Herbal adulteration – the need for advanced sample analysis

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INTRODUCTION

Bilberry (*Vaccinium mytillus)* is a commonly used herbal product which contains anthocyanins - also called anthocyanosides. These are highly coloured natural pigments (red-blue-purple) responsible for the colour of ripe bilberries and many other berries. Up to 20 or more individual anthocyanins are present in Bilberry and are thought to be the therapeutic component are used as the quality marker for the product. The British Pharmacopeal monograph and most analytical procedures use a routine UV-VIS spectrophotometric assay at one wavelength to quantify the anthocyanin content. This non-specific assay is very susceptible to adulteration issues. We have evaluated a commercial Chinese Bilberry extract certified by the supplier to contain ~25% anthocyanins by this spectrophotometric method and by more advanced sample analysis such as HPLC-DAD, UV-spectral comparison and LC/MS as well as profiling by HPTLC. An unkown component was separated and characterised by ^{13}C NMR and MS.

Analytical Results

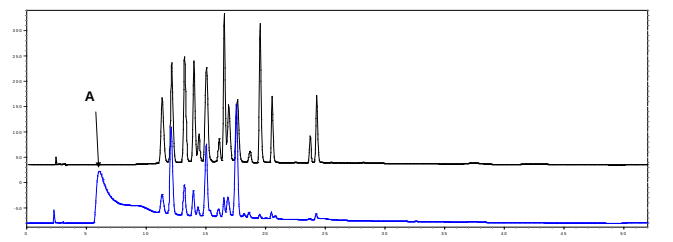

Figure 1: HPLC Traces for Bilberry Extracts
Quality extract (black) and Chinese extract (blue)

The Chinese extract inconsistent with the reference extract, exhibiting a profile dominated by an early eluting, large, broad, poorly-shaped peak (A). This peak remains unchanged after an acid hydrolysis step which is routinely used for Bilberry quantitation procedures (the sugars are removed to form 5-6 anthocyanidins and results in a simpler chromatogram for analysis).

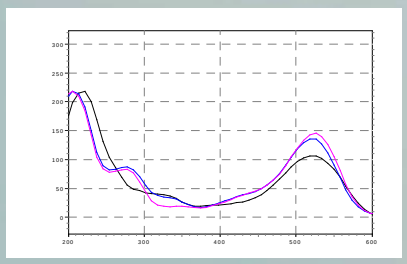

Figure 2: UV-VIS Spectra
black spectra : peak A; other spectra: typical anthocyanins

Peak A possessed a UV-VIS spectrum similar to that seen for anthocyanins (Figure 2) with its maxima at the same wavelength measured for the anthocyanins using the single wavelength protocol as stated in the BP method.

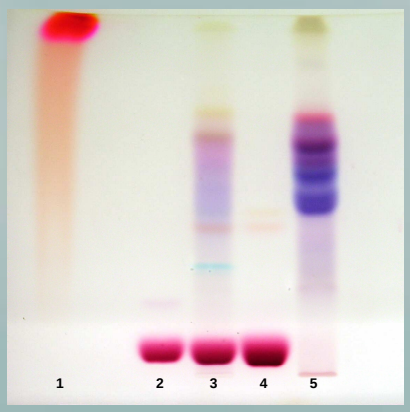

Figure 3: Bilberry HPTLC chromatograms
1 = erythrosine; 2 = isolated peak A ; 3 = Chinese extract; 4 = amaranth; 5 = quality bilberry extract

HPTLC highlighted the differences between the two extracts. Using column chromatography a fraction was isolated from the Chinese extract. After separation the unkown was similar to the chromatogram obtained for amaranth in Track 4. NMR data (^{1}H and ^{13}C) indicated naphthyl-like features and this along with the UV-VIS information was used in a literature search that yielded amaranth as a possible match.

LCMS analysis yielded retention time and mass spectral match of amaranth to the isolated material from the Chinese Extract (APCI negative ion mode displaying M-H peaks, adducts and similar fragmentations patterns).

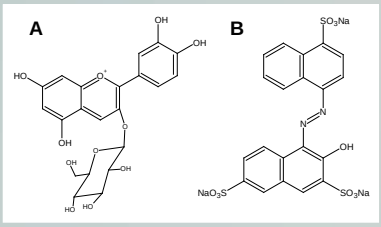

Figure 4: Compound Structures
A = an anthocyanin (Cyanidin-3-glucoside); B = Amaranth

Sample	% Anthocyanins	
	BP Method	pH Differential Method
Reference Bilberry	24.6%	24.6%
Chinese Extract	24.3%	6.6%
Erthyrosine		-48.5%
Amaranth		-0.1%

Table 1: UV-VIS calculation of anthocyanin content using the photometric methods

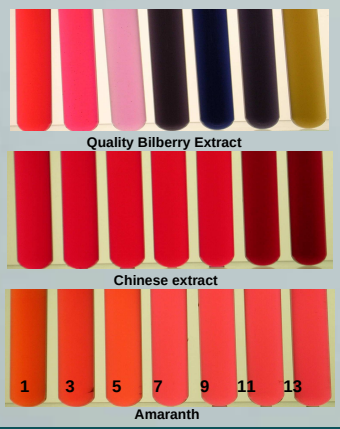

Figure 6: pH colour dependence of Anthocyanins but not amaranth
(numbers are pH values)

This technique reflects the known dependence on pH for the colour and stability of anthocyanins in solution (Figure 6). Amaranth and the Chinese extract do not display these same pH colour dependencies. Various equilibria as well as irreversible structural changes account for the colour transformations seen at different pH.

Summary

The sophistication of the adulteration is evident in that when following the British Pharmacopeal method, the extract yields the expected anthocyanin content. The UV-VIS scan profile of the adulterated extract closely mimics the profile of an anthocyanin-containing solution. The sample is also unlikely to be true bilberry due to incorrect profile of anthyocyanins.

This highlights the need for advanced sample analysis when dealing with Herbal Medicinal Products.

REFERENCE: Anthocyanin content in bilberry by pH-differential spectrophotometry - Institute of Nutraceutical Advancement (INA) method 116.000 (http://www.nsf.org/business/ina/bilberry.asp)

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