

The original bilberry extract





1. Indena: beyond quality, naturally

There is a substantial amount of evidence supporting the strong connection between health and nutrition.

Life expectancy is continuously increasing together with a higher attention to quality, this becoming a key factor for both consumers and the food industry. Consumers are more and more aware of what is contained in food, they look for safe and effective ingredients and the presence of available evidence supporting new ingredients offered in terms of benefits and safety of their dietary supplementation may heavily influence their buying decision. On the other hand, the food industry is expected to meet consumer demands, bringing to the market safe and effective products containing safe and certified ingredients.

Natural products in various forms have been used all over the world since time immemorial for the treatment of pathological conditions or for health benefits.¹ Plants represent a rich source of new active principles and botanical extracts can be used in different markets including health foods and supplements. The overall quality is essential, together with clinical substantiation to guarantee the necessary safety and efficacy profiles.

¹ Bombardelli E., Bombardelli V., Twenty years experience in the botanical health food market, Fitoterapia 76, 495-507, 2006.

In western countries where an allopathic system of medicine substituted almost completely the use of crude extracts, **plant derived compounds** constitute **25%** of the existing drugs.

Setting aside their often unsatisfactory biological and clinical documentation, many extracts today are poorly chemically characterized with their active constituents un identified, and their constancy and stability erratic. For these reasons, many extracts with totally different compositions claim to possess the same therapeutic properties. It would emerge from this scenario, that to obtain biological data, reproducible in terms of safety and efficacy, the active ingredients must be the same over time; they must be stable and devoid of unpredictable toxicity or side effects. The answer can be found only in the preparation of standardized extracts, identifying the active constituents and controlling the unknown substances in terms of stability and constancy. Standardization is a hard task which should begin from the biomass obtained through cultivation or collecting the plant in the wild in line with Good Agricultural Practice (GAP); this is followed by chemical isolation and the characterization of active reference substances, the setting up of validated analytical methods and finally applying Good Manufacturing Practice (GMP) to the production of the final ingredient.

A large number of ingredients with a distinctive high quality profile has been developed by Indena, starting out from a state-of-the-art clinical design study with the aim of meeting and possibly exceeding market expectations.

Mirtoselect[®] is a stunning prime example of this.





2. The precious bilberry

Many red fruits are full of healthy antioxidants, in particular anthocyanins, and may be helpful as an adjuvant in the prevention of several conditions.

Anthocyanins are found naturally in a number of foods - in red wine, certain varieties of cereals, and certain leafy and root vegetables (aubergines, cabbage, beans, onions, radishes) - but are most abundant in coloured fruits like bilberries.²

Furthermore, Prior et al.³ reported that *Vaccinium myrtillus* L. contain higher amounts of anthocyanins than any other berries.

Vaccinium myrtillus L. is a small edible berry which grows on small wiry shrubs on hillsides throughout Central and Northern Europe.⁴⁻⁸ Among the 450 species⁹ belonging to the genus Vaccinium, the traditional medicinal use of *Vaccinium myrtillus* L. has been documented since the Middle Ages, when its fruits were recommended for a variety of conditions, and from the 16th century the plant has been systematically mentioned in all major herbal treatises. ²Nutrient U.S. Department of Agriculture, A.R.S., USDA Database for the Flavonoid Content of Selected Foods, Release 3.0 2011.

³ Prior R.L. et al., Antioxidant Capacity As Influenced by Total Phenolic and Anthocyanin Content, Maturity, and Variety of Vaccinium Species, J. Agric. Food Chem. 46, 2686 (1998).

⁴ Tutin T.G., Diapensiaceae to Myoporaceae, Flora Europaea Vol. 3, Cambridge University Press, Cambridge, 1972, pp 12-13.

 ⁵ Hutchinson J., British Wild Flowers Vol.
1, David and Charles, Newton Abbot, 1972, p 226.

⁶Benigni R., Piante Medicinali - Chimica, farmacologia e terapia, Vol. II, Inverni della Beffa, Milano, 1962, pp 951-958.

⁷ Hegi G., Illustrierte Flora von Mittel Europa, Band 5/3 Teil, C. Hanser Verlag, Munchen, 1927 (Reprint 1966), pp 1667-1681.

⁸ Upton R. American Herbal Pharmacopoeia and Therapeutic Compendium – Bilberry Fruit 2001. Unlike most other berries, *Vaccinium myrtillus* L. is extremely difficult to grow; it does not produce clusters of berries, but single or, more rarely, pairs of berries, in limited numbers considering the biomass of the plant. Bilberries are softer and juicier than most other berries, and therefore more susceptible to damage and more difficult to transport. Bilberries cannot be cultivated, and are therefore picked from wild plants growing on publicly accessible lands; nor can they be processed unfrozen, since tissue damage triggers the deglycosidation of anthocyanins, with detrimental effects on their chemical stability.

A number of compounds belonging to different chemical classes have been isolated from the berries of *Vaccinium myrtillus* L., particularly the anthocyanins (also called anthocyanosides) which have been extensively documented in scientific literature.

In the early 1970s, Indena developed a standardized bilberry extract whose efficacy has been extensively clinically evaluated in vascular health, with over 60 positive studies, including

at least 30 controlled or double-blind studies published in peer-reviewed titles.¹⁰

⁹ Mabberly D.J., The Plant-Book II Ed., Cambridge University Press, 1997.

¹⁰ Morazzoni P., *Vaccinium myrtillus* L., Fitoterapia 1996, 67, 3-29.

Bilberry is one of the richest sources of **anthocyanins**



on a fresh weight basis

The anthocyanin profile of bilberry is combinatorial

•••••5 anthocyanins•••

The profile of all other sources is additive and characterized by 1-3 major compounds



3. **From bilberry** to Mirtoselect®

The freshly collected berries of Vaccinium myrtillus L. are frozen in ventilated tunnels at -35°C. Once frozen, the berries are machineprocessed, separated into single berries according to size, (small berries are removed), weighted (in order to remove stones), and any leaves discarded.

Any red lingonberries (Vaccinium vitis-idaea) which may be present are removed through a system called Niagara. A belt moves the berries up to a point where they fall and sensors detect any falling berries which are out of the colour range. Manual checks are also carried out before the cleaned berries are either packaged or temporarily stored in large boxes.



The process from bilberry to Mirtoselect®

Mirtoselect®

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



Authentic bilberry has a very specific **"fingerprint"** identified through HPLC analysis¹¹⁻¹²



Authentic bilberry contains ≥36% anthocyanins, plus the full range of the non-anthocyanin components, critical for its efficacy¹¹⁻¹²

Indena has developed a specific and indisputable analytical method for the identification of bilberry anthocyanins and anthocyanidins.

The HPLC based method defines the anthocyanins content of the standardized bilberry extract as 36% anthocyanins (equivalent to 25% anthocyanidins by UV). It has become a standard reference for most Pharmacopoeias in western countries.¹³⁻¹⁷

The Indena extract is the most studied bilberry ingredient on the market

The major applications investigated so far for Mirtoselect[®] are in the realms of vascular health and ophthalmology (retinal health), but animal models and pilot clinical studies suggest a broader clinical profile for anthocyanins that encompasses memory improvement, gastrointestinal and cardiovascular health, metabolic syndrome and obesity.

It shows consistent clinical benefits especially for eye health. This consistency is the result of careful biomass collection, storage and reproducible batch to batch industrial production. Clinical findings are also linked with an optimal tolerability profile.

Mirtoselect[®] is a dark red-violet powder and may be utilized in a variety of supplement formulations. Recommended dose: 160-320 mg/day.

¹¹ Gizzi C. et al., Bilberry extracts are not created equal: the role of non anthocyanin fraction. Discovering the "dark side of the force" in a preliminary study, European Review for Medical and Pharmacological Sciences, 2016 Jun; 20(11):2418-24.

¹² Motohashi N., Occurrences, Structure, Biosynthesis and Health Benefits based on their evidences of medicinal phytochemicals in vegetables and fruits -Volume 5 – 2016.

¹³ Cassinese C., New liquid chromatography method with ultraviolet detection for analysis of anthocyanins and anthocyanidins in Vaccinium myrtillus fruit dry extracts and commercial preparations., J. AOAC Int. 2007, 90 (4), 911-919.

¹⁴ Baj E. et al, Qualitative and quantitative evaluation of Vaccinium myrtillus anthocyanins by HRGC and HPLC., J. Chromatogr. 1983, 279, 365-372.

¹⁵ European Pharmacopoeia 6th Ed. Suppl. 6.2 Fresh bilberry Fruit Dry Extract Refined and Standardised, 3745-3747.

¹⁶ FU Mirtillo Nero Estratto Secco Purificato e Titolato XI Ed. Suppl. 1, 2005, 1469-1472

¹⁷ USP 31–NF 26 - Second Supplement Powdered Bilberry Extract (Identification and Assay).



The major applications investigated so far for Mirtoselect[®] are in the realms of **vascular health** and **ophthalmology** (eye protection at the retina level), but epidemiological surveys, animal models and pilot clinical studies suggest a broader clinical profile of anthocyanins encompassing memory improvement, gastrointestinal and cardiovascular health. In particular, preliminary trials have shown potential metabolic syndrome and glucose metabolism management activity with the use of Mirtoselect[®], which may represent an opportunity for future applications following further research into its use in Eye Health.¹⁸⁻²²

Through the beneficial effect on vascular circulation, Mirtoselect[®] can improve oxygen and blood delivery to the eye, restoring the functionality of tear secretion. Furthermore, the free radical scavenging properties counteract oxidative stress, one of the major risk factors of dry eye discomfort.

Mirtoselect[®] is the only bilberry extract, void of any known side effect, whose properties have been clinically and pharmacologically demonstrated. ¹⁸ Krikorian R. et al., Blueberry supplementation improves memory in older adults., J Agric Food Chem. 2010 Apr 14;58(7):3996-4000.

¹⁹ Karlsen A., Anthocyanins inhibit nuclear factor-kappaB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults. J. Nutr. 2007, 137, 1951-1954.

²⁰ Mink, P. J. Flavonoid intake and cardiovascular disease mortality: a prospective study in postmenopausal women. Am. J. Clin. Nutr. 2007, 85, 895-909.

²¹ Campbell, F. M., et al., Decrease in plasma protein markers of oxidative stress and inflammation in overweight/obese type 2 diabetics after supplementation with bilberry extract., In: Obesity facts 2012, 5 (s1) p198.

²² Hoggard N. et al., A single supplement of a standardised bilberry (*Vaccinium myrtillus* L.) extract (36 % wet weight anthocyanins) modifies glycaemic response in individuals with type 2 diabetes controlled by diet and lifestyle. Journal of Nutritional Science, 2013, vol. 2, e22, page 1 of 9.

Studies have shown that Mirtoselect® can:





Improve contrast sensitivity in retinal health²³

Attenuate **free radical** damage associated with school-age children²⁴

Support eye health in **retinal challenges²⁵**





Improve tear secretion in dry eye²⁶

More than 60 positive studies, including at least 30 controlled or double-blind studies published in peer-reviewed titles, have validated the efficacy of Indena's bilberry extract in **vascular health**²⁴

New positive data has recently emerged regarding the supportive role of Mirtoselect[®] in eye health, in particular dry eye conditions. The new randomized, double-blinded, placebo-controlled clinical study²⁶ offers further proof of the efficacy and safety profile of Mirtoselect[®], where preserving adequate tear levels and maintaining the ocular surface healthy may possibly help in attenuating discomfort and visual disturbance.

Today Mirtoselect® is the only bilberry extract (*Vaccinium myrtillus* L.) supported by clinical data on dry eye condition. ²³ Kim E.S., Clinical Evaluation of Patients with Nonproliferative Diabetic Retinopathy Following Medication of Anthocyanoside: Multicenter Study, J. Korean Ophthalmology Soc., 2008;49(10):1629-1633.

²⁴ Kajimoto S. et al., Therapeutic Effect of Blueberry Extract in School Children with Pseudomyopia at the Age of Cramming for Exam to Restore Their Vision, J. New Rem. Clin., 2000, Vol.49 N.1, 72-79.

²⁵ Mazzolani F. et al., Minerva Oftalmol 2017 June;59(2)_38-41.

²⁴ Riva A. et al, The effect of a natural, standardized bilberry extract (Mirtoselect®) in dry eye: a randomized, double blinded, placebo controlled trial, European Review for Medical and Pharmacological Sciences, 2017;21: 2518-2525.



5. Almost half a century of bilberry extract

Indena has been working on bilberry extract since the early seventies. Over the years, our interest in the stunning properties of the precious small blue berry has risen and our standardized extract Mirtoselect[®] is today not only the most extensively studied bilberry extract available, but also the market leader and benchmark.

Indena brings to the market, to customers and to final consumers a level of quality that few others are able to guarantee; Mirtoselect[®] is one of the finest examples of how Indena pioneers the development of botanical derived dietary ingredients, and proof of the company's expertise in innovation and quality.

Milestones in the story of Mirtoselect®



Indena began research into a standardized bilberry extract, first named Myrtocyan® "Bilberry 25% dry extract".

1976

Indena registered a medicinal specialty soon to become the world's best-selling OTC bilberry product.

1980

The Indena product included in dietary supplements market under the Mirtoselect® brand name and used as a benchmark for the bilberry extract market.

2004

Indena's new dedicated state-of-the-art production facility for Mirtoselect® was inaugurated at the Settala site. It processes up to 26,000kg of bilberries a day, and has an annual production capacity of more than 45 tons of extract.

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2003

The European Scientific Cooperative On Phytotherapy (ESCOP) published a monograph on Indena's bilberry extract in "The Scientific Foundation for Herbal Medicinal Products".

2005

Indena updated an analytical method, already used for the release of its first registered product in Italy.¹⁴ This new validated HPLC method for bilberry quantifies all the anthocyanins present in both plant material and in extracts and is able to detect anthocyanin extracts produced from different plant materials (raspberry, blackberry, black currant, elderberry, etc.). This method represents a standard reference for the main pharmacopoeias.

2006

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Indena filed a new logo to promote the consistency and quality of Mirtoselect®. This may also be used by Indena customers to reassure consumers about the quality of the active ingredients they use.

2008

Widespread official welcome for the company's efforts against adulterated bilberry extracts. Indena continues to actively contribute to the collection of available bilberry extract monographs in the European, Italian and US pharmacopoeia.²⁸⁻³⁰

2017

To date, almost 60 peer-reviewed papers have been published on Indena's standardized bilberry extract since the early seventies, including at least 30 controlled or double-blind studies published in peer-reviewed titles. Confirmation indeed of its quality, safety and efficacy.

2007

Indena published its internal method of analysis for bilberry extract¹³, applicable also to finished products, for the benefit of the Industry. In the same year, Indena denounced the presence on the market of fraudulent bilberry extracts and improperly labelled finished products.²⁷ The new Mirtoselect® web site went online.

> ²⁷ C. Artaria, R. Pace, G. Maramaldi, G. Appendino., "DIFFERENT BRANDS OF BILBERRY EXTRACT – A comparison of selected components", NUTRAfoods 2007 6(4) 13-18.

> ²⁸ European Pharmacopoeia 6th Edition – Supplement 6.2 – «Fresh bilberry Fruit Dry Extract Refined and Standardised».

²⁹ FU XI Ed, 1st Suppl. 2005, «Mirtillo Nero Estratto Secco Purificato e Titolato».

³⁰ USP 31–NF 26 – Second Supplement, «Powdered Bilberry Extract» (Identification and Assay).

