ABC AHP NCNPR
Botanical Adulterants Program
BOTANICAL ADULTERANTS BULLETIN

on Bilberry (*Vaccinium myrtillus*) Extracts

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Bilberry *Vaccinium myrtillus*
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Goal: The goal of this bulletin is to provide information and/or updates on issues of adulteration of bilberry extract to the international herbal industry and extended natural products community in general. It is intended to complement the previously published works with information on bilberry extract adulteration, e.g., the *American Herbal Pharmacopeia* monograph published by Upton et al,[1] and the article by Foster and Blumenthal in *HerbalGram*[2] by presenting new data on the occurrence of adulteration, the market situation, and consequences for the consumer and the industry.

1 General Information

1.1 Common name: Bilberry[3]

1.2 Other common names:

English: European blueberry, whortleberry, huckleberry[4]

Chinese: *Hei guo yue ju* (黑果越桔)

French: Myrtille, ambroche, ambreselle, brimbelle, gueule-noire, raisin des bois, vigne des montagnes

German: Heidelbeere, Blaubeere, Schwarzbeere, Waldbeere, Bickbeere, Moosbeere

Italian: Mirtillo, ampulette, asaire, bagole, baggiole, cesarelle, giasine, lambrune, mirtillo nero, murucule

Spanish: Arándano azul, mirtilo

1.3 Accepted Latin binomial: *Vaccinium myrtillus*[5,6]

1.4 Synonyms: *Vaccinium myrtillus* var. *oreophilum*, *Vaccinium myrtillus* subsp. *oreophilum*, *Vaccinium oreophilum*.[5]

1.5 Botanical Family: Ericaceae

1.6 Plant part and form: Bilberry extracts are made from fresh bilberry fruit.[1] The extracts are often standardized to 25% anthocyanidins or 36% anthocyanins. Products that claim these levels of compounds may in actuality contain the same amount as the differences are most often due to different quantitative values obtained from using different analytical techniques. In the case of bilberry, high-performance liquid chromatography (HPLC) provides a lower quantitative value than ultraviolet-visible spectrophotometry (UV/Vis).[7] Also found on the market are bilberry leaf extracts, or combinations of bilberry fruit and leaf extracts. Extracts made entirely from bilberry leaves and properly labeled as such are not within the scope of this document.

1.7 General use(s): The indications for bilberry fruit include the symptomatic treatment of dysmenorrhea associated with premenstrual syndrome, circulatory disorders in patients with capillary leakage or peripheral vascular insufficiency, and ophthalmic disorders.[1,4,8] In addition, bilberry fruit is used topically for mild inflammations of the oral mucosa.[4,8]

2 Market

2.1 Importance in the trade: In the United States, bilberry was among the top 20 herbal supplements between 2007 and 2012 in the food, drug and mass market with annual sales above US $1.4 million in this channel.[9-13] The market volume has been fairly steady in recent years (D. Stanek oral communication, January 23, 2015).

2.2 Supply sources: Commercial bilberries are sourced mainly from Scandinavian and Eastern European countries. The World Blueberry Acreage & Production report lists Poland as the major producer of bilberry, followed by Russia, Ukraine and Scandinavia. Commercial bilberry for the production of bilberry extracts is sourced mainly from Eastern European (Lithuania, Romania, Poland, Russia and Ukraine) and Scandinavian (Sweden, Finland, Norway) countries, but also from France, Italy, and the Netherlands. (D. Stanek oral communication, January 23, 2015; E. M. Martinelli e-mail, November 16, 2015)

2.3 Raw material forms: Bilberry extracts are prepared from fresh berries that are frozen and then extracted with aqueous ethanol or aqueous methanol, and further concentrated according to the manufacturer's proprietary processes. Generally, the extract yield is approximately 100 times lower than the initial weight of raw material, i.e., 1 kg of fresh bilberry will provide ca. 10 grams of bilberry dry extract (D. Stanek oral communication, January 23, 2015). Dry extract materials using other manufacturing processes are also available.

2.4 Market dynamics: Tracking the bilberry harvest volume is difficult as the fruit is generally harvested by local people (i.e., wildcrafted) who then sell the fruit to brokers. A report from the North American Blueberry Council indicates that the total harvest for wild *Vaccinium* species (mostly bilberry, but other *Vaccinium* species, e.g., bog bilberry [*V. uliginosum*], are collected as well) in Europe was 11,340 metric tons (25 million lbs.) in 2008, 7,710 metric tons (17 million lbs.) in 2010, and 19,500 metric tons (43 million lbs.) in 2012.[14]

For 2012, the harvest in Poland was around 10,000 metric tons, but the harvest was mainly destined for the domestic market for confectionary and baked goods. Russia, Ukraine, and Scandinavia provided at least an additional 6,000 metric tons to the overall harvest.[14] According to the report, the harvest in 2012 was particularly strong despite unfavorable weather conditions; since the economy was depressed in European countries, many people harvested wild blueberries for additional revenue.

3 Adulteration

3.1 Known adulterants: Materials that are used to adulterate bilberry extracts typically have an intensive dark blue color, such as anthocyanidin-containing extracts or red food coloring such as amaranth dye (FD&C Red 2, E 123). Anthocyanidin-rich extracts known to be used as substitute for bilberry include those from bog bilberry (*Vaccinium uliginosum*), lingonberry (*V. vitis-idaea*), blueberry species (*V. angustifolium, V. corymbosum, V. floribundum*), cranberry (*V. oxycoccos and V. macrocarpon*), raspberries (*Rubus* spp., Rosaceae), wild cherry (*Prunus avium*, Rosaceae), black chokeberry (*Aronia melanocarpa*, Rosaceae), European elder (*Sambucus nigra*, Adoxaceae) berry, black soybean (*Glycine max*, Fabaceae) hull, black rice (*Oryza sativa*, Poaceae), and mulberry species (*Morus australis, M. nigra*, Moraceae).[2,15-18]

The common names "blueberry" and "wild blueberry" have different meanings depending on the geographical location. In the US dietary supplement trade, the name "blueberry" is restricted to three species, *Vaccinium angustifolium*, *V. corymbosum*, and *V. pallidum*.[3] In Europe, *V. myrtillus* is often called blueberry, though bilberry is the English word which refers to this species in the trade.[3] The hybrid cultivated blueberries from which the majority of the commercial food supply is derived are generally called blueberries. North American wild blueberry, common blueberry, common lowbush blueberry, low sweet blueberry, and lowbush blueberry refer to *V. angustifolium* which grows in the Northeastern U.S. and is commercially harvested in its habitat. Velvet leaf blueberry (*V. myrtilloides*) is also traded as "wild blueberry," and is mostly wild-harvested in the Canadian maritime provinces. It is safe to assume that "wild blueberry" in a commercial sense refers to both *V. angustifolium* and *V. myrtilloides* (Steven Foster, e-mail, July 1, 2015).

3.2 Sources of information confirming adulteration: There have been a number of publications on bilberry adulteration, such as the reviews by Foster and Blumenthal,[2] Giacomelli et al.,[16] Upton et al.,[1] the presentation by Pace et al.,[19] and the doctoral thesis by Primetta.[20] Foster and Blumenthal distinguish between the deliberate adulteration of bilberry extracts by addition of extracts from anthocyanin-rich sources such as blueberry, cranberry, European elder, sweet cherry, and others, and the adulteration occurring in the markets in China, where extracts of lingonberry and bog bilberry are wild-harvested and offered as "home-

made Chinese bilberry" and "Chinese domestic bilberry" extracts at prices as low as $10/kg.[2] This is in contrast to the much more expensive authentic bilberry extract (see section 3.3 below). Two additional studies regarding adulteration of commercial bilberry extracts were published in 2013 and 2014.[15,21] The investigation into the quality of 20 dietary supplements purchased in a store or over the Internet in Japan by ultra high-performance liquid chromatography (UHPLC) with detection by visible light at 535 nm provided evidence for adulteration in one sample. The product in question was labeled to contain a mixture of bilberry and black currant (*Ribes nigrum*, Grossulariaceae) extracts, but instead consisted entirely of black currant.[21] Gardana et al. analyzed 26 commercial bilberry materials including 14 bulk extracts, six food supplements, and six juices by UV/Vis spectrophotometry and by UHPLC using a photodiode array detector (DAD). The samples were purchased either directly from the supplier (bulk extracts), or from herbal shops and local markets in Italy. The authors found an admixture of black mulberry in five samples (two extracts, three food supplements), and substitution of bilberry with chokeberry in two extracts and with a material tentatively identified as blackberry (*Rubus* spp.) in one extract. One food supplement did not contain any anthocyanins at all. Five of the juices were consistent with products made from bilberry fruit, while one juice did not contain measurable amounts of anthocyanins.[15]

3.3 Accidental or intentional adulteration: The limited supply and high commodity prices of bilberry raw materials have created an incentive for economically-motivated adulteration. In 2015 authentic bilberry extracts sold for a price of US $600-800/kg extract (D. Stanek oral communication, January 23, 2015). More easily accessible anthocyanidin-containing fruit species can be collected in larger amounts than bilberry in a comparatively short time since many of these are readily available from cultivated rather than more expensive wild sources. The fruit from these other species can be made into extracts at a significantly lower cost than bilberry and can be priced below bilberry market rates while producing a profit for the producer/seller.

3.4 Frequency of occurrence: There are very little data on the extent of adulteration of commercial bilberry extracts and dietary supplements. A presentation by Pace et al. suggested that "adulteration of bilberry is rampant";[19] Roberto Pace, PhD, Director of Quality Control at Indena S.p.A. (Milan, Italy), the world's leading marketer of bilberry extract whose use has been confirmed in clinical trials, commented that "bilberry is one of the most economically adulterated ingredients of the health food market" (R. Pace oral communication, November 5, 2015). As noted above, one study included results of 20 commercial products purchased in Japan, with only 13 of the products labeled to contain bilberry. Of these 13 products, one was found to be entirely composed of black currant, while the remainder did contain authentic bilberry.[21] Another study looked at bilberry extracts from different manufacturers and supplements purchased on local markets in Italy. The results indicated that six out of 14 extracts, and four out of six supplements were adulterated.[15] Lee analyzed 15 commercial bilberry supplements purchased in the states of Washington and Idaho in the United States. Five products contained authentic bilberry material, and two samples could not be evaluated because they were composed of a mixture of anthocyanin-containing fruits, including bilberry. The remaining eight products were found to be adulterated.[18] Based on the limited published data avail-

Bilberry *Vaccinium myrtillus*
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able, it is not possible to come to any conclusions about the frequency of bilberry adulteration, but it is accurate to say that it is not uncommon.

3.5 Possible safety/therapeutic issues: Most of the known adulterants of bilberry have a long-standing history of safe use in food and therefore do not represent a safety concern. However, adulteration of bilberry extracts with amaranth dye is of possible safety concern. Amaranth dye has been prohibited for use by the US Food and Drug Administration (FDA) since 1976 due to concerns about carcinogenicity, which are mainly based on two controversial studies in rats.[22,23] The use of amaranth dye is still permitted in Europe, but is limited to 0.15 mg/kg per day.[24] Most botanical adulterants are cultivated plants, and might be potentially contaminated by pesticides, largely used in the growing of berries. Conversely, pesticides are not an issue with wild-harvested bilberry.

3.6 Analytical methods to detect adulteration: The most suitable way to authenticate bilberry extracts and detect adulteration is by chemical analysis based on the specific anthocyanin fingerprint using either high-performance thin layer chromatography (HPTLC) or high-performance liquid chromatography (HPLC) with ultraviolet/visible (UV/Vis) and/or mass spectrometric (MS) detection. The monograph of the *United States Pharmacopeia* (USP)[26] recommends use of HPLC, while that of the *American Herbal Pharmacopoeia* (AHP)[1] and *European Pharmacopoeia* (EP)[27] utilize the UV/Vis method after adequate identification tests have been performed. The use of UV/Vis spectrophotometry alone will allow measurement of total anthocyanins in bilberry extracts, but is not specific enough as an identity test. It can be applied as a quantitative tool only after the identity of the raw material has been assured. A comprehensive evaluation of 39 publicly available methods for the authentication and detection of adulterants in bilberry extracts, the Bilberry Extract Laboratory Guidance Document, is available through the ABC-AHP-NCNPR Botanical Adulterants Program.[25]

3.7 Perspectives: Don Stanek, US Sales Director at Linnea Inc., a European producer of bilberry extract, commented that adulteration of bilberry extracts is likely to persist, since the authentic material is so expensive (oral communication, January 23, 2015). Furthermore, explains Giovanni Appendino, PhD, professor of pharmaceutical sciences at the University of Eastern Piedmont (Novara, Italy) and consultant to Indena, the leading producer of bilberry extract, the price of the berries is subjected to significant variations due to the effect of climate on the labor-intensive harvest (e-mail, July 20, 2015).

4 Conclusions: The adulteration of bilberry extracts continues to afflict the natural products industry. Since the raw material is harvested in the wild, the control of the supply chain is more challenging and adequate testing of the incoming raw material is crucial. Test methods outlined, e.g., in the *USP*,[26] the *EP*,[27] or in the *AHP*[1] are able to detect adulteration of bilberry extracts and should be in place in every quality control laboratory to prevent manufacturers of bilberry extract products from becoming victims of companies providing fraudulent ingredients.

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REVISION SUMMARY

Version # , Author,	Date Revised	Section Revised	List of Changes
Version 1, S. Gafner new	n/a	n/a	none