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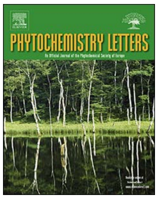

Phytochemical variation in the plant-part specific phenols of wild crowberry (*Empetrum hermaphroditum* Hagerup) populations

Anu Lavola*, Anneli Salonen, Virpi Virjamo, Riitta Julkunen-Tiitto

Department of Environmental and Biological Sciences, Natural Product Research Laboratories, University of Eastern Finland (UEF), P.O. Box 111 FI-80101 Joensuu, Finland

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ABSTRACT

The phytochemical content in plants can develop variably according to e.g. the microclimate and the soil conditions of the habitat. The evergreen dwarf shrub species *Empetrum* sp. occur widely in the northern hemisphere, where it is suggested to have its ecological contribution to the growth environment by a chemical interference with high constitutive levels of phenolic metabolites. We determined by HPLC and UHPLC-qtof-MS the individual phenolic compounds of fruits, leaves, stems and roots in *Empetrum hermaphroditum* plants growing on three different lake districts and under differing microclimate and soil conditions in order to find out natural, habitat related differences in the accumulation of phenols among the populations. The phytochemical content in *E. hermaphroditum* turned out to be diverse, plant-part specific and to vary a lot among the populations. Anthocyanins and flavanols were the most abundant phenolic compounds of fruits. Of the thirty eight compounds identified in leaves, stilbenoids were quantitatively in the majority but also a flavanone, pinocembrin was found in high quantities in some populations. Stems and roots contained mainly catechins, procyanidins and proanthocyanidins. Despite of the plant-part and habitat specific variation, some groupings of populations could be formed according to their phenolic constituents by PCA.

1. Introduction

Crowberry, *Empetrum* is an ericaceous dwarf-shrub species that occur widely in arctic and boreal areas of the northern hemisphere: In northern Europe, Eurasia and Canada. It is an important ground-layer species, dominating landscape in dry and low-nutrient condition forest types. These slow-growing plants are ecologically regarded as nutrient-conserving and stress-tolerant species, i.e. the plants which may allocate a great part of their recourses to secondary metabolites (e.g. Väisänen et al., 2013; Shevtsova et al., 2005). In the subarctic tundra ecosystems, crowberry colonies have shown to exert some ecological pressure due to their chemical interference with high constitutive levels of phenolic metabolites (González et al., 2015; Tybirk et al., 2000; Gallet et al., 1999). Accordingly, its edible berries are considered pro-healthy because of high concentrations of anthocyanins and other antioxidants (Jurikova et al., 2016; Ogawa et al., 2008; Kähkönen et al., 2001).

It is widely recognized, that the secondary metabolites play an important role in plants' adaptation to the growth environment. Phenolic acids, flavonoids, and tannins are the phenolic secondary compounds of woody shrubs and trees, which may respond by fluctuating concentrations to different kinds of environmental growth factors and stress conditions, such as enhancement in light (including UV-radiation), CO_2 concentration, temperature, soil nutrition, water deficit/drought and grazing (Lavola et al., 2013; Väisänen et al., 2013; Gwynn-Jones et al., 2012; Eichholz et al., 2011; Deluc et al., 2009; Heinäaho et al., 2006).

It is the fruits of crowberries that have been most intensively studied and which phenolic profiles have been previously determined (Laaksonen et al., 2011; Ogawa et al., 2008; Häkkinen et al., 1999a). Of the two closely related subspecies, the southern and diploid *Empetrum nigrum* L. has been shown to contain constitutively lower phenolic levels in fruits compared to the northern one, tetraploid *Empetrum hermaphroditum* spp. *hermaphroditum* Hagerup (Määttä-Riihinen et al., 2004; Häkkinen et al., 1999b). In the previous studies concerning the communities of *E. hermaphroditum*, it has been observed that the phenol content of fruits in varies geographically among and within different populations (Kellogg et al., 2010; Koskela et al., 2010), and the phenol content of leaves varies temporarily among different habitats and environmental conditions (Väisänen et al., 2013; Shevtsova et al., 2005; Gallet et al., 1999). However, the information about the natural variation in the phytochemical levels of wild, shrubby

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* Corresponding author.
E-mail addresses: anu.lavola@uef.fi (A. Lavola), anneli.salonen@uef.fi (A. Salonen), virpi.virjamo@uef.fi (V. Virjamo), riitta.julkunen-tiitto@uef.fi (R. Julkunen-Tiitto).

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berry species and the environmental effects on the other plant parts than fruits is rather limited, although the phenols in leaves, stems or flowers of some wild berry species have been analyzed (Lätti et al., 2011; Hokkanen et al., 2009; Harris et al., 2007; Witzell et al., 2003).

In the lake districts of Eastern Finland, the populations of northern crowberry, *E. hermaphroditum*, grow in the southern parts of their distribution area. The populations are found both at hot, sunny and rocky habitats on open lake islands and at more shady, forested mainland habitats. As *Empetrum* sp. are wind-pollinated species and seeds are also spread by birds, it is supposed that the genetic variation among the populations within a lake district is not very high and the variation in the secondary chemical levels of populations is mainly caused by different microclimate and soil conditions. In order to clarify the phytochemistry of *E. hermaphroditum* and find out the natural, habitat related variation among the populations we collected plant material from three different lake districts and there, from two differing growing sites, the rocky islands at the open lakes and the forested mainland areas of the lakesides. The concentrations of individual phenolic compounds were determined from different plant parts (fruits, leaves, stems and roots) by liquid chromatography (HPLC). The data was further analyzed by using statistical multivariate techniques, the principal component analysis (PCA), to find out the phytochemical based differences among populations.

2. Results and discussion

2.1. Phenolic phytochemicals in fruits

The phytochemical content of *E. hermaphroditum* fruits consisted mainly of flavonoids (Fig. 2A,B). Of the soluble phenolic compounds detected, 15 were identified as anthocyanins (delphinidin, cyanidin, petunidin, peonidin and malvidin glycosides) and 14 as flavonols (myricetin and quercetin glycosides) (Table 1). Methanol-soluble tannins (proanthocyanidins) were the second abundant component that comprised about the other half of the phenolic content of fruits (37–43%). The phenolic acids of fruits were benzoic, cinnamic and chlorogenic acid derivatives, which were in the minority (Fig. 3, Table 1). The total amount of the phenolic compounds in fruits varied among populations and accounted for 0.6 to 1% of the fresh weight, on average (Fig. 4A).

In crowberry fruits, the composition of phenolic compounds is diverse and the flavonoid concentrations are high compared to many other berries (Lavola et al., 2012; Kähkönen et al., 2001; Häkkinen et al., 1999a, 1999b). In particular, the anthocyanin content is very high and complex and resembles of that of the bilberry (*Vaccinium myrtillus* L.) (Ogawa et al., 2008; Harris et al., 2007; Määttä-Riihinen et al., 2004). Although the fruits of *Empetrum* are edible and found in large amounts in northern hemisphere forests, they are poorly utilized natural recourse in spite of that they could have a great potential for example in food industry. Because the fruits contain high amounts of flavonoids with multiplicity of specific anthocyanins, they may be regarded as important sources of health-promoting bioactive compounds and could be more widely used for example in functional food processing (e.g. Jurikova et al., 2016; Törrönen et al., 2012; Huttunen et al., 2011 and Laaksonen et al., 2011).

The phytochemical concentrations of fruits varied among the collection sites among different populations (Fig. 4A) and also, some lake district-specific differences in the phytochemical composition were detected. The populations of Lake Pielinen had the lowest amounts of phenolic acids in fruits (Fig. 3, Table 1). The amounts of flavonoids and proanthocyanidins were the highest in fruits from Lake Rikkavesi populations and the lowest from Lake Koitere populations (Fig. 3). Furthermore, there were differences in the proportions of anthocyanins according to the lake-districts, while the proportions of flavonols in the fruits were more similar (Table 1).

The amounts of flavonoids and especially, anthocyanins in wild grown and cultivated berries have been shown to fluctuate according to growing site and to be influenced by both biogeographical and microclimatical factors (Lavola et al., 2012; Lätti et al., 2010; Giovanelli and Buratti, 2009; Sellappan et al., 2002). In the wild berry species of Alaska, site-specific variations in anthocyanin and proanthocyanidin contents of fruits have been observed, and the highest anthocyanin contents were detected in *E. hermaphroditum* populations growing under most extreme annual climate variations (Kellogg et al., 2010). Studies of grapes have shown that light, temperature, water deficit and availability of nutrients affect significantly the maturation of the fruits as well as the composition and the content of phenolic compounds during ripening (Downey et al., 2006; Deluc et al., 2009). Thus, compositional differences in the phenol concentrations may

Table 1
The amounts of small molecular weight phenols in fruits (mg 100 g^{-1} fr. wt ± s.e.) and the proportions of different flavonoids ($^{1)}$ % of flavonols, $^{2)}$ % of anthocyanins) in Lake Rikkavesi, Lake Koitere and Lake Pielinen populations. Statistically significant differences among lakes are marked with bold. The results of post-hoc test are indicated with different letters ($p < 0.05$), and transformations to fullfil assymptions of ANOVA are marked in superscript. Compounds tested with non-parametric *Kruskal-Wallis* test are marked with asterisks.

Fruit phenols	Lake Rikkavesi	Lake Koitere	Lake Pielinen
Protocatechuic acid log10(x)	**0.26 ± 0.02^{a}**	**0.28 ± 0.02^{a}**	**0.18 ± 0.01^{b}**
Neochlorogenic acid log10(x)	**2.71 ± 0.18^{a}**	**3.90 ± 0.02^{b}**	**2.32 ± 0.32^{a}**
p-Hydroxycinnamic acid 1/x	**11.2 ± 0.83^{a}**	**14.83 ± 2.20^{a}**	**6.17 ± 0.45^{b}**
Myricetin derivative	7.21 ± 0.41	6.21 ± 0.35	6.54 ± 0.34
Myricetin 3-galactoside	**0.43 ± 0.03^{a}**	**0.36 ± 0.03^{b}**	**0.34 ± 0.02^{b}**
Myricetin 3-rhamnoside	1.33 ± 0.06	1.19 ± 0.07	1.23 ± 0.07
Myricetin	**0.39 ± 0.02^{a}**	**0.31 ± 0.02^{b}**	**0.36 ± 0.03^{b}**
Myricetins%$^{1)}$	37.6	37.6	38.7
Quercetin 3-galactoside	5.66 ± 0.43	5.04 ± 0.46	4.85 ± 0.39
Quercetin 3-glucoside	**3.73 ± 0.21^{a}**	**3.12 ± 0.20^{b}**	**3.21 ± 0.18^{b}**
Quercetin 3-arabinopyranoside 1/x	**0.26 ± 0.21^{a}**	**0.25 ± 0.02^{a}**	**0.19 ± 0.01^{b}**
Quercetin 3-arabinofuranoside 1/x	0.19 ± 0.01	0.17 ± 0.01	0.16 ± 0.01
Quercetin 3-rhamnoside	1.07 ± 0.07	0.99 ± 0.07	0.99 ± 0.05
Quercetin derivative 1 log10(x)	**1.71 ± 0.20^{b}**	**2.10 ± 0.19^{a}**	**0.38 ± 0.04^{c}**
Quercetin derivative 2*	**2.50 ± 0.36**	**1.33 ± 0.22**	**3.42 ± 0.20**
Quercetin log10(x)	**0.43 ± 0.05^{a}**	**0.40 ± 0.06^{a}**	**0.50 ± 0.02^{b}**
Flavonoid derivative 1*	**0.54 ± 0.08**	**0.28 ± 0.07**	**0.69 ± 0.04**
Flavonoid derivative 2*	0.54 ± 0.04	0.47 ± 0.04	0.50 ± 0.02
Quercetins%$^{1)}$	62.4	62.4	61.3
Delphinidin 3-galactoside	**117.9 ± 2.51^{a}**	**102.7 ± 5.04^{b}**	**106.9 ± 6.42^{ab}**
Delphinidin 3-glucoside x^2	0.74 ± 0.07	0.59 ± 0.03	0.67 ± 0.05
Delphinidin 3-arabinoside	13.2 ± 0.27	12.2 ± 0.65	12.4 ± 0.74
Delphinidins%$^{2)}$	25.5	26.4	26.1
Cyanidin 3,5-diglucoside	**0.18 ± 0.01^{a}**	**0.12 ± 0.02^{b}**	**0.13 ± 0.02^{b}**
Cyanidin 3-galactoside	**111.1 ± 3.13^{a}**	**93.6 ± 6.84^{b}**	**93.0 ± 7.75^{b}**
Cyanidin 3-glucoside	**1.48 ± 0.07^{a}**	**1.21 ± 0.13^{b}**	**1.21 ± 0.14^{b}**
Cyanidin 3-arabinoside*	**24.3 ± 0.60**	**21.1 ± 1.57**	**20.7 ± 1.53**
Cyanidins%$^{2)}$	26.5	26.6	25.0
Petunidin 3-galactoside	**48.4 ± 0.81^{a}**	**40.4 ± 2.56^{b}**	**44.1 ± 2.08^{ab}**
Petunidin 3-arabinoside	**5.22 ± 0.10^{a}**	**4.62 ± 0.23^{b}**	**4.85 ± 0.25^{ab}**
Petunidins%$^{2)}$	10.4	10.3	10.6**1.71^{b}**
Peonidin 3-galactoside	**41.7 ± 1.35^{a}**	**34.3 ± 2.78^{b}**	**34.9 ±**
Peonidin 3-glucoside	**2.68 ± 0.16^{a}**	**2.06 ± 0.21^{b}**	**2.09 ± 0.24^{b}**
Peonidin 3-arabinoside	**1.91 ± 0.04^{a}**	**1.50 ± 0.07^{b}**	**1.63 ± 0.05^{b}**
Peonidins%$^{2)}$	8.9	8.7	8.4
Malvinidin 3-galactoside	**135.0 ± 3.50^{a}**	**110.8 ± 3.76^{c}**	**124.5 ± 2.03^{b}**
Malvinidin 3-glucoside*	0.14 ± 0.03	0.08 ± 0.03	0.10 ± 0.03
Malvinidin 3-arabinoside	**13.8 ± 0.38^{a}**	**11.6 ± 0.40^{b}**	**13.0 ± 0.30^{a}**
Malvidins%$^{2)}$	28.8	28.0	29.9
TOTAL	557.9 ± 16.0	478.2 ± 28.9	491.2 ± 25.6