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Microscopy for Quality Assessment of Bilberry Fruit (*Vaccinium myrtillus* L.)

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ABSTRACT

Bilberry is a traditional plant from which the berries have been eaten as a fresh fruit, made into jam, and included in some baked products for centuries. More recently, bilberries have become a popular dietary supplement and are among the best-selling fruit in the US market. Adulteration of bilberries in the marketplace, however, can occur due to misidentification or mixing with other species during harvesting and processing. Intentional adulteration also occurs through the purposeful addition of foreign materials to increase the apparent quality or reduce the cost of the final product.

Quality control is thus a critical aspect in the use of the species as a medicinally active plant. Chromatographic techniques, such as TLC, GC, and HPLC, are useful for chemical profiling and verification of the presence of medicinally active constituents, but are quite limited in their ability to detect non-chemical contaminants, such as dirt, insects, and molds and other adulterants, such as wood, sand, and plant materials. The current study investigated and compared the use of botanical microscopy and chemical analysis for identifying adulterants in bilberry fruit. The experimental results demonstrated that chemical analysis was insufficient, while microscopy readily detected contaminating materials in bilberry fruit.

INTRODUCTION

Bilberry (*Vaccinium myrtillus* L., Ericaceae), a native shrub of Europe, is a common ingredient in food and health products. Indeed, the fruit and leaves of bilberry have been used in popular medicine in Europe since the Middle Ages, but did not become frequently named by herbalists until the 16th century (Morazzoni and Bombardelli, 1996). Bilberry fruit, also known as the European blueberry, has been traditionally used for astringent, tonic, and antiseptic properties, and the bilberry leaves have been used for hypoglycemic and anti-inflammatory properties (Morazzoni and Bombardelli, 1996). Bilberry has been popularized in recent times thanks to a growing interest in health-conscious consumers.

The bilberry fruit has been suggested to have preventive action against cardiovascular diseases and to improve vision (Morazzoni and Bombardelli, 1996; Canter and Ernst, 2004; Szajdek and Borowska, 2008). The fruit of bilberry contains a number of phytochemicals, such as anthocyanins that have biological activities and health promoting benefits (Kong et al., 2003; Elks et al., 2013; Nile and Park, 2014; Kalt and Dufour, 1997). Extracts of bilberry fruit exhibit potent antioxidant activities (Morazzoni and Bombardelli, 1996; Faria et al., 2005; Zafra-Stone et al., 2007; Bao et al., 2008), anti-carcinogenic activity (Bomser, et al., 1996; Katsube et al., 2003), anti-inflammatory activity (Luo et al., 2014), anti-

hyperglycemic activity (Stefanut et al., 2013), and protective effects against neurodegenerative diseases (Subash et al., 2014) and retinal neuronal damage (Matsunaga et al., 2009).

The health promoting effects of bilberry fruit, have been associated with their potent antioxidant activity (Szajdek and Borowska, 2008; He and Giusti, 2010). The antioxidant activity has potential value in the treatment or prevention of conditions associated with inflammation, dyslipidemia, hyperglycemia, increased oxidative stress, cardiovascular disease, cancer, diabetes, and other age related diseases (Chu et al., 2011; Elks et al., 2013). Reproducible results in treatment are, however, problematic, being intrinsically in part linked to inconsistent and non-reproducible quality (Brinckmann, 2011). In an analysis of 40 commercial bilberry preparations from a number of countries, only 15% of the products provided dosage comparable to those demonstrated to be clinically effective (Cassinese et al., 2007).

In the United States, bilberry is a popular dietary supplement and is among the best selling in the market (Foster and Blumenthal, 2012). The American Herbal Products Association has classified bilberry as a class 1 herb, indicating the fruit is safe to consume (Upton, 2001). Due to the unique chemical composition, the wide range of health promoting benefits, and the high demand and price, bilberry is undoubtedly the most economically adulterated ingredient of the health food market (Pace et al., 2010). Thus, quality control of bilberry is extremely important.

Penman, et al. (2006) have reported bilberry being adulterated with synthetic anthocyanins, anthocyanins from different species, and amaranth dye that has a similar spectrophotometric absorbance to that of anthocyanins. Adulteration with other plant species has also been detected, whether intentionally or due to taxonomic misidentification, adulteration leads ultimately to low consumer confidence and thus limits commercialization (Foster and Blumenthal, 2012).

To address the adulteration problem multiple analytical methods for determining the chemical profile of bilberry have been developed (Hong and Wrolstad, 1990; Zhang et al., 2004; Cassinese et al.,

2007; Penman et al., 2006). Relying only on chemical analysis for quality assessment of bilberry, however, is insufficient to detect adulteration with foreign part plants, plant species, or inorganic materials that may not produce a detectable signal by HPLC/MS. Thus, microscopic characterizations of botanical medicines constitute an important tool for assessing the authenticity and quality of the herbal products (Upton et al., 2011).

Microscopic authentication is a particularly valuable tool to identify foreign materials in herbs or mixtures of herbs in powder form. Moreover, microscopy is rapid, inexpensive, and requires minimal sampling material (Zao et al., 2005). The objective of this study was to test the importance of microscopy for quality assessment of bilberry fruit.

MATERIALS AND METHODS

Plant material. Commercial samples of bilberry fruit (*Vaccinium myrtillus* L., Ericaceae) adulterated with elderberry (*Sambucus nigra* L., Caprifoliaceae) were analyzed using light microscopy and HPLC/UV/MS. In addition, an authentic sample of *V. myrtillus* and *S. nigra* obtained from Herbalist and Alchemist (Washington, NJ) were analyzed microscopically and chemically for comparison purposes.

Microscopy. Microscopic analysis was done using a fine powder of authenticated botanical tissue of bilberry and elderberry fruit. The tissues were each spread on a separate microscope slide containing two drops of Visikol™ clearing solution (Phytosys LLC, New Brunswick, NJ) and a cover slip. The slides with the authenticated botanical tissues were subsequently placed on a hot plate and heated for 30-60 seconds as previously described (Villani et al., 2013). Microscopic images of the tissue were made using a Nikon Eclipse 80i microscope, equipped with a NIS-Elements D 3.00 SP7 imaging software (Nikon, Tokyo, Japan). Differences and similarities in diagnostic features for each tissue sample and control were recorded.

Chemical analysis. Chemical analysis was done using 50 mg bilberry fruit powder extracted by sonication in 10 mL 60% (v/v) ethanol in water with 1% (v/v) HCl. The acidic ethanolic extract was then

passed through 0.45 μm Nylon syringe filter and placed in an amber autosampler vial for HPLC analysis. HPLC separation was done on a Polaris amide-C18 column, 250 x 4.6 mm, 5 μm (Varian Inc., Palo Alto, CA). For LC-MS analysis, a Hewlett Packard Agilent 1100 Series LC/MS (Agilent Technologies, Waldbronn, Germany) equipped with autosampler, quaternary pump system, DAD detector, degasser, MSD trap with an electrospray ion source (ESI) was applied, and software for data processing was HP ChemStation, Bruker Daltonics 4.2 and Data Analysis 4.2.

HPLC separation was done with the mobile phase containing solvent A (2% formic acid in water) and B (2% formic acid in acetonitrile) in gradient: 0-20 min, linear gradient from 10 % to 20 % B; 20-30 min, linear gradient from 20% to 30% B; 30-40 min, isocratic elution at 30% B; 40-50 min, linear gradient from 30% to 50%; 50-60 min, linear gradient from 50% to 60%. The flow rate was set at 1.0 mL/min with a 1:10 splitter after the output of the UV detector (set at 254, 280, 370, 520 nm) leading ~ 100 $\mu\text{L}/\text{min}$ to the ESI-MS.

The eluent was monitored by electrospray ion mass spectrometer (ESI-MS) scanned from m/z 100 to 1200. ESI was done using a needle voltage of ± 3.5 KV (positive and negative mode). High purity nitrogen (99.999%) was used as dry gas at a flow rate of 8 L/min and capillary temperature was at 350°C. Nitrogen was used as the nebulizer at 45 psi, and helium as the collision gas. Compounds were tentatively identified by their mass spectra and UV-Vis spectra when compared with reported data (Zhang, et al., 2004).

RESULTS

The microscopic analyses used to assess the taxonomic identity of bilberry fruit and elderberry fruit was used to develop criteria to distinguish samples of bilberry fruit from adulterants and to determine the content of foreign materials in bilberry fruit samples. Samples of the authenticated bilberry were a violet-brown color and showed characteristic anatomical features when viewed microscopically (Figure 1).

The dry colored bilberry fruit consisted of a

reddish-brown epicarp having polygonal cells with thickened walls (Fig. 1A), violet pink elongated sclereids with thick cell walls from the endocarp (usually observed in groups) (Fig. 1B), and solitary mesocarp sclereids (Fig. 1C). In addition, characteristic brownish–yellow testa–seed fragments with elongated cells having U-shaped thickened walls (Fig. 1 D). The elderberry fruit were characterized by fragments of sclerenchyma fiber layers arranged perpendicular to each other (Fig. 1 E and F).

The chemical analysis of bilberry extracts by HPLC/UV/MS focused on the anthocyanins in the bilberry fruit. The UV detector was set to 520 nm for the detection of anthocyanins, and a positive ion source was selected for ionization. A total of five major compounds and several minor compounds were detected (Figure 2a). The UV-Vis spectrum of these compounds was consistent with the spectrum of known anthocyanin compounds.

A tentative identification of the major compounds was assigned to each constituent using the mass spectrum (Figure 2). The most abundant compound was cyanidin-3-glucoside (Figure 2B), with the characteristic mass spectrum at 449 m/z (Figure 3A). The delphinidin-3-glucoside mass spectrum (Figures 2C & 3B) and the mass spectrums for malvidin-3-glucoside (Figures 2D & 3C), peonidin (Figures 2E & 3D), and petunidin (Figures 2F & 3E) were detected, but at lower concentrations as compared with the predominant cyanidin-3-glucoside.

No discernable chemical differences between the authentic bilberry fruit and bilberry adulterated with elderberry could be detected by the use of HPLC/UV/MS. Being identical, the chemical profiles of bilberry and elderberry using the described chemical analysis could not be used to ascertain adulteration of bilberry fruit with elderberry (Figure 4).

DISCUSSION

Plant species are typically identified by a specific combination of morphological and chemical characteristics. The described microscopic observations of bilberry and elderberry fruit in the present study are in agreement with earlier diagnostic identifications of dry samples of bilberry (Upton et

al., 2011) and elderberry (AHPA, 2015). Morphological identification constituted the most accurate

and rigorous means of confirming the taxonomic identity of a specimen (Applequist and Miller, 2013).

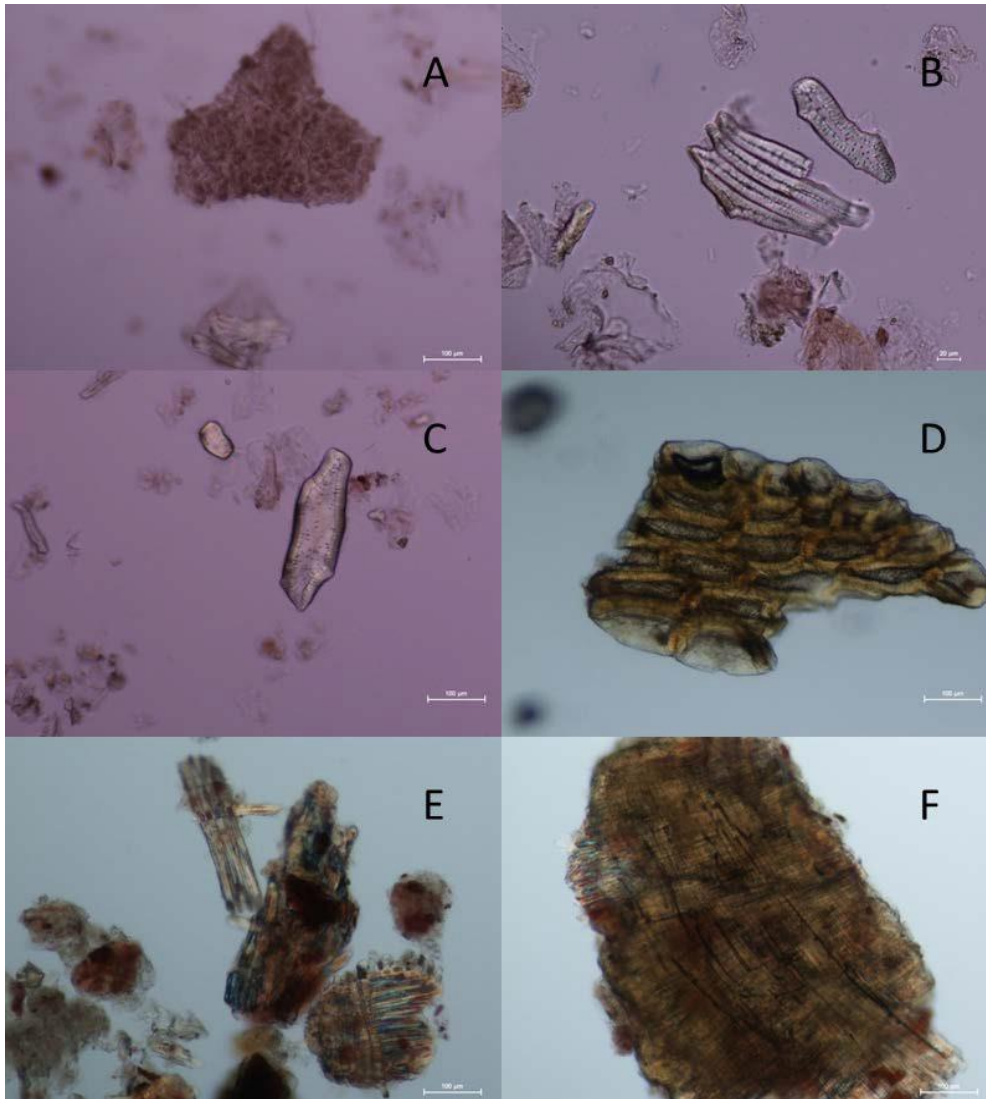


Figure 1. Light micrographs of dry and powdered botanical specimens cleared.

A-D = bilberry fruit (*Vaccinium myrtillus*); E-F = elderberry fruit (*Sambucus nigra*).

- A. Epicarp tissue with characteristic polygonal cells.
- B. Group of elongated sclereids from the endocarp.
- C. Isolated mesocarp sclereids.
- D. Testa epidermis with elongated cells with thick cell walls.
- E. Elderberry fruit with intersecting sclerenchyma fibers.
- F. Close-up details of elderberry fruit tissue.

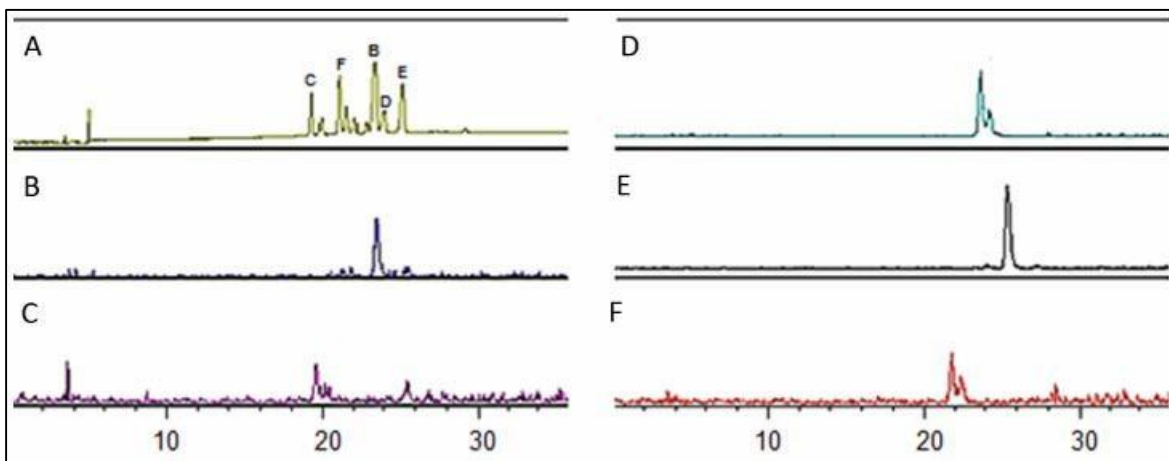


Figure 2. HPLC/UV/MS chromatograms of authentic bilberry fruit (*Vaccinium myrtillus*).

- A. 520 nm chromatogram. B. Extracted ion chromatogram (EIC) Cyanidin-3-glucoside (449 m/z).
 C. EIC Delphinidin-3-glucoside (465 m/z). D. EIC Malvidin-glucoside (493 m/z).
 E. EIC Peonidin-glucoside (463 m/z). F. EIC Petunidin-glucoside (479 m/z);

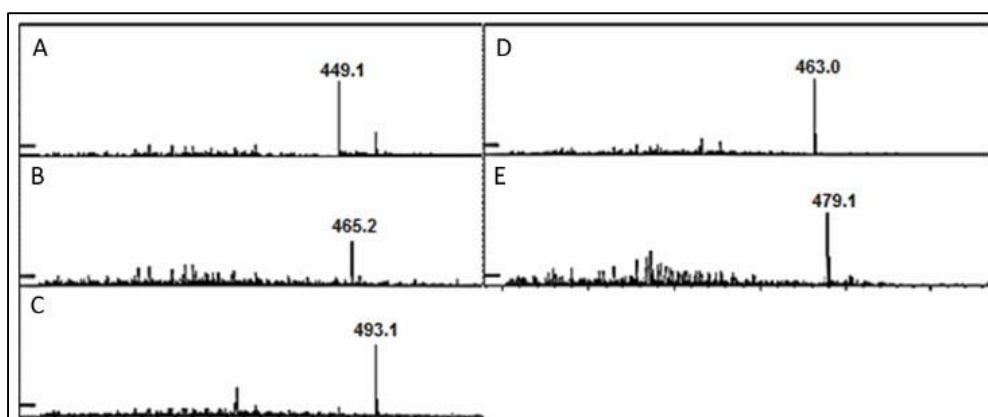


Figure 3. Mass spectra depicting compounds in bilberry extract.

- A. Cyanidin-glucoside B. Delphinidin-glucoside C. Malvidin-glucoside
 D. Peonidin-glucoside E. Petunidin-glucoside

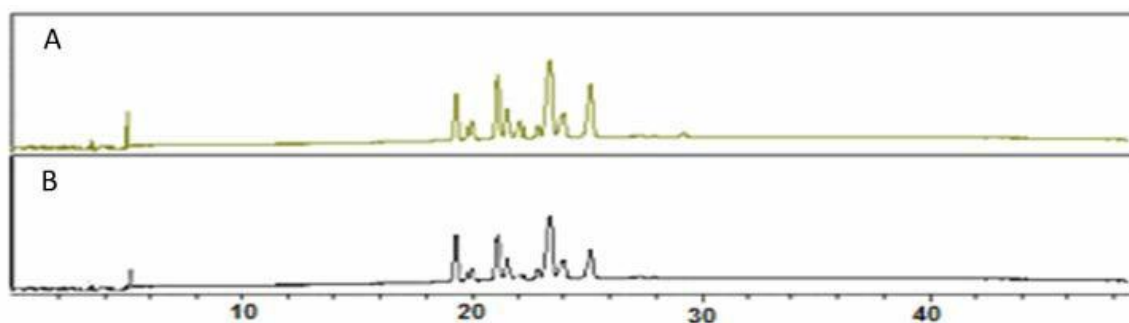


Figure 4. Comparison of 520 nm chromatogram of extracts.

- A. Authentic bilberry fruit (*Vaccinium myrtillus*).
 B. Bilberry fruit (*V. myrtillus*) adulterated with elderberry (*Sambucus nigra*).

For an accurate microscopic identification and authentication, the microscopist must ensure that the expected characteristic anatomical features are present, and importantly, the unexpected ones are absent (Applequist and Miller, 2013). Bilberry fruit samples adulterated with elderberry will contain characteristic layers of sclerenchyma fibers arranged in a perpendicular formation (Fig 1 E and F).

The lack of distinguishing chemical characteristics between chromatograms (bilberry sample and bilberry adulterated with elderberry) demonstrates the shortcoming in utilization of HPLC/UV/MS to detect adulteration with foreign plant matter. Chromatographic techniques are only capable of detecting adulteration when evidenced by differences in chemistry.

If adulteration is due to plant material with a low content of extractable and/or detectable chemical constituents, then HPLC/UV/MS cannot easily discern adulterated material from authentic material. While HPLC-based methods can provide detailed access to chemical composition, the procedures require experience, skill, and expensive equipment, and thus are only available to dedicated analytical laboratories with specialized technicians. In addition, chromatographic techniques are limited to the identification of known adulterants with a unique and previously characterized chemical signature.

As such, contamination or adulteration of a plant product with unknown materials or species cannot be reliably determined by chromatographic techniques. Thus, in the current study the pharmacopeial protocols for identification of anthocyanins in commercial bilberry extracts by HPLC were not sufficient to separate the bilberry extract from the bilberry adulterated by an extract from elderberry. The data indicate that current chemical analyses are not useful enough for the commercial marketplace (Govindaraghavan, 2014).

Due to the increase in the demand for botanical

products and corresponding increase in the number of entities involved in the botanical marketplace, rapid screening techniques are needed for the reliable determination of contaminants and adulterants in bilberry products. Our observations with adulteration of bilberry demonstrated the importance of botanical microscopy. Once an integral part of pharmacognosy training, microscopy is now in rapid decline due to the over-reliance on chemistry (Upton, 2006). Microscopy can easily detect foreign matter, such as dirt, sand, fungus, and insect-parts, in a product at a low level that would be easily missed by other techniques. In our study, microscopy proved to be the fastest, simplest, and least expensive method for a quality assessment of bilberry, suggesting that microscopy should always be used as a first-screen for contaminants before any subsequent chemical screens.

Ensuring the quality of bilberry remains a key step in the commercialization of this fruit. Taking into consideration the various aspects described above, a stepwise quality assessment process is recommended for the assessment of bilberry and other botanical products (Figure 5). First, a macroscopic examination (when available) should be done to evaluate that the proper part of the plant is sampled, possesses the proper color, and checked for any obvious signs of mold, insects, dirt, or spoilage. Secondly, a microscopic analysis should be made in which characteristic anatomical features specific for the fruit are identified and in which the absence of other organ parts or other plants, fungi or animal species and inorganic matter is confirmed.

Finally, chemical analyses could be used to confirm the presence of active ingredients at relevant concentrations. Assessments on the quality of bilberry and other botanicals are necessary to determine the efficacy and safety of the plant material, and thus is a critical aspect for the industry of medicinally active plants.

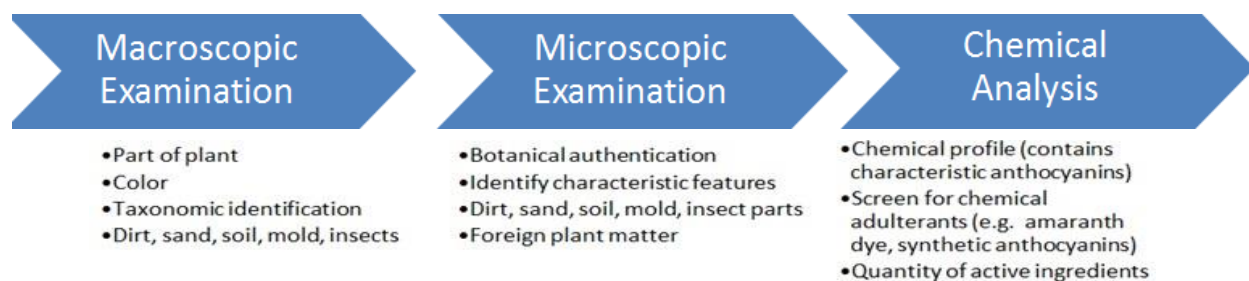


Figure 5. Recommended stepwise process for quality assessment of bilberry fruit.

CONCLUSION

Chromatographic techniques are expensive and require substantial technical expertise to develop and perform methodologies. Microscopy, in contrast, excels in the detection of non-chemical contaminants and a skilled microscopist can easily use taxonomical features to identify the species of material, but cannot elucidate the identity and content of medicinally active compounds. Microscopy is a rapid, inexpensive technique, and can be utilized with little technical experience. Ample resources are available in botanical monograph that can be used to verify the taxonomical identity of plant materials. As such, microscopy works complementary to chromatographic techniques, and should always be done in addition, not as a substitute for chromatographic techniques.

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REFERENCES

- AHPA (American Herbal Product Association). 2015. *Sambucus nigra* fruit. http://www.botanicalauthentication.org/index.php/Sambucus_nigra_fruit. Visited January, 2015.
- Applequist, W.L. and J. S. Miller. 2013. Selection and authentication of botanical materials for the development of analytical methods. *Analytical and Bioanalytical Chemistry* 405: 4419-4428.
- Bao, L., X.S. Yao, C.C. Yau, D. Tsi, C.S. Chia, H. Nagai and H. Kurihara. 2008. Protective effects of bilberry (*Vaccinium myrtillus* L.) extract on restraint stress-induced liver damage in mice. *J. Agric. Food Chem.* 56: 7803-7807.
- Bomsler, J., D.L. Madhavi, K. Singletary, and M.A.L. Smith. 1996. *In vitro* anticancer activity of fruit extracts from *Vaccinium* species. *Planta Medica* 62: 212-216.
- Brinckmann, J. 2011. Reproducible efficacy and safety depend on reproducible quality: their intended uses in cosmetics, dietary supplements, foods and medicines. *HerbalGram* 91:40-55.
- Canter, P.H. and E. Ernst. 2004. Anthocyanosides of *Vaccinium myrtillus* (Bilberry) for night vision- A systematic review of placebo –controlled trials. *Survey of ophthalmology* 49:38-50.
- Cassinese, C, E. de Combarieu, M. Falzoni, N. Fuzzati, R. Pace and N. Sardone. 2007. New liquid chromatography method with ultraviolet detection for analysis of anthocyanins and anthocyanidins in *Vaccinium myrtillus* fruit dry extracts and commercial preparations. *J. AOAC International* 90:911-919.
- Chu, W.K., S.C.M. Cheung, R.A.W. Lau, and I.F.F. Benzie. 2011. Bilberry (*Vaccinium myrtillus* L.) In: *Herbal Medicine: Biomolecular and clinical aspects*. Benzie IFF, S. Wachtel Galore eds. 2nd Edition. Boca Raton (FL): CRC Press.
- Elks, CM, J. Francis, J. AJ Stull, W.T. Cefalu, B. Shukitt-Hale and D.K. Ingram. 2013. Overview of the Health Properties of Blueberries. In: *Bioactives in Fruit: Health Benefits and Functional Foods*. M. Skinner & D. Hunter, eds. pp 251-271. John Wiley & Sons, New York.
- Faria, A., J. Oliveira, P. Neves, P. Gameiro, C. Santos-Buelga, V. de Freitas, and N. Mateus. 2005. Antioxidant properties of prepared blueberry (*Vaccinium myrtillus*) extracts. *J. Agric. and Food Chem.* 53: 6896-6902.
- Foster, S. and M. Blumenthal. 2012. The adulteration of commercial Bilberry extracts. *HerbalGram* 96:64-73.
- Govindaraghavan, S. 2014. Pharmacopeial HPLC identification methods are not sufficient to detect adulterations in commercial bilberry (*Vaccinium myrtillus*) extracts. Anthocyanin profile provides additional clues. *Fitoterapia* 99:124-138.
- He, J. and M.M. Giusti. 2010. Anthocyaninins: Natural colorants with health- promoting properties. *Ann. Rev. Food Sci. Technol.* 1:163-187.

- Hong, V., and R.E. Wrolstad. 1990. Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection. *J. Agric. Food Chem.* 38(3):698-708.
- Kalt W. and D. Dufour. 1997. Health functionality of blueberries. *Horttechnol.* 7: 216-221.
- Katsube, N., K. Iwashita, T. Tsushida, K. Yamaki, and M. Kobori. 2003. Induction of apoptosis in cancer cells by bilberry (*Vaccinium myrtillus*) and the anthocyanins. *J. Agric. Food Chem.* 51:68-75.
- Kong, J.M., L.S. Chia, N.K. Goh, T.F. Chia and R. Brouillard. 2003. Analysis and biological activities of anthocyanins. *Phytochemistry* 64: 923-933.
- Luo, H., X.D. Lv, G.E. Wang, Y.F. Li, H. Kurihara and R.R. He. 2014. Anti-inflammatory effects of anthocyanins-rich extract from bilberry (*Vaccinium myrtillus* L.) on croton oil-induced ear edema and *Propionibacterium acnes* plus LPS-induced liver damage in mice. *Int. J. Food Sci. Nutr.* 65:594-601.
- Matsunaga, N., S. Imai, Y. Inokuchi, M. Shimazawa, S. Yokota, Y. Araki, and H. Hara. 2009. Bilberry and its main constituents have neuroprotective effects against retinal neuronal damage *in vitro* and *in vivo*. *Mol. Nutr. Food Res.* 53:869-877.
- Morazzoni P. and E. Bombardelli 1996. *Vaccinium myrtillus* L. *Fitoterapia* 68:3-29.
- Nile, S.H. and S.W. Park. 2014. Edible berries: Bioactive components and their effect on human health. *Nutrition* 30:134-144.
- Pace, R., P. Morazzoni, and G. Appendino. 2010. Omne ignotum pro magnificum (Ignorance makes everything look magnificent): Getting bilberry out of the adulteration swamp. *Planta Medica* 76-S41.
- Penman, K.G., C.W. Halstead, A. Matthias, J.J. De Voss, J.M. Stuthe, K.M. Bone, and R.P. Lehmann. 2006. Bilberry adulteration using the food dye amaranth. *J. Agric. Food Chem.* 54:7378-7382.
- Stefanut, M.N., A. Cata, R. Pop, C. Tanasie, D. Boc, I. Ienascu and V. Ordodi. 2013. Anti- and mulberry ultrasonic extracts on diabetic hyperglycemic effect of bilberry, blackberry rats. *Plant Foods for Human Nutr.* 68:378-384
- Subash, S., M.M. Essa, S. Al-Adawi, M.A. Memon, T. Manivasagam, and M. Akbar. 2014. Neuroprotective effects of berry fruits on neurodegenerative diseases. *Neural Regen. Res.* 9:1557-1566.
- Szajdek, A. and E.J. Borowska. 2008. Bioactive compounds and health-promoting properties of berry fruits: A review. *Plant Foods for Human Nutr.* 63:147-156.
- Upton, R. 2006. Authentication and quality assessment of botanicals and botanical products used in clinical research. In: *Proceeding of the Fourth International Conference on Quality and Safety Issues related to Botanicals*. Ed. I.A. Khan, T.J. Smillie, L.E. Craker, and Z.E. Gardner. *Acta Hort.* 720:39-48.
- Upton, R. 2001. *Bilberry fruit Vaccinium myrtillus* L. Standards of Analysis, Quality Control, and Therapeutics. Santa Cruz, CA: American Herbal Pharmacopoeia and Therapeutic Compendium.
- Upton, R., A. Graff, G. Jolliffe, R. Langer and E. Williamson. 2011. *American Herbal Pharmacopoeia: Botanical Pharmacognosy: Microscopic Characterization of Botanical Medicines*. CRC Press, Taylor & Francis Group, Boca Raton, Florida, USA.
- Villani, T. S., A.R. Korocho, and J.E. Simon. 2013. An improved clearing and mounting solution to replace chloral hydrate in microscopic applications. *Applicat. in Plant Sci.* 1(5).
- Zafra-Stone, S., T. Yasmin, M. Bagchi, A. Chatterjee, J.A. Vinson and D. Bagchi. 2007. Berry anthocyanins as novel antioxidants in human health and disease prevention. *Mol. Nutr. Food Res.* 51:675-683.
- Zhang, Z., X. Kou, K. Fugal, and J. McLaughlin. 2004. Comparison of HPLC methods for determination of anthocyanins and anthocyanidins in bilberry extracts. *J. Agric. Food Chem.* 52(4):688-691.
- Zhao, Z.Z., Y.N. Hu, Y.W. Wong, W.C.G. Wong, K. Wu, Z.H. Jiang and T. Kang. 2005. Application of microscopy in authentication of Chinese patent medicine—Bo Ying compound. *Microscopy Res. and Tech.* 67:305-315.