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DIFFERENT BRANDS OF BILBERRY EXTRACT
A comparison of selected components

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SUMMARY

Forthcoming recommendations on the use of botanical extracts for the maintenance of health and the prevention of some disorders require a careful characterization of each ingredient. On the other hand, great differences currently exist among products on the market.
In this study, ten commercial bilberry (*Vaccinium myrtillus* L.) extracts were analyzed with a USP/NF Pharmacopoaeia methods for labeling compliance in terms of anthocyanins content and significant deviations were detected.
These results highlight the need to develop standardized methods to evaluate the quality of herbal products.

INTRODUCTION

Herbal therapies for the prevention and/or the treatment of various disorders have been the first medicine available to mankind, and are still popular today. Their use is expected to grow further with the aging of the population since herbal products may be useful to treat a host of senile-related diseases. One of them is capillary fragility, one of the indications of bilberry.

Bilberry is a rich source of anthocyanins (also known as anthocyanosides), and its extracts [hereinafter called *Vm*E (*Vaccinium myrtillus* Extracts)] are extensively used in food/dietary supplements and pharmaceutical products for the treatment of vascular and vision disorders.

The term anthocyanin was initially coined to indicate the substance responsible for the color of cornflower: it derives from the Greek term *anthos* which means flower, and *kuanos*, meaning blue, and refers to a group of water-soluble pigments responsible for red, pink, mauve, purple, blue, or violet color of most flower and fruits. Chemically the pigments of the anthocyanins occur as glycosides (conjugated with a sugar), and their aglycones (the anthocyanidins) are derived from the 2-phenylbenzopyrylium cation, more commonly referred to as flavylium cation, a name that emphasizes the fact that these molecules belong to the vast group of flavonoids.
Vaccinium myrtillus fruits are one of the major dietary sources of anthocyanins, and drugs and supplements based on *Vm*E are extensively used to treat the symptoms linked to capillary and venous

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fragility especially in ophthalmology (**1**).

The astringent and antiseptic properties of bilberry have been documented since the beginning of the twentieth century. During the Second World War, RAF pilots were rumored to consume large amounts of bilberry jams to improve their night vision, and the ophthalmic properties of *Vm*E were well documented (**2**) in the second half of the past century.

Chemical and pharmacological studies on *Vm*E identified anthocyanosides as the major components involved in the host of biological properties documented for bilberry (antioxidant (**3**,**4**), antiplatelet aggregation (**5**), inhibition of phosphodiesterases (**6**), interaction with collagen, phospholipids and proteoglycans (**7-9**), relaxing effect on vascular smooth muscle (**10-11**), arteriolar vasomotion (**12**) stimulation).

These translate into interesting applications in the realm of ophthalmology (**13**), vasoprotection (**14**), inflammation, ulcer (**15**) and atherosclerosis (**16**).

The pharmacological results obtained with *Vm*E, especially those with a standardized 35% anthocyanins content, suggested a clinical application in the treatment of capillary fragility and altered permeability, either primary or secondary to arterial hypertension, diabetes or arteriosclerosis.

Since 1964, several clinical trials have demonstrated the efficacy of bilberry extracts in the therapy of peripheral vascular diseases and venous-sensitivity or to alteration of microcirculation of the retina, and of post-operative clinical symptoms (**17-21**).

Owing to their high diffusion, bilberry extracts are often sophisticated with other anthocyanin-rich plants because of the lack of a reliable official method of analysis. Only recently, a suitable HPLC method([1])# has been published in USP European and Italian Pharmacopoeia.

Making use of this method, we have analyzed some of the most common brands of bilberry in the US market for their compliance with the reported anthocyanin content, and their actual origin from bilberry.

MATERIALS AND METHODS

HPLC determination

The anthocyanins and anthocyanidins present in bilberry extracts and products were identified and quantified by HPLC as described by Cassanese *et al* (**22**). The quantification procedure foresees cyanidin-3-*O*-glucoside as external standard, and the content of each individual anthocyanin is evaluated by the means of a molecular weight correction factor. The samples collected will remain unnamed but are identified by number (1 to 10) as shown in ***Table 1***.

UV determination

The most common analytical methods use UV-visible spectrophotometry that allows the quantification of anthocyanins in the visible region.

RESULTS AND DISCUSSION

The results mentioned below comprise a total of ten commercially available products on the US market belonging to 8 different brands: they have been compared for their *Supplement Facts* indication and for their content using the above-mentioned HPLC and UV methods.

Supplement Facts summary

The ten products show bewildering differences in labeling. This, in general, is often confusing. It was found that:

1 the identity of the botanical specie used (*Vaccinium myrtillus*) is reported in only 6 of the 10 products considered for analysis.

Comment: The need to identify the species for Vaccinium myrtillus is critical. In fact, the genus Vaccinium comprises 450 species, encompassing deciduous or evergreen shrubs, small trees and

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Table 1 *Summary of label Supplement Facts indications*

No	*Supplement Facts on the label/active*	*mg*	*Directions*	*[Dosage]*
1	Bilberry extract (berry)	40	1 cps/*die*	40 mg extract
	25% Anthocyanosides	10		(10mg anthocyanosides)
2	Bilberry extract (25% Anthocyanidins)	80	1-3 cps/*die*	80-240 mg extract
	(100% Scandinavian derived)			(bilberry)
	Edelberry	140	1-3 cps/*die*	140-420 mg extract
				(edelberry)
3	Bilberry	37.5	1 cps x 3-6/*die*	112.5-225 mg extract
	(*Vaccinium myrtillus*) (fruit)			
	(from 37,5 mg of 10:1 Extract)			
4	Bilberry	40	1 cps x 6/*die*	240 mg extract
	(*Vaccinium myrtillus*) (fruit) (4:1 Extract)			
	equivalent to 160mg bilberry fruit			
5	Elderberry (berry)	125	1-2 cps x 3/*die*	375-750 mg extract
				(edelberry)
	Bilberry Dry Extract (berry)	80	1-2 cps x 3/*die*	240-480 mg extract
	25% Anthocyanins			(bilberry)
6	Bilberry Fruit extract	60	2 cps x 3/*die*	230 mg extract
	(*Vaccinium myrtillus*)			
	Anthocyanosides	15		
7	Bilberry (fruit)	60	1 cps x 1-3/*die*	60-180 mg extract
	25% Anthocyanosides	15		15-45 mg anthocyanosides
8	Bilberry (*Vaccinium myrtillus*) berry extract	60	1 cps x 2-4/*die*	120-240 mg extract
	(Guaranteed 21.6 mg [36%] Anthocyanosides)			
9	Bilberry (*Vaccinium myrtillus*) powdered extract	160	1 cps/*die*	160 mg extract
	(Guaranteed 21.6 mg [36%] anthocyanosides)			
10	Bilberry (*Vaccinium myrtillus*), powdered extract 25% (fruit)	40	1 cps x 1-3/*die*	40-120 mg extract
	Anthocyanidins	10		10-30 mg anthocyanidins
	Bilberry (*Vaccinium myrtillus*) powdered extract 5:1 (fruit)	305	1 cps x 1-3/*die*	

lianes, many with edible fruits, and some cultivated as ornamental plants.
Only *Vaccinium myrtillus* has a sound tradition of medicinal use, and has properties well documented in the scientific literature (**14-22**).

2 The part of the plant used ('fruit'), is reported in only 7 out of the 10 products.

Comment: The fruits and leaves of bilberries have been used in the traditional medicine of many European countries for different indications, with leaves being mainly used as remedy for diabetes, and, locally, to treat skin infections and burns. This is not surprising, since the leaves of bilberry contain a negligible content of anthocyanosides, and are, instead, rich of tannins. It is therefore important to specify the exact botanical source and plant part used.

3 The presence of an active principle is reported in 7 out of 10 products. The active principle is not univocally defined, as three different definitions (corresponding to two different forms of actives) are displayed: anthocyanosides, or anthocyanins, and anthocyanidins.

Comment: the main active constituents of bilberry are, chemically, anthocyanins, also known as anthocyanosides. They are natural pigments responsible for the red, pink, mauve, purple, blue or violet hue of many flowers and fruits. They are the result of the conjugation of a flavonoid to a sugar, either monomeric or oligomeric. These compounds act as potent antioxidants and phosphodiesterase

inhibitors, and are therefore particularly effective in the amelioration of capillary resistance and permeability. Anthocyanidins are anthocyanins stripped of their sugar moiety and are usually present in a very low amount in the fresh fruits.

4 The active principles are quantified in terms of mg or percentage concentration.

Comment: These differences make it difficult for consumers to compare the various products.

5 The recommended dosage of extract varies from 40 mg/*die* to 480 mg/die (12 times higher!)

Comment: The recommended dosage is also very different. The broad variability in the suggested dosage mirrors the high variability in quality within the various products. As a result, it is very difficult to compare the products in terms of active principles delivered. Consumers are clearly confused by this labeling system, and can not benefit from a clear and univocal information to compare different products.

AN ANALITYCAL OVERVIEW

The 25% (anthocyanindins) or 36% (anthocyanins or anthocyanosides) percentages can be related to the way the extracts are standardized, 25% by UV and 36% by HPLC.
The most common analytical method for evaluating anthocyanins is based on UV-visible spectrophotometry, which allows the quantification of anthocyanins by detecting them in the visible region of the spectrum.
The UV-visible spectrophotometry can be performed directly or, for practicality, the original extract is subjected to acidic hydrolysis in order to convert all anthocyanins into anthocyanidins, removing their sugar decoration.
These methods are far from satisfactory, lacking in specificity and being unable to identify any single anthocyanin.
As a result, it is impossible to distinguish anthocyanins extracts produced from different plant materials (raspberry, blackberry, black currant, elder_berry, etc.), and whose anthocyanosidic composition is very different from bilberry.
Furthermore, the acidic hydrolysis overestimates the anthocyanosidic content, since, under these conditions, proanthocyanidins are de-polymerized and turned into anthocyanidines. A host of plants are rich in proanthocyanidins, and they are 'seen' as bilberry if only this method is applied.
Another analytical method which is often applied is high-performance liquid chromatography (HPLC). Also in this case acidic hydrolysis modification is often applied.
Again, as anthocyanidins are formed by hydrolysis, HPLC may provide an overestimation of anthocyanin content, if any proanthocyanidins are present in the extracts, and this procedure does not allow the quantification of the real content of free anthocyanidins, which are markers of extract degradation.

The direct HPLC method recently introduced in the USP European and Italian Pharmacopoeia overcomes all these analytical issues.
As a result of its good reproducibility and its high specificity, the method is able to identify unequivocally the botanical raw materials used for manufacturing the extract, evaluating its composition and providing a high degree of consistency and quality in the finished products.

The analysis of bilberry is clearly a complicated issue, compounded by the poor specification of the label. For instance, what does the indication: 25% anthocyanosides 10mg' mean? And the percentage of 25% is obtained using which method (UV or HPLC)? Was it a direct analysis or an acidic hydrolysis modification that has been performed?

The variability in bilberry extract analysis, as well as the differences among available commercial extracts, are summarized in *Table 2*.
Even with the limitations highlighted above, compliance to the declared content is not always observed.

Table 2 *Summary of bilberry analysis and compliance to label: variability in bilberry extract analysis and the differences among available commercial extracts. Compliance with the declared content is not always observed*

No	Batch	Anthocyanins (HPLC) (%)	Anthocyanins (UV/Visible) (%)	Label	Compliance to label
6	0C10704	44.5	28.3	15mg/2cps (25%)	compliant
6	0L10333	41.1	28.1	15mg/2cps (25%)	compliant
10	81028	40.6	27.9	10mg (25%)	compliant
5	421101	39.0	27.3	25% - 80mg anthocyanins	compliant
9	92701	37.9	24.2	36% anthocyanosides	compliant
10	88046	37.3	25.5	25% anthocyanidins	compliant
8	90402	37.0	25.7	36% anthocyanosides	compliant
5	340435	27.7	27.8	25% - 80mg anthocyanins	compliant
1	2011616	25.8	27.0	25% anthocyanosides	compliant
2	50304	21.3	17.5	25% anthocyanidins	non compliant
3	64981-06	2.2	3.3	n.d.	not applicable
7	410127	1.7	2.2	25%anthocyanosides	non compliant
7	402178	0.7	1.3	25% anthocyanosides	non compliant
4	JJ08/A	0.0	0.0	n.d.	not appl; null

On the basis of the obtained results the examined *Vm*E can be divided into three main categories:

i. extracts with a content of anthocyanins (by HPLC) between 30% and 45% (products 6, 10, 5, 9, 8);

ii. extracts with a content of anthocyanins (by HPLC) between 10% and 30% (products 5 -different batch-, 1, 2);

iii. extracts with a content of anthocyanins (by HPLC) lower than 5% or without anthocyanins (products 3, 7 both batches tested, 4).

CONCLUSIONS

The bilberry extracts in the products we have analyzed show differences in content as well as in variations between actual and reported concentrations, with one samples even totally lacking anthocyanosides. Clearly, the presence on the market of products of this type undermine customers's reliance on herbal extracts and, in particular, on the beneficial properties of bilberry. Furthermore, even for products actually containing bilberry anthocyanosides, labeling was often uninformative, misleading or both. The scenario emerging from this analysis is not unprecedented in the unregulated herbal market, even for extracts cheaper than bilberry (**23**), and considerable efforts will have to be paid to the development reliable analytical methods to analyze finished products, benefiting consumers and reliable producers as well.

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