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Short communication

Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: Antioxidant and enzyme inhibitory effects

Helle Wangensteen[a,*], *Marie Bräunlich*[a], *Viktoria Nikolic*[a], *Karl Egil Malterud*[a] *Rune Slimestad*[b], *Hilde Barsett*[a]

[a]*Department of Pharmaceutical Chemistry, School of Pharmacy, University of Oslo, P.O. Box 1068 Blindern, N-0316 Oslo, Norway*
[b]*PlantChem, Sørheim Research Center, N-4353 Klepp Station, Norway*

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ABSTRACT

Aronia berries are known for their high content of anthocyanin and proanthocyanidin. Four different cultivars of aronia berries, *Aronia melanocarpa* 'Moskva', 'Hugin', 'Nero' and *Aronia prunifolia*, were studied with respect to their phenolic composition, antioxidant and enzyme inhibitory activities. Quantification of anthocyanins was determined by HPLC and separation was accomplished in less than 4 min. Cyanidin 3-galactoside was the major anthocyanin in all cultivars, with the highest content in *A. prunifolia* (497 ± 20 mg/100 g FW). *A. prunifolia* was also found to have the highest content of polyphenols (2996 ± 172 mg gallic acid equivalents/100 g FW) and proanthocyanidins (4.79 g procyanidin B2 equivalents/100 g FW). As antioxidants and enzyme inhibitors, the differences between extracts from the tested berries were minor. Berries from *A. prunifolia* constitute the richest source of polyphenols and might be the species of choice in order to attain berries with a high content of anthocyanin and proanthocyanidin.

1. Introduction

In recent years, substantial interest has been expressed in fruits and berries due to their potential favourable health effects and high content of polyphenols as flavonoids and anthocyanins (Miller & Shukitt-Hale, 2012; Tsuda, 2012; Wallace, 2011). The anthocyanins are a subgroup of the flavonoids, and they occur naturally in plants as glycosides. Anthocyanins have been suggested to exert positive effects against obesity, diabetes and cardiovascular disease, as well as to have a positive effect on cognitive function (Miller & Shukitt-Hale, 2012; Norberto et al., 2013; Tsuda, 2012). These effects have been attributed to the high antioxidant capacity of the anthocyanins, and the effects can more precisely be ascribed to molecular mechanisms, such as scavenging of free radicals, inhibition of radical forming and peroxidative enzymes, upregulation of antioxidant enzymes, regulation of signaling pathways and vasodilatory mechanisms (Mladenka,

* *Corresponding author*. Tel.: +47 22856567; fax: +47 22857505.
E-mail address: helle.wangensteen@farmasi.uio.no (H. Wangensteen).
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Zatloukalová, Filipský, & Hrdina, 2010; Sies, 2010). Meanwhile, proanthocyanidins have been widely investigated with respect to their beneficial effects on cardiovascular diseases. Consumption of proanthocyanidin-rich foods such as red wine and cocoa (chocolate) seems to decrease blood pressure and insulin resistance and reverse endothelial dysfunction (Grassi et al., 2005; Heiss et al., 2005, 2007; Taubert, Roesen, Lehmann, Jung, & Schömig, 2007).

The genus *Aronia* (Rosaceae family) includes two species of shrubs, native to eastern North America and Eastern Canada: *Aronia melanocarpa* (Michx.) Ell., known as black chokeberry and *Aronia arbutifolia* (L.) Pers. (red chokeberry) (Kokotkiewicz, Jaremicz, & Luczkiewicz, 2010; Kulling & Rawel, 2008). *Aronia prunifolia* (purple chokeberry) is regarded as a hybrid between *A. melanocarpa* and *A. arbutifolia* (Kokotkiewicz et al., 2010), but it is however ambiguous if it is a separate species. *A. prunifolia* (Marshall) Rehder is indexed in The International Plant Names Index (2012). Cultivars used for fruit production are from the species *A. melanocarpa* (e.g. berries are used for juice, jam and wine production). Commercially important cultivars in Europe and the United States include 'Aron', 'Nero', 'Viking', 'Hugin' and 'Rubina'. In Norway, the cultivar 'Moskva' is most common. The aronia berries contain high levels of flavonoids, mostly proanthocyanidins and anthocyanins, and *in vitro* and *in vivo* studies indicate that the berries may have potential health benefits, e.g. hepatoprotective effects, cardioprotective effects, and antidiabetes effect (reviewed by Denev, Kratchanov, Ciz, Lojek, & Kratchanova, 2012; Kulling & Rawel, 2008). Thus, aronia berries should have a potential as functional food ingredients. In order to have a systemic biological effect and impact on health, sufficient amounts of bioactive compounds have to be absorbed through the gastrointestinal tract and reach the systemic circulation. Generally, the bioavailability of anthocyanins is rather low. The bioavailability of anthocyanins from aronia juice has been investigated in humans (Wiczkowski, Romaszko, & Piskula, 2010), and it was shown that both cyanidin glycosides and their methylated and/or glucuronidated derivatives were present in plasma. Also, it appears likely that anthocyanin metabolites produced by the intestinal microflora can be absorbed and may contribute to the beneficial health effects of anthocyanins (Manach, Williamson, Morand, Scalbert, & Rémésy, 2005; Williamson & Clifford, 2010). In addition, the bioavailability of proanthocyanidins is not fully understood. However, dimers and trimers of A- and B-type procyanidins have been detected in plasma of rats (Appeldoorn, Vincken, Gruppen, & Hollman, 2009; Shoji et al., 2006). Chokeberries are a rich source of B-type proanthocyanidins with contents above 5 g/100 g dry matter (Oszmiański & Wojdylo, 2005).

Increased popularity of aronia products as functional food is expected in the future (Kulling & Rawel, 2008). New functional food products containing aronia berries or aronia juice are popular products due to their assumed health beneficial effects. In a recent study, we found that chokeberries were powerful antioxidants and enzyme inhibitors, and anthocyanins and proanthocyanidin-rich fractions from the berries could be responsible for the effects (Bräunlich et al., 2013). However, to the best of our knowledge, no scientific papers have described the differences in anthocyanin composition, in antioxidant capacity and α-glucosidase inhibitory activity between the *A. melanocarpa* cultivars 'Moskva', 'Hugin', 'Nero' and *A. prunifolia*. In addition, a dietary supplementation with aronia juice has been reported to ameliorate diabetes in humans (Simeonov et al., 2002). The aim of this study was to investigate the chemical composition (quantification of anthocyanins and content of total phenolics and proanthocyanidins) as well as the biological activities (1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and inhibition of 15-lipoxygenase (15-LO), xanthine oxidase (XO) and α-glucosidase) of these four berry sources.

2. Materials and methods

2.1. Plant material

Three *A. melanocarpa* cultivars ('Moskva', 'Hugin' and 'Nero') and an *A. prunifolia* cultivar were included in this study. The cultivar 'Moskva' was collected at Klepp, Norway (August 2010), 'Hugin' was collected in Tøyen Botanical garden, Oslo, Norway (August 2011), the cultivar 'Nero' was collected at Obstbau GbR, Coswig, Germany (September 2011) and *A. prunifolia* was collected in a private garden in Schönefeld, Germany (September 2011). The diameter of the berries was measured; results are shown as the average diameter of five berries of each cultivar. The berries were kept at −20 °C until extraction. Voucher specimens are deposited at School of Pharmacy, University of Oslo, Norway ('Moskva' MB201201; 'Hugin' MB201202; 'Nero' MB201203; *A. prunifolia* MB201204).

2.2. Extraction of phenolics

Frozen aronia berries, 'Moskva', 'Hugin', 'Nero' and *A. prunifolia*, (500 g of each) were extracted with boiling 80% ethanol with a reflux condenser for 2 h. The extraction was repeated twice. The extracts were filtered and combined, and then concentrated on a rotary evaporator followed by lyophilisation to give an 80% ethanol crude extract for each of the aronia cultivars.

For anthocyanin analysis, 10 g berries of each cultivar were weighed and frozen at −20 °C. The frozen berries were cut in small pieces, placed into a screw cap tube and freeze dried before extraction with 10 mL of methanol/0.5% trifluoroacetic acid (TFA) for 40 min at 4 °C using a magnetic stirrer. The extraction was repeated four times, and the extracts were combined after filtration. Extraction was done in five parallels for each aronia cultivar.

2.3. HPLC analysis of anthocyanins

Analysis was performed on a LaChrom Elite HPLC system (VWR-Hitachi) equipped with an L-2455 diode array detector. A Chromolith Performance RP18e 100 × 4.6 mm column (Merck, Darmstadt, Germany) was used for separation. Elution was performed using a gradient of mobile phase A (0.5% TFA in water) and mobile phase B (0.5% TFA in acetonitrile) with the following time schedule: 10% B, 0–1 min; 10–20% B, 1–3 min; 20–85% B, 3–4 min; 85–10% B, 4–5 min; and finally 10% B, 5–6 min for reconditioning of the column. The flow rate was 3.0 mL/min and injection volume was