogenesis was observed *in vitro.* Epicatechin[76] and anthocyanins, especially delphinidin, may preserve endothelium integrity whose damage can lead to the development of atherosclerosis and also cancer[97]. Some *L. caerulea* compounds can block mutagenesis by chemical carcinogens and endogenous mutagens and have been shown to modify the process of uncontrolled cell proliferation and apoptosis *in vitro*[119]. These phytochemicals may exert their **anticarcinogenic effect** by modulating the enzyme systems that metabolize carcinogens or procarcinogens to genotoxins. For example CYP activity can be induced or inhibited by flavonoids. Ellagic acid inhibits mutagenesis and carcinogenesis by acting on both CYP xenobiotic metabolism and several phase 2 detoxifying enzymes[120]. Berry extracts can protect against carcinogenesis also in animal models[121]. Numerous reports shows quercetin ability to inhibit proliferation of cancer cell lines *in vitro*, including breast, colon, pancreas cancer, and leukemia[122]. Its mechanism of action includes induction of apoptosis[123], inhibition of epidermal growth factor receptor expression and associated tyrosine kinase activity[122], reduced expression of Ras protein in colon cancer cells and primary colorectal tumors[124], increased expression of endogenous inhibitors

of matrix metalloproteinases[125] and phytoestrogenic activity involving interaction with the estrogen α- and β-receptors of human breast cancer MCF-7 cells[68]. Cyanidin and its 3-glycoside reduce oxidant-induced DNA strand breakage in normal human lymphocytes *ex vivo* and are as potent chemoprotectants as the flavonols, quercetin and myricetin. Cyanidin-3-rutinoside and cyanidin-3-glucoside suppress cancer cell metastasis by inhibiting the motility adhesiveness and invasiveness of metastatic human lung cancer cell lines A579 (ref.[126]). Cyanidin and a mixture of several of its glycosides dose-dependently inhibited HCT 116 and HT 29 colon cancer cell growth[127]. Delphinidin, malvidin and petunidin also inhibited proliferation of cancer cells derived from various tissues including colon, breast, blood and lung at high micromolar concentrations[128]. Peonidin has shown potent inhibitory and pro-apoptotic effects on cancer cells *in vitro*, notably human metastatic breast cancer cells[99]. *p*-Coumaric acid is believed to reduce the risk of stomach cancer by reducing the formation of carcinogenic nitrosamines[56]. Also caffeic acid and epicatechin have been shown to act as an inhibitor of carcinogenesis[48,76]. Wild and cultivated berry proanthocyanidin fractions demonstrated antiproliferative effects on two models of prostate cancer: an androgen-sensitive (LNCaP) and more aggressive androgen-insensitive cell line (DU145) (ref.[129]).

Several berry derivatives have also potent anti-**angiogenesis** properties *in vitro* by altering vascular endothelial growth factor (VEGF) expression and invasiveness. Berry extracts can inhibit hydrogen peroxide and TNF-α induced VEGF expression in these cells and also inhibit angiogenesis in animals[130]. For example apigenin inhibits expression of VEGF in human ovarian cancer cells[74].

Polyphenolic fractions from plants can display **insulin-like effects** by reducing blood glucose levels after food intake[131]. The main effect may be due to inhibition of starch degradation within the gastrointestinal tract (GIT) by inhibiting α-glucosidase/maltase activity[132]. It has been shown, that anthocyanins, especially diacylated forms owing to intestinal pH, inhibit α-glucosidase activity and can reduce blood glucose levels after starch-rich meals, a proven clinical treatment for controlling **diabetes mellitus** type II (ref.[132, 133]). Anthocyanins can directly induce insulin secretion from pancreatic cells in *ex vivo* models, but this effect may be disregarded because of its low serum bioavailability[31, 134]. Cyanidin-3-glucoside, quercetin, ferulic acid, peonidin-3-glucoside and tocopherol, in this order, showed significant inhibitory activity against aldose reductase activity[135]. Cyanidin-3-rutinoside inhibited α-glucosidase from baker's yeast in a dose-dependent manner[136]. It can lead to a reduction in glucose absorption and therefore the rise of postprandial hyperglycemia can be attenuated[94]. Berry anthocyanins appear to benefit **vision** in several ways in diabetes, including improving night vision by enhanced generation of retinal pigment, increasing circulation within the capillaries of the retina, decreasing macular degeneration and diabetic retinopathy, and improving or preventing glaucoma and cataracts[137].

A range of berry polyphenols can inhibit protease activities at levels which may affect protein digestion in the GIT. Soluble proanthocyanidins can inhibit pancreatic and gastric lipase activity and therefore would be a target in the treatment of obesity[133]. Berries have also been shown to reverse age-related and oxidative stress-induced decline in brain function in rats[138].

CONCLUSION

Lonicera caerulea berries contain 7.20 % saccharides, 1.52 % lipids, 14.62 % dry matter, 12.2 % organic acids and 4 % phenolics, containing 33.5 % of phenolics, including anthocyanins (18.5 %), flavonoids and phenolic acids. The major anthocyanins in *L. caerulea* fruit are glucosides and rutinosides of cyanidin, peonidin, dephinidin and pelargonidin. These berries seem to be prospective sources of health supporting phytochemicals that exhibit beneficial activities such as anti-adherence, antioxidant and chemoprotective, thus they may provide protection against a number of chronic conditions, e.g. cancer, diabetes mellitus, tumor growth or cardiovascular diseases. These plants can be cultivated in European climatic conditions and therefore are a suitable source of economically accessible nutraceutical preparations.

ACKNOWLEDGEMENTS

This study was supported by grants MSM 6198959216 and FT-TA3/024 that are gratefully acknowledged.

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