Vaccinium myrtillus L. is a small deciduous shrub growing on hilly heaths and underbrush throughout Central and Northern Europe. The origin of the name Vaccinium is uncertain, and it could derive from the Latin words *vacca* = cow, or *bacca* = berry. The name myrtillus derives instead from its similarity with the fruits and leaves of myrtle. Among the 450 species belonging to genus Vaccinium, the traditional medicinal use of Vaccinium myrtillus is documented since the Middle Ages, when its fruits were recommended to promote menstruation, and from the 16th century the plant has been systematically mentioned in all major herbal treatises. Several compounds belonging to different chemical classes have been isolated from the berries of Vaccinium myrtillus, particularly anthocyanins (also called anthocyanosides) have been extensively documented in the scientific literature. Anthocyanins are found naturally in a number of foods. They can be found in red wine, certain varieties of cereals, and certain leafy and root vegetables (aubergines, cabbage, beans, onions, radishes), but are most abundant in colored fruits like bilberries. Despite the difficulties in calculating the exact daily exposure, several attempts to estimate anthocyanidins intake in European, US, Australian and Japanese populations have been done. Total anthocyanidin mean intake ranged from 19.83 to 64.88 (± 1.86) mg/day in the most recent European study, while much lower values have been instead reported in the US, Australian and Japanese population, where anthocyanidin intakes of 3.1 mg/day in the USA, 2.9 mg/day in Australia and 11.3 mg/day in Japan have been calculated. In the early Seventies, Indena developed a standardized bilberry extract whose efficacy has been extensively clinically evaluated in vascular health, with over 50 positive studies, including at least 25 controlled or double-blind studies. The original titre “Bilberry 25%” referring to the anthocyanidin content by UV analysis, became a globally recognized reference for bilberry extracts. Over the past decade, the specification “Bilberry 25%” has been applied to products that do not conform to the quality of Indena’s bilberry extract. For this very reason, Indena has developed a specific and indisputable analytical method for the identification and quantification of bilberry anthocyanins and anthocyanidins. The method, originally used for the release of Indena’s first registered product in Italy, has been ultimately released to the market. This HPLC-based method defines the anthocyanins content of the standardized bilberry extract as 36% anthocyanins (the glycosilated compounds) and this titer corresponds to the “old” 25% specification by UV analysis, that was based on anthocyanidins (the aglycones). This method, more suitable to quantify accurately the anthocyanosidic content and profile of bilberry, has now become a standard reference for most Pharmacopoeias of the Western countries.
Studies show that bilberry extract, rich in anthocyanins, has positive influences on a variety of health conditions. Probably most known activity is related to a traditional story that during World War II the ingestion of bilberry jam (made from *Vaccinium myrtillus* berries) improved the sight of Royal Air Force pilots on night flights. Although the veracity of this story has been questioned, the effect of anthocyanins in improving vision has been validated by instrumental techniques, like computerized perimetry, suggesting that a standardized bilberry extract containing 36% anthocyanins (Mirtoselect®) can play an important role in improving retinal sensitivity. Other activities, somehow explaining the activity at the eye level, have been deeply studied. Primarily many pharmacological trials have confirmed the efficacy of 36% anthocyanin bilberry extract on microvascular health as well as in phlebology and its efficacy in the treatment of symptoms associated with venous health has been demonstrated in randomized, double-blind, placebo-controlled clinical trials carried out on hundreds of patients. Furthermore anthocyanins of bilberry are emerging as an alternative dietary strategy to support healthy blood sugar levels and its complications associated to inflammation and oxidative stress.

**Clinical studies on Mirtoselect® in ophthalmology**

**Mirtoselect® and eyesight fatigue**

Mirtoselect® has been shown to help support healthy visual function due to eye fatigue. In a crossover, double-blind, placebo-controlled study on 20 patients, four weeks of daily treatment with 250 mg of Mirtoselect®, showed an improvement in subjective symptoms like the occurrence of vision sparks (80% of subjects), eyesight dimming (73% of subjects) and ocular fatigue (70% of subjects) in computer operators. Other improved symptoms were lacrimation and heavy headedness. Flicker values improved significantly following the administration of bilberry anthocyanins, and an overall improvement of eye fatigue could be established.

**Mirtoselect® and healthy eyesight**

Prolonged intake of Mirtoselect® has been associated with significant changes in visual acuity. An 8-weeks trial with a daily dosage of 150 mg of Mirtoselect® was carried out on 63 school children. This suggests that Mirtoselect® could be a powerful tool in the support of ocular health related to increased eye accommodation associated with prolonged reading and computer work, activities that are typical of school education. The anthocyanins in Mirtoselect® have an affinity for small blood vessels and have been shown to improve the blood flow in the eye bulb tissues, activating the nutrition supply.

Anthocyanins have been reported to help maintain healthy synthesis of retinal rhodopsin and attenuate free radical impacts associated with accommodation and its muscular burden.
**Pharmacokinetics**

After oral administration of a single dose of 400 mg/kg in rats, Indena’s bilberry extract was rapidly absorbed from the gastrointestinal tract, reaching a Cmax value of 2.5 µg/mL anthocyanins in plasma within 15 minutes. Anthocyanins disappeared from systemic circulation within 2 hours, and underwent a rapid distribution in highly vascularized tissues due to the specific affinity to endothelial membranes, a property that has great relevance for the vaso-protective activity of anthocyanins. The elimination of bilberry anthocyanins occurs in 24 hours, mainly through the bile. Similar results have been observed also in a human pharmacokinetic study with anthocyanins found in portal blood 5 hours after ingestion and under the detection level after 8 hours, while the stomach has been reported the predominant site of Mirtoselect® anthocyanins absorption in humans. After a single intraperitoneal dose of 36% anthocyanin bilberry extract (200 mg/kg), anthocyanins were detected in rats kidneys, skin, liver and in the heart respectively, while in another study, modelling human familial adenomatous polyposis in mice, anthocyanins were detected in intestinal mucosa at a 8.1 ng/mL concentration after oral administration of Mirtoselect®.

**Bilberry extracts and night vision**

Although a critical review of the available clinical studies under reduced light conditions (1964-2001) has recently been published, its negative results are confounded by several factors: different products having different phytochemical compositions were used in the trials analyzed; the amount of anthocyanins administered varied considerably within the trials, and the negative outcomes were generally associated with the lowest dosages. These considerations highlight the relevance of standardizing bilberry extracts and using them at clinically effective dosages.

**Mirtoselect® in supporting healthy visual function**

Several clinical investigations have been carried out with Indena’s bilberry extract. In particular, in a multicentric study, daily use of 510 mg (three 170 mg capsules/die) of Mirtoselect® over a period of one year on 88 patients was associated with remarkable improvement of contrast sensitivity and stabilization of visual acuity leading to improved visual quality. Additionally prolonged administration (6 to 53 months) of Mirtoselect® (120 mg/day divided in two capsules of 60 mg) in 132 patients, resulted in an amelioration of the best-corrected visual acuity (measured as logMAR) and of the Visual Field (Humphrey Visual Field test) compared to the initial conditions. Furthermore the amelioration of the best-corrected visual acuity was statistical significant also compared the control (97 patients) and Ginkgo biloba (103 patients) treated group, while the visual field increase could not reach statistical significance amelioration when compared to the Gingko biloba treated group. In a recent registry study evaluating the effects of Mirtoselect® in different types of eye challenges 140 subjects spontaneously decided to join one of the following groups: standard management (SM) only (n=38); SM associated with 160 mg/day of Mirtoselect® supplementation (n=47); SM associated with the same amount of generic bilberry extract supplementation (n=55). After 6 months, inter-group comparison revealed a statistical advantage in all tested parameters for Mirtoselect® supplementation groups. No side effects or tolerability concerns were reported, suggesting that the natural and peculiar standardization of Mirtoselect® containing the full range of the non-anthocyanin components, is critical for its the efficacy.

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*Comparison of image with different contrast sensitivity. A: image with high contrast sensitivity; B: image with low contrast sensitivity.*

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Mirtoselect® is a standardized bilberry extract containing 36% anthocyanins, and is characterized by a very specific and consistent HPLC profile that represents the “fingerprint” of the extract. Mirtoselect® is obtained exclusively from bilberry (Vaccinium myrtillus L.) fresh fruits harvested when ripe, between July and September.

Chemical profile

Anthocyanins and anthocyanidins

Anthocyanins (anthocyanosides),59 the water-soluble pigments responsible for the deep blue color of the berries, represent the main active fraction of the numerous biological properties of Mirtoselect®. Anthocyanins occur in nature as glycosides and their aglycones, the anthocyanidins, are derived from the 2-phenylbenzopyrylium cation, more commonly referred to as flavylium cation. This name emphasizes the close relationship with flavonoids, a class of natural products to which also anthocyanins belong.

Bilberry anthocyanins are 3-O glucosides, galactosides and arabinosides of five anthocyanidins (cyanidin, delphinidin, malvidin, peonidin and petunidin). Anthocyanidins, anthocyanins without the sugar moiety, are trace components of Mirtoselect® (≤1%) and should be considered as anthocyanins degradation products. Anthocyanidins are diagnostic of an incorrect manufacturing and/or storage of the extract.

The analytical method

A new high-performance liquid chromatography method was developed and validated to identify and quantify both anthocyanins and anthocyanidins present in bilberry extract and products.18 The quantification procedure employs cyanidin-3-glucoside as external standard and the content of each individual anthocyanin is evaluated by means of a weight-correction factor.

The method is highly reproducible and, due to its high specificity, is suitable to the univocal identification of raw materials, as well as the evaluation of the phytochemical pattern of bilberry extracts, securing a high degree of product consistency and quality.

The reliability of this innovative HPLC-based method is such that it has become the official method for analyzing bilberry’s identification and chemical composition in the European, Italian and US Pharmacopoeias.22-24
Mirtoselect® 36% anthocyanin bilberry extract has demonstrated its efficacy in:

- increasing capillary resistance
- reducing vascular permeability
- exerting antioxidant activity at vascular level
- improving arteriolar vasomotion
- exerting healthy inflammatory response function activity

Vasoprotection

The vasoprotective activity of bilberry anthocyanins is related to their ability to reduce capillary permeability and increase capillary resistance via several mechanisms, including stimulation of mucopolysaccharides biosynthesis, inhibition of proteolytic enzymes involved in the degradation of the extravascular matrix components of the blood vessels, and the interaction with collagen metabolism. The stimulation of polysaccharides production is considered critical for vasoprotective activity, since mucopolysaccharides play crucial role in the physiology of perivascular tissues.

Effect on arteriolar vasomotion

Arteriolar vasomotion, the rhythmic variation of arteriolar diameter in the microvascular network, modulates the mechanism underlying the formation of interstitial fluid. Indena’s standardized 36% anthocyanin bilberry extract was proven to induce arteriolar vasomotion and increase vasomotion frequency, indicating that bilberry extract may prevent or control the formation of interstitial fluid, with an overall improvement of blood flow redistribution into microvascular network.

Inflammation response function

A recent study on microarray-based gene expression, has shown that, in an inflammatory response function model, Mirtoselect® anthocyanins can attenuate expression levels of numerous relevant genes, while supporting other relevant genes expression levels. These observations provide a rationale for support of a healthy inflammatory response function of bilberry anthocyanins.

Concluding remarks

The major applications investigated so far for Mirtoselect® are in the realms of vascular health and ophthalmology (eye protection at the retina level), but animal models and pilot clinical studies suggest a broader clinical profile of anthocyanins encompassing memory improvement, cardiovascular health, and the interaction with collagen metabolism. In particular, on Mirtoselect® new clinical studies are showing potential metabolic syndrome and glucose metabolism management. Mirtoselect® is used worldwide as a functional ingredient and has also been authorized as a functional food with a specific claim in Korea.