

# American Herbal Pharmacopoeia™ *and Therapeutic Compendium*

## *Bilberry Fruit*

*Vaccinium myrtillus* L.

Standards of Analysis, Quality Control,  
and Therapeutics

2001

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### Cover Photograph

Bilberry *Vaccinium myrtillus* L.  
Photograph courtesy of Indena SpA,  
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# NOMENCLATURE

## Botanical Nomenclature

*Vaccinium myrtillus* L.

## Botanical Family

Ericaceae

## Definition

Bilberry fruit consists of the fruits of *Vaccinium myrtillus* L. conforming to the methods of identification provided and yielding not less than 0.2% anthocyanins, calculated as cyanidin-3-glucoside as determined by spectrophotometric assay.

## Common Names

United States: Bilberry (McGuffin and others 2000), European blueberry, huckleberry, whortleberry.  
France: Airelle myrtille.  
Germany: Heidelbeere, Blaubeere, Bickbeere.  
Holland: Blaubessen.  
Italy: Mirtillo nero.  
United Kingdom: Bilberry, whortleberry.

# HISTORY

Bilberry (*Vaccinium myrtillus*) belongs to a large genus of plants that have provided humans with a primary source of edible berries, among which the common blueberry (*Vaccinium corymbosum*) is one. Bilberry fruit was reported in the writings of ancient medical authorities such as Pliny and Theophrastus. However, little mention was given to its medicinal activity. Because the common name of bilberry has historically been applied to several different species in the *Vaccinium* genus, the exact species referred to by these authorities is unknown (Gerard 1633). The leaves have also been widely used in medicine and have been written about as frequently as the fruits.

The common name bilberry is reported to be derived from the Danish word *bollebar* which means “dark berry” (Cunio 1993). The genus name *Vaccinium*, reportedly first used by Pliny (Keville 1991), is thought to be derived from the Latin *bacca*, meaning “olive”, “berry”, or “any round fruit”, or alternatively, *vacca*, meaning “cow”. The species name *myrtillus* is Latin for “little myrtle” owing to the resemblance of the leaves and fruits to those of myrtle trees (*Myrtus* spp.) (Benigni and others 1964). The medicinal activity of bilberry has been written about since at least the Middle Ages. Saint Hildegard from Bingen (AD 1098-1179) is reported to have recommended the fruits for promoting menstruation (Morazzoni and Bombardelli 1996). The 16th century herbalist Culpeper reported on the use of bilberry for the liver and stomach, as an astringent, and for chronic coughs and diseases of the lungs (Culpeper 1826). Green, in his *Universal Herbal* of 1820, noted the use of bilberry fruits

as an astringent for diarrhea and dysentery, a practice that was widespread throughout Europe (Benigni and others 1964).

Despite their use throughout Europe, bilberry fruits do not appear to have been incorporated into the medical practices of American physicians. According to Grieve, the fruits of bilberry were used to treat dysentery, diarrhea, gastrointestinal inflammation, hemorrhoids, vaginal discharges, scurvy, urinary complaints, and to dry up breast milk (Grieve 1994). The most noted medicinal use of bilberry in recent times may be the common consumption of bilberry jam by World War II pilots to improve their night vision when going out on night missions. Today, in addition to its continued use as a delicious food, bilberry is widely used throughout Europe and the United States for its reported ability to improve vision and for its positive effects in decreasing vascular permeability and capillary fragility. These uses have been attributed to the presence of flavonoids and anthocyanins (Morazzoni and Bombardelli 1996). It is also often dispensed for its reported antioxidant activity. Bilberry is currently included in the pharmacopoeias of Austria, Europe, Germany, and Switzerland.



**Figure 1** Bilberry (*Vaccinium myrtillus*)  
**Source:** Chaumeton, *Flore Medicale* (1814)

**Table 1 Historical timeline of the medical use of bilberry fruit (*Vaccinium myrtillus*)**

1098-1179	Saint Hildegard from Bingen recommended the fruits for promoting menstruation.
1826	Nicholas Culpeper used the fruits for liver and stomach complaints, as an astringent tonifier, and for chronic coughs.
1983-present	Bilberry fruit widely studied for its effects in treating diabetic retinopathy, other ophthalmological conditions, and vascular insufficiency.
1998-present	United States Department of Agriculture investigates and supports the antioxidant effects of bilberry and blueberry fruit anthocyanidins.

## IDENTIFICATION

### Botanical Identification

*Vaccinium myrtillus* L. Trailing shrub forming large colonies from creeping rhizomes, 1-6 dm tall; twigs green, glabrous, 3-angled. **Leaves:** Deciduous, alternate, short petiolete; blade broadly elliptic to ovate, 6-18 mm wide, 10-30 mm long, apex acute to obtuse, base rounded; margin serrulate; bright green, lower surface sparsely glandular with prominent venation. **Inflorescence:** Flowers solitary or paired in leaf axils, bracts 2. **Flowers:** Perfect, radially symmetric, 5-lobed; calyx lobes very short to almost absent; corolla pale green or white to pink, broadly urceolate to globose, 4-7 mm wide, 3-5 mm long, lobes very short and revolute; stamens 10, filaments glabrous, anthers awned, dehiscent by terminal pores; ovary inferior, style usually included. **Fruit:** Berry, oblate-globose, 5-9 mm diameter, blue to black, rarely glaucous, many-seeded. Chromosome number:  $2x = 2n = 24$ .

**Distribution:** Heaths, meadows, moist coniferous forests; southern populations found in montane and subalpine meadows. Flowers May to June. Circumboreal from Europe to Asia with disjunct populations centered in the American and Canadian Rocky Mountains. The population in Greenland is considered an ancient introduction (Hitchcock and Cronquist 1976; Linnaeus 1753 [original citation]; Popova 1972; Scott 1995; Vander Kloet 1988). The geographically isolated Rocky Mountain populations are considered by some to be ssp. *oreophilum* (Rydb.) A. Löve and others (Scott 1995).



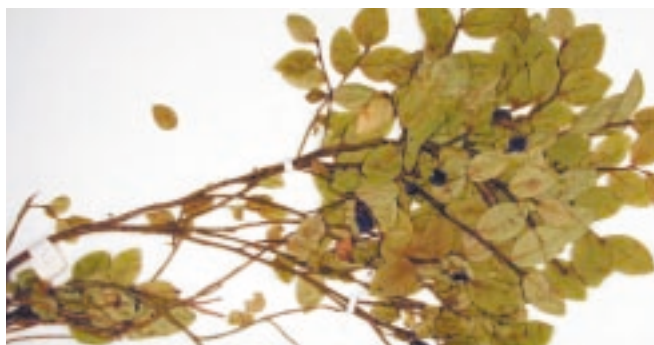
**Figure 2b Bilberry (*Vaccinium myrtillus*) flowering**

Photograph courtesy of Reinhard Länger, University of Vienna



**Figure 2c Bilberry (*Vaccinium myrtillus*)**

Photograph courtesy of Indena SpA, Milan, Italy



**Figure 2a Bilberry (*Vaccinium myrtillus*) pressed specimen**

Scan courtesy of Herbarium of the Institute of Pharmacognosy, University of Vienna



**Figure 2d Bilberry (*Vaccinium myrtillus*)**

Photograph courtesy of Indena SpA, Milan, Italy



**Figure 3a** Macroscopic characteristics of whole bilberry fruits (*Vaccinium myrtillus*)

Photograph courtesy of Reinhard Länger, University of Vienna



**Figure 3b** Macroscopic comparison of blueberry (*Vaccinium corymbosum*), left; lingonberry (*V. vitis-idaea*), center; and bilberry (*V. myrtillus*), right

Photograph ©2001, courtesy of Roy Upton, Soquel, CA

## Macroscopic Identification

Bilberry is sold as fresh, frozen, or dried whole berries. The dried berries are oblate-globose, 4-8 mm in diameter, with a bluish-black and coarsely wrinkled exocarp. The pedicel may be attached or detached, and often the remains of the style, nectar disk, and calyx are persistent at the apex of the berry, with the calyx appearing as a circular fold. The mesocarp is purple. There are 4-5 locules, each containing many seeds; each seed is approximately 1 mm long with a yellowish-brown dimpled surface. For the macroscopic characteristics of the fresh berry, see Botanical Description.

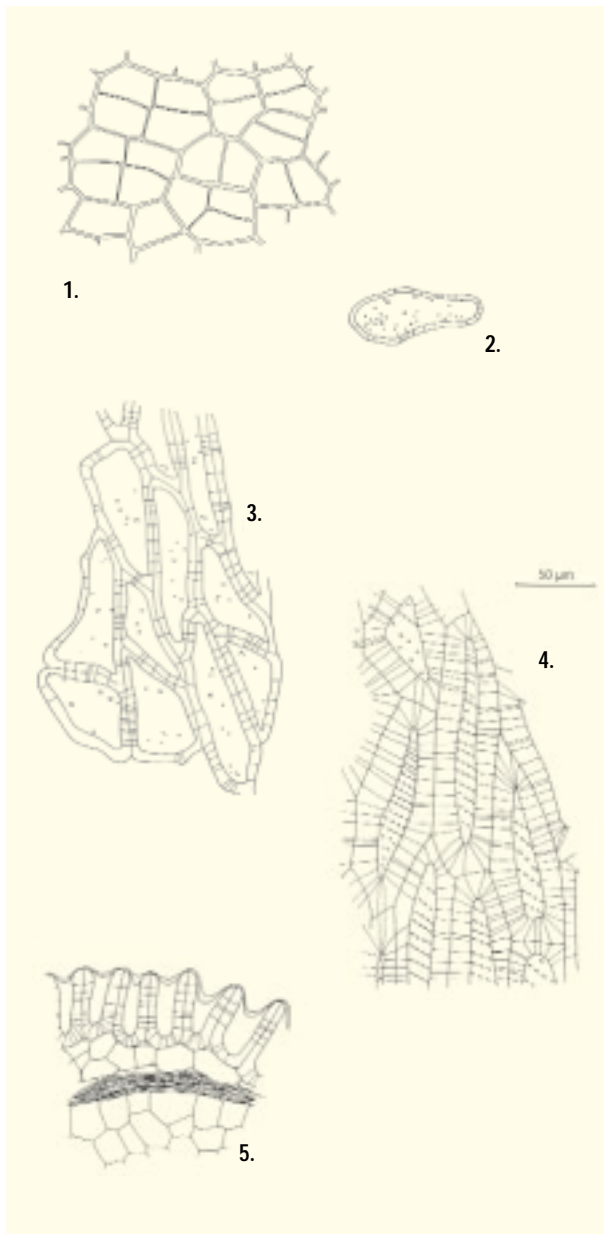
**Aroma:** No characteristic smell. **Taste:** Slightly astringent, pleasant, sour to sweet, mildly acidic.

**Powder:** Dark violet, violet-brown (DAC 1998; Moeck 1994; PharmEuropa 1998; Wichtl 1994).

**Table 2** Macroscopic and microscopic differentiation of the fruits of bilberry (*V. myrtillus*), bog bilberry (*V. uliginosum*), and lingonberry (*V. vitis-idaea*)

Character	Bilberry	Bog bilberry	Lingonberry
Dried fruit color (external)	blue to black or dark red, not glaucous	light blue-gray, glaucous	dark red, not glaucous
Juice color	clear dark blue or purple	colorless to greenish	—
Calcium oxalate crystals <sup>1</sup>	occasional in all tissues	absent	absent
Sclereids <sup>1</sup>	present in the mesocarp, endocarp, and testa	absent	absent
Endocarp mother cell length <sup>1</sup>	up to 140 µm	up to 350 µm	—
Epidermal cells of the testa in cross section <sup>1</sup>	U-shaped secondary walls	secondary walls not U-shaped*	U-shaped secondary walls
Chromosome number (2n)	24	48	24

<sup>1</sup>Data from Reinhard Länger, University of Vienna. \* = data from Moeck 1994.



**Figure 4a** Microscopic characteristics of bilberry fruit (*Vaccinium myrtillus*)

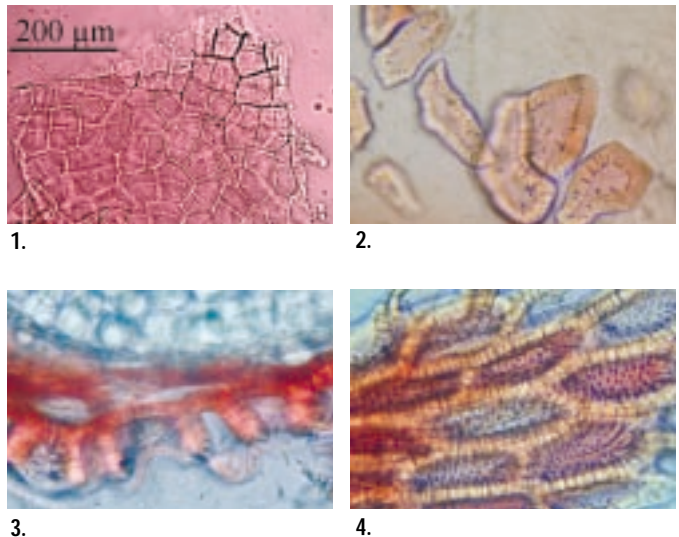
1. Exocarp (surface view).
2. Solitary sclereid from the mesocarp.
3. Group of sclereids from the endocarp.
4. Testa (surface view).
5. Testa showing U-shaped secondary walls (cross section).

Microscopic drawings courtesy of Reinhard Länger, University of Vienna.

## Microscopic Identification

The exocarp consists of polygonal, rectangular, or quadratic cells with slightly pitted tangential walls. Groups of 2-4 cells occur, each group surrounded by a thick wall, while within the groups the walls are considerably thinner. The mesocarp consists of large parenchymatous cells with scattered solitary sclereids and vascular bundles containing vessels with spiral or helical secondary wall thickenings. The endocarp is composed largely of groups of sclereids similar to those in the mesocarp and having an elongated or nearly quadratic shape. The outer layer of the testa consists of elongated, heavily thickened and pitted sclereids. In cross section these cells have U-shaped secondary walls with the unthickened side occurring on the outer tangential wall. The endosperm cells are thin-walled and contain droplets of fixed oil. Calcium oxalate crystals may occur occasionally in all tissues.

**Powder:** Intensely violet colored with sclereids from the mesocarp, endocarp, and testa. Also present are parenchyma cells, vascular bundles, the polygonal cells of the exocarp, and oil droplets.



**Figure 4b** Microscopic characteristics of bilberry fruit (*Vaccinium myrtillus*)

1. Endocarp cells.
2. Sclereids of the endocarp.
3. Testa (cross section).
4. Testa (surface view).

Microscopic images courtesy of Sidney Sudberg, Alkemists Pharmaceuticals, Costa Mesa, CA (1) and Reinhard Länger, University of Vienna (2-4).

## COMMERCIAL SOURCES AND HANDLING

The primary commercial supply of bilberry comes from wild sources in Poland, Romania, Bulgaria, Russia, Albania, the former Yugoslavia (Moeck 1994), Sweden, and other Scandinavian countries.

### Collection

Bilberry fruit is harvested when ripe, usually from July to September. In one study, the highest berry yields came from plants growing in somewhat exposed areas with moderate shade and moderately humid ground (Gozin 1972, cited in Vanhaelen and others 1991). It has been determined that as the fruit ripens, the concentration of the flavonols and pro-cyanidins decreases while the concentration of anthocyanins increases (Brenneisen and Steinegger 1981a; Moeck 1994; Morazzoni and Bombardelli 1996). Increased maturity and the resultant increase in anthocyanins have also been positively correlated with increased antioxidant activity (Prior and others 1998). One source maintains that there is geographic variation in constituent profile: cyanidin glycosides make up most of the anthocyanins in berries coming from northern regions, such as Norway and Sweden, while delphinidin glucosides predominate in berries coming from Italy, Poland, and Romania (Moeck 1994). In another study, geographic source was not found to affect either anthocyanin content or antioxidant activity (Prior and others 1998).

Bilberry grows well in polluted environments. The fruits have been shown to accumulate heavy metals in direct proportion to their concentration in the soil or as a function of distance from the contaminant source. Additionally, one study found that the ascorbic acid content of fruit from plants grown on industrial sites was lower than that of fruits from plants grown in a rural environment. Bilberry fruits have also been shown to accumulate pesticide residues in direct proportion to the rate of application (Cunio 1993). Therefore, berries should be collected away from contaminated sources and appropriate analyses should be used to ensure that contaminants, if present, are within acceptable limits.

### Cultivation

Bilberry prefers damp acidic soils (pH 4-5) which are high in organic matter. Propagation is by rooted stem cuttings.

### Handling and Processing

Bilberries should be handled with care. Anthocyanins have been found to concentrate in the skin of most blueberry (*V. corymbosum*) cultivars. If the skin is damaged during handling these compounds may oxidize. There is very limited data suggesting an effect of heat on the bioactivity of bilberry extract. One review reported that the bacteriostatic activity of the aqueous extract against *Staphylococci* increased after boiling or autoclaving at 110 °C for 25 minutes. No such change occurred in aqueous extracts obtained from an acidic menstruum (Benigni and others 1964).

### Drying

According to the European pharmacopoeia (PharmEuropa 1998), bilberry should be dried for 1 hour at 100 °C -105 °C. High temperatures should be avoided since anthocyanins are not stable when heated (Cunio 1993). In Europe, berries are typically air-dried in the shade or subjected to artificial heat (Moeck 1994).

### Storage

Follow general guidelines for storage by packing in airtight containers protected from light, heat, moisture, and insect infestation. Storage in a cool environment of 18 °C - 25 °C is particularly important because anthocyanins are not stable when heated (Cunio 1993).

### Qualitative Differentiation

Dried fruits should be soft and pliable. Berries that have been stored too long desiccate and turn hard (Wichtl 1994). Fermented, moldy, or insect-damaged fruits should be discarded.

### Adulterants

Historically, bog bilberry (*V. uliginosum*) and lingonberry (*V. vitis-idaea*) have been noted as adulterants (Moeck 1994), though this is considered to have been rare. The micro- and macroscopic characters that differentiate the fruit of these two species from *V. myrtillus* are given in Table 2.

### Preparations

Commercial bilberry extracts are generally standardized to 36% anthocyanins, calculated as and equivalent to 25% anthocyanidins. Prior and Cao (1999) found that total anthocyanin content (expressed as mg cyanidin-3-glucoside equivalent per g dried extract) of 15 commercial bilberry products ranged from 2.0-204.4 mg/g with a mean ( $\pm$  SD) of  $56.24 \pm 64.84$  mg/g. Recent studies have demonstrated antioxidant activity of aqueous extracts (Joseph and others 1999). According to one patent, the suspension of the extract in a lipophilic carrier (fractionated coconut oil [4-8% w/w]) results in greater oral bioavailability compared to aqueous solutions of the extract (Seghizzi and others 1998).

The following guidelines for preparations have been reported in the literature:

#### Cold Macerate\*:

Soak 5-10 g of crushed dried fruit in 150 mL of cold water for 2 hours, allowing the fruit to swell, then strain (Meyer-Buchtela 1999).

#### Decoction\*:

Place 5-10 g of crushed dried berries in 150 mL of cold water. Bring to a boil for approximately 10 minutes, then strain (Meyer-Buchtela 1999; Wichtl 1994).

#### Dry Extract:

The fresh frozen berries are extracted with ethanol or methanol at 10 °C - 60 °C and then filtered. The resulting solution is diluted with water and then further concentrated using a chromatographic system such as a col-

umn chromatograph. Various manufacturers may have proprietary processes for accomplishing this.

Fluid Extract (1:1 g/mL)\*:

Use a 1:1 drug to extract ratio (Anderhuber 1991).

Topical Solution\*:

10% decoction (Blumenthal and others 1998).

\* These preparations reflect the use of crude bilberry for its astringent properties in the treatment of diarrhea and local inflammations.

## CONSTITUENTS

The constituents considered to be of primary importance in bilberry fruits are the flavonoids, specifically the anthocyanins. Anthocyanins are the natural pigments responsible for the red, blue, and purple colors of the fruit and, along with phenolic compounds, have been directly correlated with antioxidant activity. One study found that bilberry contains the highest concentration of anthocyanins compared to *V. angustifolium*, *V. ashei*, and *V. corymbosum* (Prior and others 1998). The content of anthocyanins in the berries increases during the ripening process (see Commercial

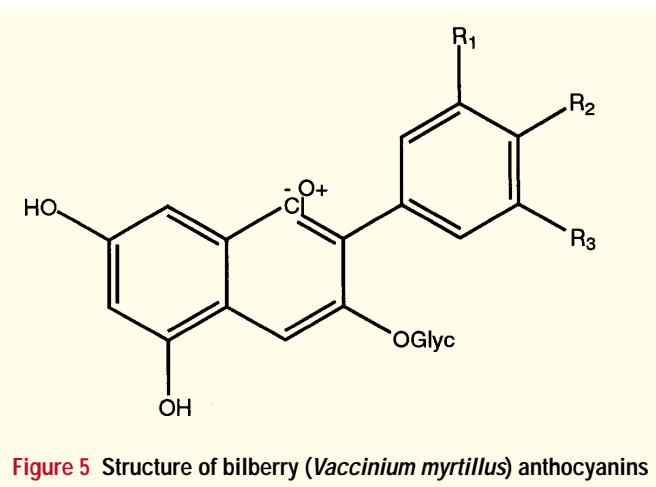


Figure 5 Structure of bilberry (*Vaccinium myrtillus*) anthocyanins

Sources and Handling).

### Flavonoids and Anthocyanins

The flavonol-O-glycosides in fresh bilberry juice and fruit include quercetin-3-rhamnoside (quercitrin), quercetin-3-glucoside (isoquercitrin), quercetin-3-galactoside (hyperoside), and kaempferol-3-glucoside (astragalin) (Azar and others 1987; Brenneisen and Steinegger 1981b; Friedrich and Schönert 1973a; Häkkinen and Auriola 1998).

The flavonols, catechin (maximum 0.02%) and epicatechin (maximum 0.07%), as well as small amounts of gallo-catechin and epigallocatechin, have been identified in the unripe fruit (Brenneisen and Steinegger 1981a; Friedrich and Schönert 1973b). The 4-8 linked catechin and epicatechin dimers, procyanidins B-1, B-2, B-3, and B-4 are also present (Brenneisen and Steinegger 1981a).

Total anthocyanin content ranges from 300-698 mg/100 g (Mazza and Miniati 1993, cited in Prior and others 1998; Prior and others 1998). Fifteen anthocyanins have been identified in bilberry fruit, juice, and extract (Baj and others 1983; Brenneisen and Steinegger 1981b; Goiffon and others 1991; Krawczyk and others 1991; Krawczyk and Petri 1992; Petri and others 1994, 1997; Simard and others 1980). These are the 3-O-arabinosides, 3-O-glucosides, and 3-O-galactosides of the five anthocyanidins: cyanidin, delphinidin, malvidin, peonidin, and petunidin (Baj and others 1983; Martinelli and others 1986) (Table 3). According to one study, cyanidin and delphinidin glycosides account for 64% of the total anthocyanin content of bilberry (Prior and Cao 1999). Of these, cyanidin-3-glucoside displayed the greatest level of antioxidant activity in at least two studies (Morazzoni and Bombardelli 1996; Prior and others 1998).

### Tannins

According to an older study using gravimetric analysis, 5-12% tannins, mostly catechol tannins, occur in bilberry fruit (Moeck 1994; Wichtl 1994).

### Phenolics

The following phenolic acids have been identified in fresh

Table 3 Primary anthocyanins of bilberry (*Vaccinium myrtillus*) and their relative concentrations in bilberry extract

Compound	OR <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Mean % content*
Delphinidin-3-O-arabinoside	OH	OH	OH	4.32
Delphinidin-3-O-glucoside	OH	OH	OH	5.81
Delphinidin-3-O-galactoside	OH	OH	OH	5.04
Cyanidin-3-O-arabinoside	OH	OH	H	2.19
Cyanidin-3-O-glucoside	OH	OH	H	3.42
Cyanidin-3-O-galactoside	OH	OH	H	2.75
Peonidin-3-O-arabinoside	OCH <sub>3</sub>	OH	H	0.22
Peonidin-3-O-glucoside	OCH <sub>3</sub>	OH	H	1.31
Peonidin-3-O-galactoside	OCH <sub>3</sub>	OH	H	0.34
Petunidin-3-O-arabinoside	OH	OH	OCH <sub>3</sub>	1.08
Petunidin-3-O-glucoside	OH	OH	OCH <sub>3</sub>	3.67
Petunidin-3-O-galactoside	OH	OH	OCH <sub>3</sub>	1.89
Malvidin-3-O-arabinoside	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	0.81
Malvidin-3-O-glucoside	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	3.35
Malvidin-3-O-galactoside	OCH <sub>3</sub>	OH	OCH <sub>3</sub>	1.27

\* Relative concentration based on a single high performance liquid chromatographic (HPLC) analysis of a commercial bilberry extract (Myrtocyan®).

Source: Baj and others (1983).



bilberry juice and unripe fruit: gallic, protocatechuic, m- and p-hydroxybenzoic, vanillic, chlorogenic, caffeic, syringic, o- m-, and p-coumaric, ferulic, and syringic acids (Azar and others 1987; Brenneisen and Steinegger 1981a; Friedrich and Schönert 1973b). Several sugar esters of phenolic acids also have been reported to be in the fruit (Reschke and Herrmann 1981). These are the 1-O-hydroxycinnamyl- $\beta$ -D-glucoses and include p-coumarylglucose, ferulylglucose, and sinapylglucose.

#### Organic Acids

Several organic acids have been reported in the juice from fresh fruit. Citric (0.8% to 0.9%) and malic acids (0.06%) are present in the highest concentrations. Present in smaller amounts are lactic, oxalic, succinic, and quinic acids (Benigni and others 1964).

#### Macronutrients

According to the older literature, fresh fruit contains an average of 86.5% water and 5.8% sugar (0.2% saccharose, 2.3% glucose, and 3.3% fructose) (Benigni and others 1964). The vitamins C (3 mg/100 g), B<sub>1</sub> (0.02-0.03 mg/100 g), B<sub>2</sub> (0.03-0.04 mg/100 g), pantothenic acid (0.08-0.16 mg/100 g), nicotinamide (0.3-0.65 mg/100 g), and  $\beta$ -carotene (100 IU/g) have been reported in fresh fruit (Benigni and others 1964; Moeck 1994).

#### Volatile Compounds

Approximately 109 volatile compounds have been identified in bilberry fruits (Sydow and Anjou 1969). These include aliphatic alcohols, aldehydes, and ketones. Also present are terpene derivatives, aromatic compounds, and esters. Three major constituents of the characteristic bilberry aroma were identified as trans-2-hexenal, ethyl-3-methylbutyrate, and ethyl-2-methylbutyrate (Sydow and others 1970).

#### Other Compounds

The iridoid glycosides monotropein and asperuloside have been reported to occur in unripe bilberry fruit (Friedrich and Schönert 1973a). The quinolizidine alkaloid myrtiline (10  $\mu$ g/g) has also been identified, though it is unclear from the publication whether it was isolated from leaf or fruit (Slosse and Hootel  1978).

## ANALYTICAL

A thin layer chromatography (TLC/HPTLC) method developed by CAMAG, Switzerland is presented as a fingerprint for the qualitative determination of bilberry raw material and powdered extract. A spectrophotometric assay for quantifying total anthocyanins in bilberry raw material was adopted from the European Pharmacopoeia (PharmEuropa 1998) and substantiated by two American Herbal Pharmacopoeia™ collaborating laboratories. This assay calculates total anthocyanin content expressed as cyanidin-3-glucoside. Because this method cannot detect adulteration, it should only be employed after appropriate methods of plant identification have ensured authenticity and purity. For quantifying total anthocyanins in bilberry extract, the pH-differential spectrophotometric assay published by the

Institute for Nutraceutical Advancement Methods Validation Program (INA-MVP) (Giusti and Wrolstad 2001) is recommended. This method is able to detect adulteration by added colorants such as FD & C Red No. 40, FD & C Red No. 3, cochineal, and beet powder.

### High Performance Thin Layer Chromatography (TLC/HPTLC) of Bilberry Anthocyanins

#### Sample Preparation

Accurately weigh 0.5 g of the powdered drug and place in a flask with 10 mL of methanol and sonicate for 15 minutes. Filter the mixture and transfer the filtrate into a vial for analysis. This is the test solution.

#### Reagent Preparation

Anisaldehyde-sulfuric acid reagent: 8 mL of 98% H<sub>2</sub>SO<sub>4</sub> are carefully added to an ice-cooled mixture of 85 mL methanol and 10 mL acetic acid. To this solution 0.5 mL anisaldehyde is added. This mixture should be kept on ice until ready to use.

#### Chromatographic Conditions

Stationary Phase:

HPTLC plates 10 x 10 cm silica gel 60 F<sub>254</sub> (EM Science or equivalent).

Mobile Phase:

Formic acid:water:1-butanol (10:15:40).

Sample Application:

5 mL of the test solution is applied as an 8 mm band. Application position should be 8 mm from the lower edge of the plate.

Development:

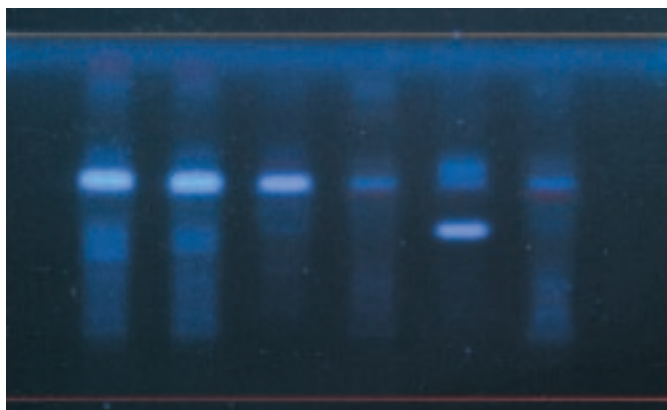
10 x 10 cm Twin Trough chamber (CAMAG or equivalent), saturated for 10 minutes, 5 mL per trough (or enough solvent to have 5 mm in each trough). Developing distance is 60 mm from the lower edge of the plate. Dry plate in a stream of cold air for 10 minutes.

Detection:

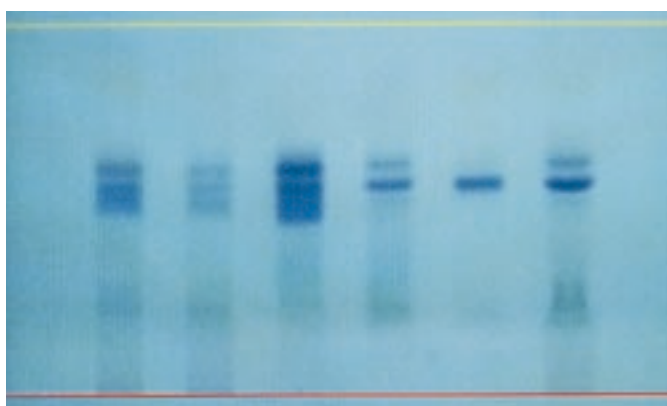
- UV 366 nm.
- White light.
- Anisaldehyde-sulfuric acid reagent: Immerse plate in reagent for 1 second, dry in a stream of cold air, heat plate at 110 °C for 5 minutes. Evaluate plate under white light.

Results:

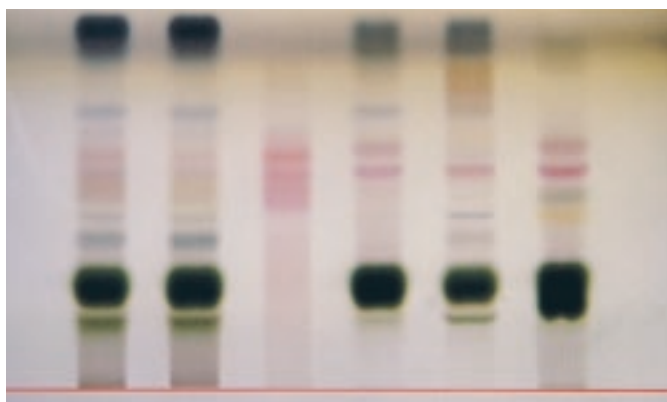
Compare to the chromatograms provided.



6a UV 366 nm



6b White light



6c Anisaldehyde-sulfuric acid reagent, white light

**Figures 6a-c** HPTLC chromatograms of bilberry fruit, bilberry fruit extract (*Vaccinium myrtillus*), and fruits of other *Vaccinium* species

- Lane 1: Dried bilberry fruit, sample A.
- Lane 2: Dried bilberry fruit, sample B.
- Lane 3: Dried extract of bilberry fruit.
- Lane 4: Dried blueberry fruit (*Vaccinium corymbosum*).
- Lane 5: Freeze-dried lingonberry fruit (*Vaccinium vitis-idaea*).
- Lane 6: 95% ethanol extract of fresh blueberry (*Vaccinium corymbosum*).

### Discussion of Chromatograms

**6a) UV 366 nm:** Bilberry (Lanes 1-3) shows a strong blue band at  $R_f$  0.6. Blueberry (Lanes 4 and 6) also shows a weak blue band at the same position while lingonberry (Lane 5) shows two bands and another bright blue band below them.

**6b) White light:** Bilberry (Lanes 1-3) shows three dark blue bands that are not completely separated between  $R_f$  0.5- 0.6. The intensity of the bands in the extract is higher and an additional band is seen. Blueberry (Lane 4 and 6) shows two dark blue bands at the same position while lingonberry (Lane 5) shows only one dark blue band.

**6c) Anisaldehyde-sulfuric acid reagent, white light:** Bilberry dried fruit (Lanes 1-2) shows a very broad dark green band at  $R_f$  0.3 and a weaker green band just below. These bands are lacking in the bilberry extract (Lane 3) but are present in the other *Vaccinium* species (Lanes 4-6). Bilberry dried fruit (Lanes 1-2) and lingonberry (Lane 5) have two sharp blue bands at  $R_f$  0.4 above the dark green zone. Dried fruit of all species (Lanes 1-2 and 4-5) show a prominent dark blue band close to the solvent front and another dark blue band at  $R_f$  0.7. Only lingonberry (Lane 5) shows two orange-red bands between those two blue bands. Bilberry (Lanes 1-3) shows four pink-red bands which are not completely separated between  $R_f$  0.5-0.6. Blueberry (Lanes 4 and 6) shows two dark pink bands at the same position while lingonberry (Lane 5) shows only one.

### Spectrophotometric Assay for the Quantification of Anthocyanins in Bilberry Raw Material

#### Sample Preparation

Accurately weigh 1.00 g of the powdered drug and add 95 mL of methanol. Sonicate for 30 minutes and filter into a 100 mL volumetric flask. Rinse the filter and adjust to volume with methanol. Prepare a 20-fold dilution of this solution in a 0.1% V/V solution of HCl in methanol.

#### Procedure

Measure the absorbance of the solution at 528 nm using a 0.1% V/V solution of HCl in methanol as the reagent blank.

#### Calculation

Calculate the content of anthocyanins, expressed as cyanidin-3-glucoside, from the expression:

$$\frac{A \times 2000}{772 \times m}$$

A = the absorbance of the solution at 528 nm.

m = the mass of the sample in grams

The specific absorbance of cyanidin-3-glucoside at 528 nm is 772.

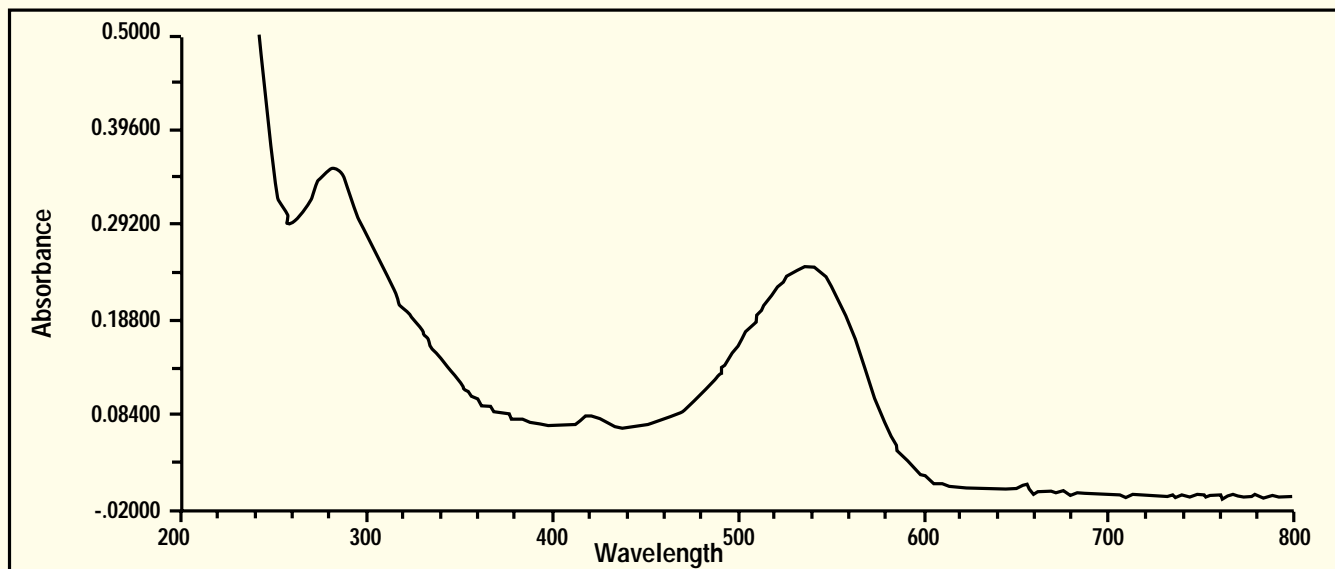


Figure 7 Absorbance spectrum of bilberry fruit total anthocyanins (*Vaccinium myrtillus*)

### Quantitative Standards

Foreign Matter:	Not to exceed 2% (DAC 1998), including leaves, twigs, and other fruits.
Total Ash:	Not to exceed 5% (Pharm-Europa 1998).
Sulfated Ash:	Not to exceed 4.5% determined on 2 g powder (Helv VII 1987).
Loss of Moisture on Drying:	Not to exceed 16% determined on 1 g powder oven-dried at 100 °C-105 °C (Pharm-Europa 1998).
Water-soluble Extractive:	Not less than 50% (ÖAB 1990).
Tannins:	Not less than 1.5% (Helv VII 1987).

### THERAPEUTICS

Numerous clinical trials on the use of bilberry fruit preparations for vascular health and vision problems have been conducted since the 1960s. Much of the available data suggest that bilberry is of benefit for many of these problems. However, the majority of clinical trials have marked methodological deficiencies including small sample populations, poor design, and lack of placebo controls. Additionally, many of the published clinical reports have not been subjected to peer review, while others remain unpublished. The majority of pharmacological studies have been conducted with the bilberry fruit extract Myrtocyan® or Tegens®, both of which contain 36% anthocyanins (equivalent to 25% by weight of anthocyanidins)\*. The potential for fruit and fruit extract toxicity appears to be very small.

In Europe, a postmarketing surveillance study of 2295 subjects who had taken Myrtocyan® found that the product was primarily used for lower limb venous insufficiency, fragility or altered permeability of capillaries, retinal degeneration, and hemorrhoids, in decreasing order of predominance. Most of the subjects took the equivalent of 160 mg of anthocyanins twice daily for 1-2 months. Physician evaluation of efficacy was rated good or very good (60%) with a very low occurrence of adverse events (4%), which is consistent with most studies (Eandi 1987, cited in Morazzoni and Bombardelli 1996). Specific indications for which there appears to be substantiating evidence from well-designed trials include diabetic and hypertensive retinopathy. Though bilberry extract is reputed to enhance night vision, the most recent well-designed trials suggest that it may be ineffective for improving night vision in healthy humans. Several observational studies, with considerable support from animal and in vitro research, suggest that bilberry extract is also useful in treating vascular insufficiency. One trial suggests that bilberry extract can significantly alleviate symptoms associated with dysmenorrhea.

Anthocyanins are thought to be responsible for most of the pharmacologic effects related to this botanical and are reported to elicit a number of actions. With respect to the vascular system, these actions include vasorelaxation, a decrease in vascular permeability, and an increase in arteriolar vasomotion. With respect to the eyes, the actions include the modulation of retinal enzymes and stabilization of the phospholipid membranes of the retina. Bilberry is considered to have general antioxidant activity.

\* Myrtocyan® (Indena SpA, Milan, Italy) is included in and is considered to be the active principle of the proprietary product Tegens® (Inverni della Beffa, Sanofi-Synthelabo).

## Pharmacokinetics

The pharmacokinetic profile of bilberry fruit extract has been studied in rats using intravenous (iv), intraperitoneal (ip), and oral (po) administration of the bilberry extract Myrtocyan® (Lietti and Forni 1976b; Morazzoni and others 1991). Lietti and Forni (1976b) administered 20-40 mg/kg iv or 25 mg/kg ip of the extract to rats. Administered by either route, anthocyanins were rapidly distributed throughout the body and eliminated primarily through the urine and bile following a three-compartment model. After 4 hours, approximately 20% of the dose was eliminated through the urine regardless of administration route, while 15% (iv) and 18% (ip) was eliminated through the bile after 24 hours (difference between routes significant;  $P < 0.05$ ). After ip administration, anthocyanins showed a stronger affinity for the kidneys and skin compared to plasma (Table 4). Morazzoni and others (1991) administered anthocyanins at doses of 20-40 mg/kg iv or 400 mg/kg po. Their results for iv doses were consistent with those of the previous study (Table 5). Following oral administration, peak blood levels of 2-3 µg/mL were detected within 15 minutes, declining rapidly thereafter (Table 6). Less than 1 µg/mL anthocyanins were detected in plasma after 2 hours. Only 4% and 1% of the oral dose was eliminated in the bile and urine, respectively. The absolute bioavailability of oral anthocyanins was quite low (1.2%; not more than 5% absorption). The authors note, however, that the plasmatic peak levels following oral administration are within the range of biological activity reported for anthocyanins. The authors of both of these studies suggest that the higher affinity of anthocyanins for the kidneys and skin compared to other organs may explain the long duration of pharmacologic actions such as the increase in capillary resistance, even after blood plasma levels are low.

According to a European patent, oral bioavailability of anthocyanins is enhanced by administering them in a fractionated coconut oil suspension (Seghizzi and others 1998). Oral doses of 400 mg/kg in rats reportedly yielded peak plasma concentrations over 10 µg/mL using this formulation, compared to 1 µg/mL with a water formulation and 1.3-6.4 µg/mL in suspensions using other oils. Relative bioavailability was reported to be 8.8 times greater than for a water formulation. Compared to controls, the coconut oil suspension

(100 mL/kg daily for 3 days) was also found to increase capillary resistance more than the aqueous preparation (200 and 400 mL/kg daily for 3 days) 6 hours after the first oral administration to guinea pigs ( $n = 6$ ); at earlier times post-treatment, both solutions were equally effective.

Additional information on the pharmacokinetics of anthocyanins can be gleaned from two recent studies using elderberry (*Sambucus* spp.) anthocyanins. A preliminary pharmacokinetic study of total anthocyanins was done in a single man who consumed 25 grams of elderberry extract containing 1.5 grams of total anthocyanins after fasting overnight (Cao and Prior 1999). Blood samples were obtained before, 30, and 60 minutes after extract consumption. After 30 minutes, plasma anthocyanin concentration reached at least 100 µg/L. Cao and others (2001) showed that anthocyanins can be absorbed in their unchanged glycosylated form. The study was done in four elderly females who consumed 12 grams of elderberry extract containing 720 mg of anthocyanins. Ten blood samples and six urine samples were obtained over 24 hours. Diet was controlled to provide no additional anthocyanins and to be low in other flavonoids throughout the study period. Using HPLC, five compounds were detected in the plasma samples and six in the urine. These included cyanidin 3-sambubioside and

**Table 4** Tissue distribution of anthocyanidins 1 hour after administration of bilberry fruit anthocyanins<sup>1</sup> to rats

	Anthocyanidins (µg/g tissue)*	Tissue/plasma ratio
Plasma (µg/mL)	25.6 ± 1.9	—
Heart	13.6 ± 2.6	0.55
Kidneys	79.0 ± 9.8	3.18
Liver	21.0 ± 4.5	0.84
Lungs	12.2 ± 0.9	0.49
Plasma (µg/mL)**	19.0 ± 1.3	—
Skin	27.4 ± 1.8	1.48

<sup>1</sup>Myrtocyan®; 200 mg/kg ip

\* Mean ± standard error of 5 rats per group

\*\* Two groups of animals are represented in this Table. In the second group, only skin and plasma anthocyanidin levels were measured, allowing a comparison between these two tissues.

**Source: Adapted from Lietti and Forni (1976b).**

**Table 5** Plasma levels and pharmacokinetic data after intrafemoral and intraportal administration of bilberry anthocyanins (40 mg/kg) to rats

Route	A µg mL <sup>-1</sup>	a min <sup>-1</sup>	B µg mL <sup>-1</sup>	b min <sup>-1</sup>	C µg mL <sup>-1</sup>	g min <sup>-1</sup>	t <sub>1/2</sub> α min	t <sub>1/2</sub> β min	t <sub>1/2</sub> γ min	AUC 0 - ∞ min·µg·mL <sup>-1</sup>	Cl <sub>p</sub> mL min <sup>-1</sup> kg <sup>-1</sup>	V <sub>c</sub> mL kg <sup>-1</sup>
Intra-femoral	37.44	0.392	9.50	0.051	14.78	0.008	1.77	13.59	86.63	2088.29	7.28	246.27
Intra-portal	44.43	0.391	11.32	0.046	17.54	0.009	1.77	15.07	77.00	2216.03	6.86	207.39

**Source: Morazzoni and others (1991).**

**Table 6** Plasma levels and pharmacokinetic data after oral administration of bilberry anthocyanins (400 mg/kg) to rats

$C_{\max}$ $\mu\text{g mL}^{-1}$	$T_{\max}$ min	$K_{\text{ab}}$ $\text{min}^{-1}$	$\alpha$ $\text{min}^{-1}$	$\beta$ $\text{min}^{-1}$	$t_{1/2 \alpha}$ min	$t_{1/2 \beta}$ min	AUC 0 - $\infty$ $\text{min} \cdot \mu\text{g} \cdot \text{mL}^{-1}$	$Cl_p$ $\text{mL min}^{-1} \text{kg}^{-1}$	Bioavailability %
2.47	15.00	0.129	0.078	0.011	8.88	63.00	129.45	14.10	1.2 (iv)

Source: Morazzoni and others (1991).

cyanidin 3-glucoside, which accounted for 92.5% of the total anthocyanins. Others included cyanidin aglycone and malvidin hexoside. The mean maximum plasma concentration ( $C_{\max}$ ) of total anthocyanins was 97.4 nmol/L and was reached within 71.3 minutes ( $T_{\max}$ ). The elimination of plasma anthocyanins appeared to follow first-order kinetics. The elimination half-life ( $t_{1/2}$ ) of plasma total anthocyanins was 132.6 minutes. The total amount of anthocyanins excreted in the urine over a 24 hour period was  $397.0 \pm 45.1 \mu\text{g}$ . Anthocyanins seem to be unique, compared to other flavonoids, in that they are absorbed intact and not conjugated with glucuronide or sulfate. However, their relative bioavailability appears to be quite low.

## Clinical Efficacy and Pharmacodynamics

### Effects on Vascular Health

Human clinical studies have been done investigating the efficacy of bilberry fruit extract in treating venous insufficiency. This can be defined as inadequacy of the venous valves and impairment of the venous return from the legs, often accompanied by increased vascular permeability leading to edema and, less often, by stasis ulcers. Animal and in vitro work has focused on elucidating the mechanisms by which bilberry elicits its therapeutic effects in these conditions. This research suggests that bilberry extract may decrease abnormal vascular permeability through an effect on vascular connective tissue (specifically by protecting collagen and elastin). In addition, the extract acts as a vasorelaxant, helping to reduce vascular permeability caused by hypertension, and it appears to stimulate arteriolar vasomotion, helping to improve microvascular circulation and decrease vascular permeability in that way.

#### Vascular Insufficiency

##### Human Clinical Studies

The use of bilberry fruit for the treatment of venous insufficiency resulting from idiopathic varices or deep vein thrombosis and lower limb varicose syndrome, was reported in at least four observational clinical studies (Coget and Merlen 1968; Corsi and others 1985; Ghiringhelli and others 1977; Tori and D'Errico 1980). In the earliest of these, the researchers investigated the ability of bilberry anthocyanins (Difrel 20<sup>®</sup>, typically administered at 4-6 tablets daily, equivalent to 100-150 mg anthocyanins daily, for 10-15 days a month for 2 months) to enhance venous health in 27 subjects suffering from varices, varicosities, and telangiectases, or who were prone to bruising (Coget and Merlen 1968). Improvements in a variety of symptoms were observed with the most positive effects being a reduction in the propensity

for bruising. No statistical analyses were presented.

Tori and D'Errico (1980) administered 480 mg of Tegens<sup>®</sup> daily for 6 months to 97 patients with lower limb stasis and varicosities. Parameters measured included heaviness and edema of the limbs, paraesthesias, cramping, and burning. Statistically significant improvements in all symptoms were reported. In the study of Corsi and others (1985), statistically significant improvement in lower limb peak blood flow was reported in 7 patients with phlebitis and varicose veins who were given 480 mg of Tegens<sup>®</sup> daily for 30 days. The small patient population limits the significance of these findings. Ghiringhelli and others (1977) administered 480 mg of Tegens<sup>®</sup> daily for 30 days to 47 patients with varicosities. Parameters investigated included edema, heaviness in the legs, pain, burning sensation, diurnal and nocturnal cramping, pruritus, hemorrhagic sub-effusions, and numbness. Statistically significant improvements in all symptoms were reported ( $P < 0.01$ ). Falls in edema scores from  $2.28 \pm 0.17$  to  $0.33 \pm 0.6$  by day 15 were especially marked ( $P < 0.001$ ).

In a clinical study of 40 patients hospitalized for various circulatory problems, a proprietary bilberry extract (Difrel 100<sup>®</sup>) was typically administered at 4 tablets daily for an average of 28 days (12-69 days) (Amouretti 1972). Though no statistical analysis was provided, the researchers reported that capillary resistance was significantly improved in 55% of subjects with the best results reported for diabetic patients and the worst results reported for those with circulatory disturbance due to liver cirrhosis.

In one observational study, 54 pregnant women showing overt signs of vascular pathologies were treated with Tegens<sup>®</sup> (equivalent to 320 mg anthocyanins daily for 60-90 days) administered beginning in the 6<sup>th</sup> month of pregnancy (Grismondi 1980). The following symptoms were present at the beginning of the study: primary varicosities (75.9% of subjects); pigmentation changes (83.3%); burning sensations and pain in lower extremities (> 50%); edema (44.4%); and skin dystrophy (7.4%). Parameters measured included edema, a feeling of heaviness, paraesthesias and itching, burning sensations, day and night cramps, and alterations in capillary fragility (propensity to bruising). Subjective evaluation of all symptoms occurred at days 0, 30, 60, and 90. Improvements in symptoms were a function of time, with the most marked results occurring at 90 days (Table 7). No adverse events were associated with treatment.

#### Animal and In Vitro Studies

The effect of bilberry anthocyanins on vascular permeability under various conditions and hypothesized mechanisms of action have been studied in animals and in vitro. One

**Table 7** Modification of subjective and objective symptomatology<sup>1</sup> of phlebitis due to stasis in pregnancy following high dosage of anthocyanins<sup>2</sup>

Symptoms in % of subjects	Mean ( $\pm$ SE) basal value (B)	Mean ( $\pm$ SE) at day 30 of treatment (I)	Mean ( $\pm$ SE) at day 60 of treatment (II)	Mean ( $\pm$ SE) at day 90 of treatment (III)	Statistical significance
Edema (44.4%)	1.48 $\pm$ 0.11	1.16 $\pm$ 0.13	0.95 $\pm$ 0.13	0.50 $\pm$ 0.12	B-II = $P < 0.01$ II-III = $P < 0.05$ B-III = $P < 0.001$
Feeling of heaviness (92.5%)	2.18 $\pm$ 0.08	1.23 $\pm$ 0.12	0.75 $\pm$ 0.13	0.52 $\pm$ 0.08	B-II = $P < 0.001$ II-III = $P < 0.05$ B-III = $P < 0.001$
Pain (57.4%)	1.20 $\pm$ 0.07	0.44 $\pm$ 0.13	0.10 $\pm$ 0.07	0	B-I = $P < 0.01$ I-II = $P < 0.01$
Paraesthesia (27.7%)	1.07 $\pm$ 0.07	0.41 $\pm$ 0.15	0.30 $\pm$ 0.15	0	B-I = $P < 0.001$ I-II = NS B-II = $P < 0.001$
Pruritus (85.1%)	1.77 $\pm$ 0.09	1.27 $\pm$ 0.13	0.78 $\pm$ 0.12	0.66 $\pm$ 0.09	B-I = $P < 0.01$ I-II = $P < 0.01$ II-III = NS
Burning sensation (59.2%)	1.50 $\pm$ 0.08	0.54 $\pm$ 0.14	0.20 $\pm$ 0.08	0	B-I = $P < 0.001$ I-II = $P < 0.05$
Cramps (day) (59.25%)	1.34 $\pm$ 0.08	0.38 $\pm$ 0.11	0.25 $\pm$ 0.11	0.11 $\pm$ 0.06	B-I = $P < 0.001$ I-II = NS II-III = NS
Cramps (night) (72.2%)	1.56 $\pm$ 0.09	0.81 $\pm$ 0.11	0.42 $\pm$ 0.10	0.38 $\pm$ 0.10	B-I = $P < 0.01$ I-II = $P < 0.01$ II-III = NS
Capillary fragility (75.9%)	1.93 $\pm$ 0.11	1.73 $\pm$ 0.11	1.43 $\pm$ 0.12	1.45 $\pm$ 0.11	B-I = NS B-II = $P < 0.001$ B-III = $P < 0.001$

<sup>1</sup> Symptoms were evaluated on a score of 0.5-3

<sup>2</sup> 2 capsules of Tegens<sup>®</sup> twice daily for 60-90 days (equivalent to 320 mg anthocyanins daily)

Key: B-I, -II, -III = comparison of means at baseline and day 30, day 60, and day 90, respectively; I-II = comparison of means at day 30 and day 60; II-III = comparison of means at day 60 and day 90; NS = not significant (Student's t-test)

**Source: Modified from Grismondi (1980).**

proposed mechanism of action is the protection of vascular connective tissue against enzymatic activity. Another mechanism may involve the stimulation of mucopolysaccharide synthesis since these compounds help stabilize both perivascular tissue and the basal membrane (see Other Effects).

Vasoprotective and anti-inflammatory effects were reported in two animal models treated with anthocyanins (Lietti and others 1976a). Inflammation always involves some increase in vascular permeability. In rabbits, chloroform, histamine, or bradykinin directly applied to skin (topically or subdermally) resulted in increased capillary permeability that peaked at 30 minutes and persisted for 120 minutes. Animals were pretreated with a 25% anthocyanidin bilberry preparation at doses from 25-100 mg/kg ip and 200-400 mg/kg po 30 minutes prior to irritation. Pretreatment with the bilberry preparation decreased treatment-induced capillary permeability in a dose- and time-dependent fashion.

For example, in the chloroform-treated group, permeability inhibition increased from 28% to 53% at 25 mg/kg ip and from 48% to 71% at 50 mg/kg ip ( $P < 0.05$ ). Oral administration of 400 mg/kg decreased capillary permeability by 66%. Similarly in rats, administration of bilberry anthocyanins (100 mg/kg im and ip and 200 mg/kg po) decreased bradykinin-induced capillary permeability by approximately 48% at the lower parenteral dose, and 39% at the higher oral dose ( $P < 0.01$ ). In the same study, administration of anthocyanins (100 mg/kg ip) increased vascular resistance in rats fed a flavonoid-deficient diet. At a dose of 200 mg/kg iv and po, anthocyanins also reduced carrageenin-induced rat paw edema by 45%. When the inflamed paw was submerged for 60 seconds in a 1% anthocyanin solution (in 70% ethanol), inflammation was reduced by approximately 40% ( $P < 0.01$ ).

An early investigation looked at the effects of bilberry

anthocyanins on the vascular permeability of the aorta, brain, and skin in experimental hypertension in rats (3 groups of 15 each) (Detre and others 1986). Hypertension was induced through the ligation of the abdominal aorta between the two renal arteries and resulted in significantly increased vascular permeability in all 3 tissues. In animals treated with anthocyanins (50 mg/100 g po for 12 days), blood-brain barrier permeability was equal to that of the normotensive controls and aortic vascular permeability was approximately 40% less than that of untreated hypertensive rats. Treatment reduced the 6-fold experimental increase in skin capillary permeability by approximately 20%. In this experiment, the greatest reduction in permeability increase was observed in brain vessels.

In another study, oral administration of Myrtocyan® (10 mg daily for 2-4 weeks) was found to significantly reduce ( $P < 0.01$ ) microcirculatory damage due to ischemic reperfusion in a hamster cheek pouch model. Symptoms measured included the number of leukocytes adhering to vessel walls, capillary permeability, and number of perfused capillaries (Bertuglia and others 1995).

Degradation of collagen and elastin is associated with an increase in vascular permeability as well as other pathologies such as atherosclerosis, emphysema, and rheumatoid arthritis. There is evidence that bilberry anthocyanins stimulate the cross-linking of collagen fibers, helping to protect them from breakdown by the enzyme collagenase. Several early studies investigated the interaction of bilberry anthocyanins and collagen in vascular tissue. In one study using Wistar rats, Robert and others (1977) studied the effects of bilberry anthocyanins on the protease- (collagenase, pronase, and  $\alpha$ -chymotrypsin) and dimethylsulfoxide (DMSO)-induced increase in permeability of the blood brain barrier to the dye trypan blue. In addition to measuring permeability, they measured the level of hydroxyproline in the cerebral spinal fluid (CSF) as a positive indicator of collagen breakdown. Bilberry fruit anthocyanins dissolved in sterile 0.9% NaCl were given at 5 mg/100 g ip daily for 5 days. Treatment decreased the permeability-enhancing effects of all agents, nearly abolishing the effects of DMSO and reducing the effect of collagenase (401 U/mg intravenicularly) by approximately 25%. The level of hydroxyproline in the CSF was also decreased by 28% in the bilberry treatment group, suggesting that bilberry anthocyanins interfered with the enzymatic degradation of collagen.

In diabetics, microangiopathy is characterized by the thickening of the capillary basal laminae and the abnormal accumulation of collagen or structural glycoproteins. In a study using microvessels extracted from normal and diabetic rats, bilberry anthocyanins resulted in a reduction in collagen accumulation. Whereas administration of insulin reduced collagen accumulation by 22%, bilberry anthocyanins (2  $\mu$ g/mL) reduced it by 58% (Robert and others 1979, cited in Boniface and others 1986).

An in vitro study found that anthocyanins extracted from bilberry have been found to inhibit elastase, a proteolytic enzyme that degrades elastin, an important component of connective tissue (Jonadet and others 1983). In the

same study, rats given the anthocyanin mixture (ip) showed a decrease in capillary permeability.

At least 3 studies have focused on the effect of bilberry fruit anthocyanins on lactate dehydrogenase (LDH) activity (Cluzel and others 1969; Marcollet and others 1969; Monboisse and others 1983). Excessive exercise can cause oxygen depletion in muscle tissue with a consequent increase in lactate dehydrogenase (LDH), causing oxidative stress to the vascular tissue which in turn can cause hemorrhaging. In the study of Marcollet and others (1969), rats subjected to forced swimming showed diminished release of lactate dehydrogenase (LDH) from the heart and in plasma when treated with anthocyanin-containing extracts. Anthocyanin doses of 8, 40, and 200 mg/kg completely inhibited the increased LDH release normally induced by the forced swimming test. The untreated rats exhibited hemorrhagic symptoms, while those treated with anthocyanins did not.

### **Vasorelaxant Effects**

#### **Ex Vivo Studies**

A series of studies investigated the hypothesis that bilberry may induce vasorelaxation by stimulating prostaglandin (PG) synthesis in vessels. In a pair of studies, Myrtocyan® blocked the barium- and serotonin-induced contractile response of vascular smooth muscle (calf splenic artery) (Bettini and others 1984a, 1984b). This effect was highly reduced in preparations pretreated with indomethacin (1  $\mu$ g/mL) or lysine acetylsalicylate (1  $\mu$ g/mL), known inhibitors of PG-synthetase. Pretreatment of the vascular tissue by the  $\beta$ -blockers propranolol or nifedipine did not counteract the vasorelaxant effect of the anthocyanins. The researchers interpreted these findings as support for their hypothesis that anthocyanins cause vascular smooth muscle relaxation by stimulating the local synthesis of vasodilating PGs rather than via a  $\beta$ -adrenergic mechanism. Ascorbic acid (1-4  $\mu$ g/mL) potentiated the effect of bilberry fruit extract in both studies. The same researchers suggested that the action of anthocyanins is similar to that of bioflavonoids with regard to their ability to stimulate PG synthesis (Bettini and others 1984c). They also found that the relaxant effect of adrenaline on calf coronary artery preparations was potentiated by Myrtocyan® in a dose-dependent manner. This effect of the extract, however, was abolished in the presence of pyrogallol, a catechol-O-methyl transferase (COMT) inhibitor, a known smooth muscle relaxant. This was interpreted as evidence that bilberry fruit extract has a direct inhibitory effect on COMT (Bettini and others 1985).

This same group extended their work on the vasoactive mechanism of Myrtocyan® using acetylcholine- and methacholine-induced contraction of isolated calf coronary artery tissue (Bettini and others 1991, 1993). In parallel with their earlier work, the extract and ascorbic acid together antagonized the contractile effect, but this effect was blocked by cyclooxygenase inhibitors. The researchers suggested that this relaxant effect was, in part, due to stimulation of prostacyclin, since the relaxant effect of bilberry anthocyanins was inhibited when prostacyclin synthesis was blocked (Bettini

and others 1991). The relaxant effect might also be due to a stimulation of endothelium-derived relaxing factor (EDRF) synthesis, since the relaxation induced by the extract was partially reduced by methylene blue, an inhibitor of EDRF (Bettini and others 1993).

### **Vasomotor Effects**

#### **Animal Studies**

The stimulation of arteriolar vasomotion, defined as the rhythmic changes in the diameter of arterioles, may improve microvascular blood flow and hence decrease the formation of interstitial fluid. The effect of bilberry fruit extract on arteriolar vasomotion was evaluated in a study on anaesthetized hamster cheek pouch ( $n = 20$ ) and unanaesthetized hamster skeletal muscle ( $n = 15$ ) models (Colantuoni and others 1991). Myrtocyan<sup>®</sup> (0.5 and 1 mg/100 g iv) increased the frequency and amplitude of vasomotion in both models ( $P < 0.05-0.01$  in skeletal muscle, depending on vessel size; statistical analysis was not presented for the results on cheek pouch tissue). These results indicate that bilberry anthocyanins may be beneficial in increasing microvascular blood flow and reducing the effects of increased vascular permeability, including edema. This mechanism was reported to be distinctly different from the action of prostaglandins on arteriolar tone.

### **Effects on the Eyes**

Diseases of the retina are the leading causes of blindness throughout the world. The use of bilberry fruit extracts for the treatment and prevention of visual problems has been widely studied. Conditions which bilberry extract has been reported to improve include diabetic and hypertensive retinopathy, mild senile cortical cataract, myopia, deficient night vision, and retinitis pigmentosa. Because oxidative damage to the retina during image processing and ischemic processes has been shown to be a significant part of the pathology of retinopathy, much of the therapeutic effect of bilberry extract is thought to be associated with the antioxidant activity of bilberry anthocyanins (see Antioxidant Effects) and their ability to increase blood supply to the retina. It has been hypothesized that bilberry anthocyanins have a particular affinity for phospholipid membranes such as those that comprise a large portion of the retina, and can help protect them from oxidative damage. Research on bilberry extract's use in slowing macular degeneration is needed given the prevalence of this disease and the lack of current medical interventions that can stop the degenerative process. Bilberry extract may be of benefit in macular degeneration given that it has confirmed in vitro antioxidant activity and that supplementation with antioxidants has been reported to be of potential benefit in this disease (Richer 1996).

#### **Human Clinical Studies**

Several trials have been carried out to determine the effectiveness of bilberry on diabetic or hypertensive retinopathy. In diabetic retinopathy, damage of the retinal arteriolar endothelium occurs. This causes increased vascular permeability and the resultant capillary dilation, microaneurisms,

transudate, and localized edema. Macroglobulins, platelets, and erythrocytes accumulate in the capillaries. This eventually leads to an impairment of normal capillary perfusion and subsequent ischemia. As retinopathy progresses, hard exudations appear at the borders of edematous zones around the microaneurisms. These exudations are formed by cholesterol crystals and histocytes which occur singly or in clusters and contain lipids and glycoproteins. After retinal edema resolves, the exudations can remain unchanged or can take several months or years to regress (Repossi and others 1987).

In a randomized, double-blind, placebo-controlled, partial crossover study, the effects of bilberry fruit extract were investigated in 40 patients with either diabetic ( $n = 35$ ) or hypertensive ( $n = 5$ ) vascular retinopathy (Perossini and others 1987). Patients were equally divided into two equal groups, one group treated with Tegens<sup>®</sup> (160 mg twice daily for 1 month), and the other administered a placebo. At the end of the month, those administered the extract were removed from the study irrespective of results. Retinopathy in all 20 patients who had been administered the placebo remained unchanged, worsened, or only slightly improved. These patients continued in the study for an additional 30 days receiving Tegens<sup>®</sup> at the same dosage in place of the placebo. Ophthalmoscopic examination was conducted prior to admission to the study and at 30 and 60 days afterwards for both groups. Prior to the trial, ophthalmoscopically detectable retinal abnormalities were confirmed in 13 members in the Tegens<sup>®</sup>-treated group. At the end of the trial, 1 of these patients was much improved, 9 were improved, and 3 were unchanged. Prior to the trial, retinal abnormalities were evident in 15 members of the placebo group. None of these patients showed improvement within the first 30 days. After 30 days on Tegens<sup>®</sup>, 12 of these patients were rated as improved and 3 had no change. In total, 79% of the patients with confirmed retinal abnormalities showed improvement following treatment with the extract.

Repossi and others (1987) used fluorangiography to evaluate diabetic patients ( $n = 40$ ) with early stage retinopathy before and after a treatment period of 12 months. Patients were divided into two groups: those receiving a "conventional" (undisclosed) treatment plus Tegens<sup>®</sup> (160 mg po twice daily) and those receiving the conventional therapy plus a placebo. Based on either a slowed progression or improvement in the extent of hard exudates, the researchers reported positive findings in the bilberry extract-treated group (50% of patients with decreased exudates) compared to the placebo group (20% with decreased exudates; no statistical analysis reported). These researchers also reported that the greatest improvement was observed in patients in the earliest stage of diabetes, suggesting that bilberry extract is most appropriate as a preventive agent.

Type II diabetics ( $n = 10$ ) with simple retinopathy were given approximately 480 mg po of bilberry fruit anthocyanins in 3 divided doses daily for 6 months (Orsucci and others 1983). All cases had improved by the end of the trial period. Assessment criteria included reduction of hemor-



rhage and alleviation of weeping exudations from the retina. The small number of patients, the lack of a placebo control, and the ability of this condition to improve spontaneously are limitations of this particular study.

In one early study of diabetic patients ( $n = 12$ ) with various stages of retinopathy, a daily dose of a bilberry anthocyanin and  $\beta$ -carotene mixture (400 mg and 20 mg, respectively) was ineffective in reversing retinal damage due to microaneurysms. However, increased capillary resistance was noted in those capillaries not affected by the aneurysms (Sevin and Cuendet 1966). Boniface and others (1986) reported on the findings of Romand (1974) in his study of diabetic retinopathy. Bilberry anthocyanins (600 mg po daily for 6 months) were administered to diabetic patients. Among 32 patients, the number of capillaries with lesions was reduced from 34% before treatment to 14% after treatment. Another study, not available for review, showed that bilberry anthocyanins promote a tendency towards a reduction in hemorrhagic retinopathy due to anticoagulant therapy (Scharer and Ober 1981).

A randomized, double-blind, placebo-controlled study of 50 outpatients (21 men, 29 women; mean age 67 years) with mild senile cortical cataract investigated the therapeutic efficacy of a bilberry extract-vitamin E preparation (180 mg and 100 mg, respectively, twice daily for 8 months; the extract was standardized to 25% anthocyanidins). No subjective or objective improvement in the condition was noted after 4 and 8 months. However, progression of lens opacity was reported to be halted in 97% of eyes of those in the treatment group ( $P \leq 0.05$  versus placebo) (Bravetti and others 1989).

A group of Japanese researchers (Dyohei and others no year available) performed a small, double-blind, placebo-controlled study to investigate the effects of bilberry fruit juice powder on healthy men experiencing eyestrain during work (subjectively determined). The otherwise healthy subjects were divided into two groups ( $n = 5$  each), one given bilberry fruit juice powder (40 mg yielding 25% anthocyanins, added to water and taken 3 times daily for 7 days), the other a placebo. Various parameters were studied, including sight, refraction degree, retinal reaction times to dark adaptation, glare, and flicker test, visual perception of moving objects, and nearsightedness. The bilberry group showed a reduction of eyestrain compared to the placebo group as measured by increased reaction and focus time of the retina to various stimuli (no statistical analyses were provided).

It has been reported that French and British Royal Air Force aviators in World War II ate bilberry jam to improve their sight on night flights. A number of studies employing subjects with no complaints of visual impairment have investigated the effects of bilberry anthocyanins on vision under conditions of reduced light and during the night. Subjects with healthy vision might not be the best patient population to use when studying the effects of bilberry extract on night visual acuity, since therapeutic effects may be confined to subjects with impaired night vision. Most of the early studies reviewed reported positive effects (Jayle and

Aubert 1964; Jayle and others 1965; Vannini and others 1986), as did one of the more recent studies (Forte and others 1996). In one study, bilberry anthocyanins (preparation uncharacterized) were orally administered to 37 healthy subjects (1 group of 18 subjects for short-term treatment; 19 subjects for long-term treatment) (Jayle and Aubert 1964). Ten additional subjects per short- and long-term treatment groups were administered a placebo control. The findings indicated a positive effect on short-term improvement of vision in reduced light and in night vision. Improvement was only reached within the first 15 minutes of administration. This parallels the pharmacokinetics in which peak plasma levels were observed, upon oral administration, within 15 minutes, with rapid metabolism. In another placebo-controlled study, the same research group reported statistically significant improvement in night vision in healthy subjects ( $n = 30$ ) treated with bilberry compared to placebo (Jayle and others 1965). The study of Vannini and others (1986) reported statistically significant ( $P < 0.05$ ) improvements in the pupillary dynamics of 27 out of 40 human subjects after administration of 240 mg of anthocyanins compared to a placebo group. The greatest improvement was observed 2 hours after administration.

In contrast to the positive results reported in the above studies, three recent, double-blind, placebo-controlled, crossover trials failed to observe any significant differences between the use of low or high dose bilberry anthocyanins and placebo for improving night vision in healthy adults (Levy and Glovinsky 1998; Muth and others 2000; Zadok and others 1999). In the study of Muth and others (2000), male subjects with healthy vision were administered 160 mg of bilberry extract (25% anthocyanins) ( $n = 7$ ) or a placebo capsule ( $n = 8$ ), each 3 times daily for 3 weeks. After treatment, there was a 4-week washout period, after which a second 21-day treatment period was initiated with a cross-over design. The study ended after a 4-week post-treatment period. Night visual acuity and night contrast sensitivity were tested four times throughout each treatment period and during the washout and post-treatment periods. No significant differences were seen in either night visual acuity or night contrast sensitivity between the bilberry and placebo groups. The results from this study should be interpreted with caution, since small sample sizes may have resulted in low statistical power.

Levy and Glovinsky (1998) investigated the effectiveness of a single, relatively low, oral dose of bilberry anthocyanins in enhancing night vision in 16 healthy males. Subjects were equally divided into four treatment groups receiving single oral doses of 12, 24, or 36 mg of anthocyanins or a placebo. After treatment, a 2-week washout period ensued, followed by another treatment at a different dosage in a cross-over design, until each group had experienced all four treatments. The anthocyanins were administered in the form of Strix<sup>®</sup> tablets (Halsoprodukter, Forserum, Sweden), which contain 12 mg anthocyanins each plus 2 mg  $\beta$ -carotene. Full-field scotopic retinal threshold, dark adaptation rate, and mesopic contrast sensitivity were measured at 0, 4, 8, and 24 hours after administration.

No significant differences were seen in any of the outcome measures between the bilberry and placebo groups. The study reported a statistical power of 0.95 to detect a 0.1 log unit improvement in scotopic retinal threshold and a 0.5 log unit improvement in mesopic contrast sensitivity.

The same research group also studied the effectiveness of multiple, relatively low, oral doses of anthocyanins on night vision (Zadok and others 1999). Eighteen healthy males were equally divided into three groups receiving 12 or 24 mg anthocyanins or a placebo, each given twice daily for 4 days. The cross-over design of the study, preparation administered, and outcome measures were identical to those used by Levy and Glovinsky (1998). Outcome measures were evaluated one day prior to treatment and at days 1, 2, 3, and 4 during the treatment period. No significant differences were seen in any of the outcome measures between the bilberry and placebo groups. This study design gave the same statistical power as reported for the previous study.

One small study investigated the ability of bilberry extract to reduce light sensitivity of the retina (Zavarise 1968). Fourteen patients were given 150 mg anthocyanins daily in 3 divided doses over a 3 month period. Light sensitivity was measured at day 0 and day 2 following the beginning of treatment, and again every 15 days up to 3 months. Light sensitivity was significantly improved by day 2 of the treatment compared to baseline ( $P < 0.001$ ). The effect remained constant without further improvement over the 3-month treatment period, declining gradually to baseline again after the treatment was terminated. None of the treated patients experienced side effects from the medication.

A bilberry fruit extract (160 mg anthocyanins) was studied for its use in the treatment of myopia. Improvement of scotopic function in all patients was reported ( $n = 26$ ). However, statistical significance was only reached in subjects with slight myopia ( $\leq 6$  diopters) (b2 wave,  $P < 0.01$ ). In subjects with slight to medium myopia, photopic function was significantly improved (critical central fusion frequency,  $P < 0.005$ ; b1 wave,  $P < 0.01$ ) (Contestabile and others 1991).

### In Vitro Studies

Early in vitro work elucidating potential mechanisms of action associated with the effects of bilberry extract on vision found that anthocyanins affect various retinal enzymes. Specifically, they inhibit the activity of phosphoglucosaminidase and 5-nucleotidase and increase the activity of lactate dehydrogenase,  $\alpha$ -hydroxybutyrate dehydrogenase, 6-phosphogluconate dehydrogenase, and  $\alpha$ -glycerophosphate dehydrogenase (Cluzel and others 1969; Cluzel and others 1970). Other researchers have reported that anthocyanins have an affinity for membrane phospholipids, such as those making up a significant portion of the retina, and have hypothesized that they might stabilize the phospholipid membrane with a strengthening effect on the endothelium barrier (Orsucci and others 1983). Based on histological studies on rats, part of bilberry's reported effect in improving vision may be due to an increase in rhodopsin in the outer segments of retinal rods (Contestabile and others 1991).

## Antioxidant Effects

### Animal and In Vitro Studies

Bilberry is commonly used for its antioxidant activity which is pertinent to its therapeutic value in treating various vascular and ophthalmological disorders. One group of researchers investigated the potential of an anthocyanin extract from bilberry fruit (not characterized, purchased from Sigma Tau) to inhibit lipid peroxidation and scavenge superoxide anion in rat liver microsomes (Martin-Aragon and others 1998). The extract inhibited both lipid peroxidation and hydroxyl radical formation ( $IC_{50} = 50.28 \mu\text{g/mL}$  for both;  $P < 0.01$ ). It also showed good activity as a superoxide scavenger ( $25 \mu\text{g/mL}$ ;  $P < 0.01$ ). The scavenging effect of the extract ( $50\text{-}100 \mu\text{g/mL}$ ;  $P < 0.01$ ) was reportedly comparable to that of superoxide dismutase. Myrtocyan<sup>®</sup> has also been found to protect apolipoprotein B from UV-induced oxidative fragmentation (Rasetti and others 1996/97). These researchers suggested bilberry extract may be valuable in the prevention of atherosclerosis but further noted the difficulty of extrapolating in vitro data to human use. Additional studies have reported on the ability of bilberry anthocyanins ( $15\text{-}20 \mu\text{g/mL}$ ) to potentially inhibit lipid peroxidation (Laplaud and others 1997; Meunier and others 1989). It should be noted that the concentrations required for the above effect are much higher than can be achieved from normal human therapeutic doses.

A number of studies were conducted to determine the potential health benefits of antioxidant-rich foods including bilberry and blueberry (Cao and others 1999; Joseph and others 1999; Prior and others 1998). Prior and others (1998) investigated the antioxidant activity of various *Vaccinium* species including *V. angustifolium*, *V. ashei*, *V. corymbosum*, and *V. myrtillus*. Free radical quenching activity was determined using an automated oxygen radical absorbance capacity assay (ORAC\*) developed by Cao and others (1993). The average total antioxidant capacity across all species was  $24 \pm 2.2 \mu\text{mol TE/g}$  of fresh berries. Bilberry and low bush blueberries (*V. angustifolium* from Nova Scotia) exhibited the most potent antioxidant activity ( $44.6 \pm 2.3$  and  $45.9 \pm 2.2$ , respectively). According to these researchers, blueberries elicited the highest antioxidant activity of all fresh fruits and vegetables tested to date showing antioxidant activity that was 2-6 times greater than the activity of ascorbic acid and glutathione. Anthocyanins and phenolics dose-dependently increased antioxidant activity, with a stronger relationship found between ORAC and phenolic concentration ( $r_{xy} = 0.85$ ) than between ORAC and anthocyanin concentration ( $r_{xy} = 0.77$ ). The researchers estimated that the consumption of 1/2 cup of blueberries per day (72.5 g) would increase antioxidant capacity by 1-3.2 mmol. The primary limitation of this study was that multiple samples of other *Vaccinium* species were assayed while only one sample of bilberry was investigated. In a different assay, the iso-

\* Using 2,2'-azobis(2-amidinopropane) dihydrochloride as a peroxy radical source, the ORAC procedure is a tool for the biological assessment of antioxidant activity. It has been proposed as a means of assessing the antioxidant effects of various foods and dietary supplements (Prior and Cao 2000).

lated bilberry anthocyanin cyanidin-3-glucoside exhibited antioxidant activity that was 3.5 times more potent than that of Trolox (vitamin E analogue) (Wang and others 1997).

Joseph and others (1999) reported that blueberry (species not indicated) incorporated into the diet of rats (18.6 g/kg dried aqueous extract) resulted in increases in various functional neuronal indices which typically decline in magnitude with age due to oxidative stress: carbachol-stimulated GTPase activity, oxotremorine-enhanced striatal dopamine release (ox-K<sup>+</sup>-ERDA), and <sup>45</sup>Ca<sup>2+</sup>-recovery. These protective effects may or may not be due to antioxidant activity. Of the 3 antioxidant-rich foods tested (spinach, strawberry, and blueberry), only the blueberry-supplemented animals showed reversals in motor behavioral deficits (Joseph and others 1999). In one additional study, only a blueberry extract was effective in alleviating hyperoxia-induced redistribution of antioxidants between tissues (Cao and others 1999).

## Platelet Aggregation Effects

### Animal and In Vitro Studies

The ability of bilberry fruit anthocyanins to inhibit smooth muscle contraction induced by angiotensin II, serotonin, and histamine led researchers to study the direct effect of Myrtocyan<sup>®</sup> on platelet aggregation since aggregation represents a contractile response. Results from the studies reported suggest that bilberry anthocyanins act as platelet aggregation inhibitors. The exact mechanism of action is still unknown, but as has been suggested for bilberry extract's vasorelaxant activity, this effect is similar to that of bioflavonoids and may be due, at least in part, to the stimulation of PG synthesis.

Myrtocyan<sup>®</sup> (480 mg) or ascorbic acid (3 g) were administered daily po for 30 or 60 days, both alone and in combination (Pulliero and others 1989). Blood samples were then drawn and used for platelet aggregation studies. The combination of Myrtocyan<sup>®</sup> and ascorbic acid dose-dependently reduced platelet aggregation induced by either collagen or ADP; the combination was more effective than either product singly. Platelet aggregation values returned to baseline in the treated groups 120 days after discontinuation of treatment (IC<sub>50</sub> = 0.36-0.81 mg/mL). No statistical evaluation was made. The researchers postulated an effect of bilberry extract on enzyme systems governing degradation of cyclic adenosine monophosphate (cAMP) and thromboxane synthesis, suggesting vasoprotective, antithrombotic, and antiatherogenic effects.

Morazzoni and Magistretti (1990) confirmed these effects both in vitro and in vivo with rats and rabbits, observing prolonged template bleeding time and inhibition of ADP-, collagen- and sodium arachidonate-induced aggregation at doses of Myrtocyan<sup>®</sup> from 5 to 400 mg/kg. No effect was seen on hematocrit or on intrinsic, extrinsic, or common pathways of blood coagulation. The three main anthocyanins (cyanidin 3-O-glucoside, delphinidin 3-O-glucoside, and malvidin 3-O-glucoside) were also studied individually with similar results, confirming that antho-

cyanins are in large part responsible for the platelet aggregation-inhibiting effect observed

In vitro studies by Bottechia and others (1987) found a 50% inhibition of clot retraction using concentrations of 75 µg/mL of bilberry fruit anthocyanins. In addition, platelet aggregation induced by ADP, collagen, and arachidonic acid was inhibited in a dose-dependent manner. The researchers found their results consistent with the hypothesis that anthocyanins stimulate the release of prostacyclin (PGI<sub>2</sub>), which has the effect of increasing the concentration of intracellular cAMP or decreasing the concentration of thromboxane A<sub>2</sub> in platelets.

## Other Effects

### Effects on the Female Reproductive System

A randomized, double-blind, placebo-controlled trial investigated the effectiveness of bilberry extract in the treatment of symptoms associated with primary dysmenorrhea (Colombo and Vescovini 1985). Women with primary dysmenorrhea received either a 25% anthocyanin extract (310 mg daily in 2 doses) or placebo for 5 days beginning 3 days before the onset of menses for 2 consecutive menstrual cycles. The intensity of symptoms was evaluated by the patients at baseline and on the first day of menses. The bilberry extract was shown to significantly reduce dysmenorrhea compared to placebo (Table 8). According to another study that was not available for review, minor side effects associated with the use of copper intrauterine devices were reportedly reduced in 48 women (Cerutti and others 1984).

### Anti-ulcer and Wound-Healing Effects

Myrtocyan<sup>®</sup> was found to have an ulcer-preventative and curative effect in rat models involving pyloric ligation, reserpine-, phenylbutazone-, restraint-, and acetic acid-induced ulcers (Cristoni and Magistretti 1987) (Table 9). The prepa-

**Table 8** The effect of bilberry fruit extract in the treatment of primary dysmenorrhea

Symptom	Control	Bilberry extract <sup>1</sup>	Placebo
Pelvic and lumbosacral pain	Basal	2.8 ± 0.1	2.9 ± 0.1
	Month 1	1.3 ± 0.1**	2.0 ± 0.2
	Month 2	0.9 ± 0.1**	2.3 ± 0.2
Mammary tension	Basal	1.6 ± 0.2	2.0 ± 0.2
	Month 1	0.8 ± 0.2**	1.6 ± 0.2
	Month 2	0.5 ± 0.2**	1.5 ± 0.2
Headache	Basal	2.3 ± 0.2	1.9 ± 0.2
	Month 1	1.1 ± 0.2**	1.6 ± 0.2
	Month 2	0.6 ± 0.1**	1.1 ± 0.2*
Sickness and emesis	Basal	2.0 ± 0.2	1.7 ± 0.2
	Month 1	1.7 ± 0.2	1.4 ± 0.2
	Month 2	0.8 ± 0.2**	1.5 ± 0.2
Heaviness of lower limbs	Basal	1.8 ± 0.2	2.1 ± 0.2
	Month 1	0.5 ± 0.1**	1.6 ± 0.2
	Month 2	0.2 ± 0.1**	1.6 ± 0.2

<sup>1</sup> Myrtocyan<sup>®</sup>, \* P < 0.05, \*\* P < 0.01 compared to baseline

**Source:** Original data from Colombo and Vescovini (1985); table from Morazzoni and Bombardelli (1996)

**Table 9** Activity of orally administered bilberry fruit anthocyanins<sup>1</sup> on pyloric ligature ulcer in rats

Treatment <sup>2</sup>	Dose (mg/kg)	No. of animals	Mean index of ulceration (± SE)	% Inhibition	No. of stomachs not ulcerated
Controls	—	10	49.00 ± 15.35	—	0
Bilberry	10	12	40.50 ± 11.49	17	1
Controls	—	11	35.36 ± 9.10	—	2
Bilberry	25	13	18.85 ± 4.77	47	0
	50	13	16.54 ± 3.71	53	1
Controls	—	13	47.46 ± 10.37	—	1
Bilberry	100	13	12.46 ± 3.89**	74	3
Controls	—	14	44.50 ± 6.83	—	1
Carbenoxolone	50	12	28.16 ± 7.02*	37	1
Carbenoxolone	100	12	28.08 ± 5.93*	37	0
Controls	—	14	43.78 ± 6.99	—	1
Cimetidine	50	13	30.15 ± 7.17	31	2
Cimetidine	100	16	31.43 ± 7.14	28	1

<sup>1</sup> Myrtocyan®<sup>2</sup> Treatment administered 50, 30, 25, and 6 hours before and immediately after pyloric ligature\*  $P < 0.05$ , \*\*  $P < 0.01$ ; (Mann-Whitney U-test)**Source: Modified from Cristoni and Magistretti (1987).**

ration both inhibited ulcer formation in pretreated animals and reduced the severity or number of already existing lesions. The anti-ulcer activity of the bilberry preparation was dose dependent and equal to or greater than that of cimetidine at high doses. According to the researchers, the activity was due to an increase in mucus production by the stomach wall rather than to inhibition of gastric secretion. Other studies have reported gastric protective effects of the bilberry anthocyanidin IdB 1027, confirming the antiulcer activity of bilberry fruit extract (Magistretti and others 1988; Mertz-Nielsen and others 1990). IdB 1027 was shown to elicit a significant rise ( $P < 0.05$ ) in luminal concentration and luminal release of PGE<sub>2</sub> ( $P < 0.02$ ) in humans (Mertz-Nielsen and others 1990).

Bilberry extract (0.5-2%) topically applied to rats for 3 days has been shown to promote the healing of skin wounds treated with prednisone to delay healing (Cristoni and Magistretti 1987). In another experiment, Myrtocyan® topically applied (5-10 mg aqueous solution) to rats similarly accelerated the natural healing process (Curri and others 1976). The authors of this study hypothesize that the healing activity is mediated by the stimulation of mucopolysaccharide synthesis. Because mucopolysaccharides are important for maintaining the integrity of the perivascular tissue and the basal membrane, this proposed mechanism of action might also help explain the vasoprotective activity of bilberry anthocyanins.

#### Effects on Hemorrhoids

Bilberry extract has also been investigated for its use in the treatment of hemorrhoids and the post-operative symptoms following hemorrhoidectomy. In a study of 51 pregnant

women, statistically significant improvement was noted in pain, itching, and burning associated with hemorrhoids after one month of treatment (Teglio and others 1987, cited in Morazzoni and Bombardelli 1996). Pezzangora and others (1984) studied 60 patients who were divided into 2 groups, one receiving standard therapies (anti-inflammatories, hip baths) and the other standard therapies plus treatment with bilberry anthocyanins (160 mg twice daily) for a period of 20 days after surgery. A significant reduction in edema ( $P < 0.05$ ) and the frequency and intensity of pruritus ( $P < 0.05$  and  $P < 0.02$ , respectively) was observed in the group treated with anthocyanins in conjunction with standard therapies. In another study, patients were similarly divided into two treatment groups (n = 40 each), one administered only standard therapies and the other administered standard therapy plus Tegens® (160 mg 3 times daily) for a period of 10 days following surgery (Oliva and others 1990). The same symptoms were assessed as in the previous study. In subjects treated with only standard therapies, pruritus, anal tension, inflammation, and edema were present in 40%, 52.5%, 50%, and 57.5% of patients, respectively, compared to 28%, 17.5%, 30%, and 25%, respectively, in subjects treated with both standard therapies and bilberry extract.

#### Anti-hemorrhagic Effects

Preoperative treatment with Myrtocyan® was evaluated for its effect on hemorrhagic complications after ear, nose, and throat surgery. A total of 181 patients, including 25 children, were allotted to placebo and treatment groups in a single-blind study. Daily doses of 160-320 mg for 10 days before the operation reduced the incidence and severity of intra- and post-operative bleeding ( $P < 0.01$  in both cases) and subse-

quent hemorrhagic complications, with no adverse effects noted. This study has not been published (Gentile 1987, cited in Morazzoni and Bombardelli 1996).

### **Anti-cancer Effects**

Bomser and others (1996) reported potential anti-cancer effects of the ethyl acetate and hexane/chloroform fractions of bilberry fruit extract due to induction of quinone reductase (QR), a phase II xenobiotic detoxification enzyme. However, neither the crude extract nor the fractions inhibited ornithine decarboxylase, an enzyme associated with cancer progression. A subsequent study demonstrated the effectiveness of a hexane/chloroform extract of bilberry fruit,  $\beta$ -sitosterol, and the carotenoids lutein and zeaxanthin in inhibiting the growth of two human breast cancer cell lines (Madhavi and others 1998).

Possible anti-cancer activity was also proposed by another group of researchers who found that bilberry fruits contain a specific glycoprotein oligosaccharide (Lewis<sup>a</sup> type determinant). These types of molecules have been shown to be effective inhibitors of cellular interactions that may be helpful in inhibiting metastasis (Melo and others 1997). What effect, if any, this has in humans is unknown.

### **Effects on Hormones**

Another research group studied the effects of Myrtocyan<sup>®</sup> on thyroid hormone availability, cerebral functional activity, and blood brain barrier transport in rats. Increased transport of [<sup>125</sup>I]L-triiodothyronine into various rat brain regions was observed after 5 days of 200 mg/kg ip administration of bilberry fruit anthocyanins. Inhibition of the conversion of L-thyroxine (T4) to 3,3',5'-triiodothyronine (T3), or binding of T3 to its specific binding protein were posited as possible explanations for the activity observed (Saija and others 1990a, 1990b).

### **Conclusion**

Bilberry fruit extract and its anthocyanins have been the subject of many pharmacological studies. The overwhelming majority of these have been conducted using either Myrtocyan<sup>®</sup> or Tegens<sup>®</sup>, each standardized to 25% anthocyanidins. Many of the early studies were observational only and were published in clinical reports rather than peer-reviewed journals. Nonetheless, considerable evidence exists overall and from more recent well-designed studies of the clinical efficacy of bilberry fruit extract for many of its reported uses. Based on a review of the available literature, bilberry fruit anthocyanins are useful in treating circulatory problems due to vascular insufficiency. They act to decrease vascular permeability, possibly by protecting the connective tissue of the vascular wall, particularly collagen and elastin. Other vascular effects include vasorelaxation which may help decrease vascular permeability due to hypertension, and the stimulation of arteriolar vasomotion which may increase microvascular circulation. In vitro work has shown that bilberry anthocyanins also act as platelet aggregation inhibitors.

Bilberry anthocyanins are also effective in treating diabetic and hypertensive retinopathy, presumably due to their

strong antioxidant activity and their ability to improve circulation. Although many studies have investigated the use of bilberry extract to improve night vision, results have been conflicting, with recent double-blind, placebo-controlled studies finding no significant effects. One double-blind placebo-controlled study identified a promising new use of bilberry extract in the treatment of primary dysmenorrhea, though this is not a primary use among modern herbal practitioners. Other studies reported that bilberry extract, both alone and in conjunction with standard therapies, effected a marked reduction in symptoms associated with hemorrhoids and hemorrhoidectomy. Other possible effects that require additional study include the prevention and treatment of gastric ulcers, the reduction of post-operative inflammation and hemorrhage, and possible anti-cancer activity.

### **Actions**

Based on clinical evidence, bilberry fruit extract decreases vascular permeability and increases capillary resistance. Based on preclinical evidence, bilberry extract decreases vascular permeability, inhibits elastase and collagenase, acts as a vasorelaxant and an antioxidant, and inhibits platelet aggregation.

### **Medical Indications Supported by Clinical Trials**

Bilberry fruit extract, equivalent in dosage and characterization to those shown to be effective in clinical studies, can be used to treat vascular insufficiency and its associated symptoms, including edema, varicosities, pain, paraesthesias, and cramping. It can decrease capillary fragility and the associated propensity to bruising. It can also be used to relieve the pain, itching, and burning associated with hemorrhoids and hemorrhoidectomy. When administered prior to ear, nose, and throat surgeries, it can reduce the incidence and severity of post-operative hemorrhagic complications. The extract can be used to slow the progression of various disorders of the eye, including the early stage of diabetic and hypertensive retinopathy. Though not a common use, bilberry extract can be used for symptomatic relief of dysmenorrhea.

### **Medical Indications Supported by Traditional or Modern Experience**

Modern herbal practitioners use bilberry preparations for many of the same indications studied in clinical trials, especially for the prophylaxis and treatment of a variety of ophthalmological and vascular conditions. In addition, the German Commission E supports the use of crude bilberry preparations in the treatment of non-specific diarrhea with the recommendation that medical assistance be sought if diarrhea persists for 3-4 days. The Commission further states that crude bilberry preparations can be used for inflammations of the mucosa of the mouth and throat (Blumenthal and others 1998). Modern herbalists also use bilberry as a general antioxidant-rich tonic.

## Substantiation for Structure and Function Claims

Based on a review of the preclinical literature, bilberry preparations may help in the stabilization of vascular connective tissue by protecting collagen and elastin (Jonadet and others 1983; Roberts and others 1977, 1979). Bilberry anthocyanins have an affinity for membrane phospholipids, such as those that make up a significant portion of the retina (Orsucci and others 1983). Bilberry anthocyanins affect the activity of retinal enzymes, specifically decreasing levels of phosphoglucosmutase and 5-nucleotidase and increasing levels of lactate dehydrogenase,  $\alpha$ -hydroxybutyrate dehydrogenase, 6-phosphogluconate dehydrogenase, and  $\alpha$ -glycerophosphatedehydrogenase (Cluzel and others 1970). Bilberry possesses marked antioxidant activity in vitro (Cao and others 1999; Joseph and others 1999; Prior and Cao 1999; Prior and others 1998).

### Dosages

- Dried berry\*: 20-60 g daily (Blumenthal and others 1998).  
Decoction\*: 1 cup several times daily (Meyer-Buchtela 1999; Wichtl 1994).  
Powdered extract\*\*: 160 to 480 mg daily in divided doses.

\* The dosages provided for the dried berry and decoction correspond to crude bilberry's use as an astringent in conditions such as diarrhea.

\*\* Powdered extract yielding 25% anthocyanidins.

## SAFETY PROFILE

### Classification of the American Herbal Products Association

The *Botanical Safety Handbook* of the American Herbal Products Association (AHPA) assigns bilberry fruit the following classification (McGuffin and others 1997):

Class 1: Herbs that can be safely consumed when used appropriately.

### Side Effects

Postmarketing surveillance data from 2295 patients taking the commercial bilberry extract Tegens<sup>®</sup> found a total of 94 adverse events in 86 patients (4%). These included gastric pain (1.1%), nausea (0.5%), and pyrosis (0.6%). The other adverse events occurred at very low frequency (< 0.25%). Physician evaluation of efficacy was rated good or very good in 60% of cases (Eandi 1987, cited in Morazzoni and Bombardelli 1996).

### Contraindications

None cited in the literature.

### Interactions

Since anthocyanins and tannins are common components of the diet, there should be minimal concern about interactions of bilberry fruit and fruit extract with conventional medications. However, plants that are rich in tannins can

negatively affect the absorption of some medications. Therefore, general cautions regarding consumption of tannin-rich substances and specific medications should be observed. Ascorbic acid has been reported to potentiate the in vitro vascular smooth muscle-relaxing effects of bilberry anthocyanins (Bettini and others 1984a, 1984b) and the combination of ascorbic acid and anthocyanins have been reported to interfere with mechanisms associated with platelet aggregation in vitro (Puillero and others 1989). One study, not available for review, reported that bilberry anthocyanins had a tendency to reduce hemorrhagic retinopathy due to anticoagulant therapy (Scharrer and Ober 1981). Bilberry extract has also been shown to reduce the incidence and severity of post-operative hemorrhagic complications (Gentile 1987, cited in Morazzoni and Bombardelli 1996).

### Pregnancy, Mutagenicity, and Reproductive Toxicity

One study of 54 pregnant women treated for vascular insufficiency with Tegens<sup>®</sup> (320 mg anthocyanins daily for 60-90 days beginning in the 6<sup>th</sup> month of pregnancy) found no adverse events associated with treatment (Grisondi 1980). Morazzoni and Bombardelli (1996) reviewed another study of 51 pregnant women that investigated the use of Myrtocyan<sup>®</sup> (160-320 mg daily for 90 days) for the treatment of vascular insufficiency and hemorrhoids (Teglio and others 1987, cited in Morazzoni and Bombardelli 1996). This review made no reference to adverse events associated with treatment. De Smet and others (1993) reported that no teratogenic activity was observed at 3-5 times the effective dosage. No mutagenic action was exhibited by the anthocyanin extract in *Saccharomyces cerevisiae*, *Salmonella typhimurium*, and *Schizosaccharomyces pombe* test systems with or without metabolic activation. Urine and feces of treated rats were not mutagenic in *S. typhimurium* with or without activation. Oral doses up to 5 g/kg in rats did not increase the number of micronuclei in bone marrow cells (Eandi 1987, cited in Morazzoni and Bombardelli 1996).

### Lactation

Data regarding the effects of bilberry use in lactation are lacking. Based on a review of the available literature, the experience of modern practitioners, and the high level of safety of bilberry when consumed as a food, no adverse effects are to be expected.

### Carcinogenicity

Anti-cancer activity has been reported for bilberry (Bomser and others 1996; Madhavi and others 1998).

### Influence on Driving

Based on the widespread consumption of bilberry and other *Vaccinium* species, no adverse effects are to be expected.

### Precautions

None cited in the literature.

## Overdose

No data are available.

## Treatment of Overdose

No data are available.

## Toxicology

There are no reports of toxicity of the extract in the available literature. Postmarketing surveillance of specific anthocyanin-rich bilberry preparations has similarly revealed no toxicity (Eandi 1987, cited in Morazzoni and Bombardelli 1996). The common occurrence of bilberry fruit's active constituents in the diet also support a high level of safety. Ongoing postmarketing surveillance should strengthen this position. The following data regarding the toxicology of bilberry fruit preparations were available.

### Acute

Myrtocyan<sup>®</sup> has been tested in mice and rats by various routes of administration. The LD<sub>50</sub> values for rat were 2.4 g/kg ip, 0.24 g/kg iv, and no deaths up to 20 g/kg po. For mice, the corresponding values were 4.1 g/kg ip, 0.84 g/kg iv, and no deaths up to 25 g/kg po (Pourrat and others 1967). Indena-sponsored studies found LD<sub>50</sub> values of over 2 g/kg po in mice and rats and 3 g/kg po in dogs without symptoms except darkening of urine and feces. These doses are far above those expected in human use (typically 5-10 mg/kg), hence the extract can be considered non-toxic (Eandi 1987, cited in Morazzoni and Bombardelli 1996).

### Subacute

Treatment of guinea pigs for 2 weeks and rats for 6 weeks with bilberry fruit extract at doses up to 43 mg/kg daily (route of administration not noted) did not produce toxic effects (Pourrat and others 1967). Subacute toxicity studies on rats (up to 36 mg/kg daily iv for 4 weeks) and dogs (12 mg/kg daily iv for 13 weeks) showed no abnormalities apart from dark blue pigmentation of the urine, skin, eyes, and, in some cases, liver, kidneys, and ovaries (Eandi 1987, cited in Morazzoni and Bombardelli 1996).

### Chronic

The chronic oral toxicity of Myrtocyan<sup>®</sup> was investigated by the manufacturer in dogs and rats. Oral administration of 125-500 mg/kg to rats and 80-320 mg/kg to dogs daily for 6 months reportedly brought about no changes in urinary, hematological, or biochemical parameters. Gross and microscopic necropsy findings were similarly unremarkable (Eandi 1987, cited in Morazzoni and Bombardelli 1996).

## INTERNATIONAL STATUS

### United States

Regulated as a dietary supplement.

### Austria

Official in *Österreichisches Arzneibuch* (1990).

### Council of Europe

Official in *PharmEuropa* (1998). The dried fruit of bilberry must not contain less than 0.2% anthocyanins expressed as cyanidin-3-glucoside (kuromanin) (*PharmEuropa* 1998).

### Germany

Official in *Deutscher Arzneimittel-Codex* 1998; *Standardzulassung* 1996; and the *German Commission E Monographs* (Blumenthal and others 1998). **Indications:** Approved for the treatment of nonspecific acute diarrhea and locally for mild inflammation of the mucous membranes of the mouth and throat (Blumenthal and others 1998).

### Switzerland

Official in *Pharmacopoea Helvetica* VII (1987). Bilberry extract is registered with the Interkantonale Kontrollstelle für Heilmittel (IKS) as a List B capillary-protective medication with sale limited to pharmacies by prescription only (Morant and Ruppanner 2001). A bilberry tincture is a List D anti-diarrhea medication with sale limited to pharmacies and drugstores, without prescription (Ruppanner and Schaefer 2000).

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Bilberry (*Vaccinium myrtillus*)  
Chaumeton, *Flore Medicale* (1814)

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