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Variability in the composition of phenolic compounds in winter-dormant *Salix pyrolifolia* in relation to plant part and age

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ABSTRACT

The phenolic phytochemicals of winter-dormant *Salix pyrolifolia* were determined from the vegetative buds, and the bark and wood of different-aged twigs by HPLC-DAD and UHPLC-QTOF-MS analyses. All the plant parts were composed of salicylate glucosides and the other *Salix*-specific, simple phenolic glucosides as well as of phenolic acids, flavonoids and the high molecular-weight condensed tannins. The flavonoid composition was most diverse in buds and they also contained a large amount of chlorogenic acid (5-caffeoylquinic acid IUPAC), while salicylate glucosides and simple phenolic glucosides predominated in bark. The wooden interior part of the twigs contained fewer components and the lowest concentrations of compounds. Salicortin was the main compound in winter-dormant *S. pyrolifolia* (over 10% of bark biomass), but the concentrations of picein, salireposide, isosalipurposide, catechin and condensed tannins were also high. The flavonoid composition was highly naringenin- and quercetin-biassed. The composition of phytochemicals was organ-specific and remained relatively similar between different-aged trees. However, there were compound-specific fluctuations in the concentrations of phytochemicals with the age of the trees and within plant parts. Generally, the one-year-old plants differed from the older trees in their high concentration of condensed tannins in all the plant parts studied and in the highest concentration of isosalipurposide in bark, while the total amounts of salicylate glucosides in plant parts, and of naringenin glucosides in buds, tended to be highest in 20 year-old-trees.

1. Introduction

In the classification system proposed by Skvortsov (1999), genus *Salix* can be divided into three subgenera, *Salix*, *Chamaetia* and *Vetrix*, within each of which there are many sections. However, the infrageneric division is still highly controversial, despite of the recent molecular-phylogenetic studies that have enlighten in particular the subgeneric classification within *Salix* and *Vetrix* (e.g. Lauron-Moreau et al., 2015; Trybush et al., 2008; Wu et al., 2015). On the morphological basis, *Salix pyrolifolia* Ledeb. has been phylogenetically grouped to section Hastatae in the subgenus *Vetrix*, which is a boreal and arctic-alpine holarctic group consisting 12–15 mostly American species, and in Eurasia only four other species are included to this section: *S. hastata, S. karelinii, S. apoda* and *S. fedtschenkoi* (Skvortsov, 1999).

In chemotaxonomy, closely related plant species and genera are assumed to share characteristic secondary compound composition, and the chemotaxonomic identification of species can be based on different types of plants' secondary metabolites, such as phenolics, alkaloids and terpenoids (e.g. Crawford and Giannasi, 1982). Since conjugated salicyl alcohol derivatives, i.e. salicylates (or salicinoids), are found widely in the bark and leaves of *Salix* species (e.g. Boeckler et al., 2011), screening of their concentrations among species has been used for the chemotaxonomic identification of the species (e.g. Julkunen-Tiitto, 1986, 1989a,b; Palo, 1984). Some of the phenolic compounds have previously been analysed from the leaves and twigs of *S. pyrolifolia* (Julkunen-Tiitto, 1989a,b). According to the cluster analysis, which was based on the analysis of salicylate glucosides and other *Salix*-specific, simple phenolic glucosides in current-growth twigs from 33 different *Salix* species (Julkunen-Tiitto, 1989a), *S. pyrolifolia* was suggested to belong to the same group as *S. hastata*, *S. daphnoides* and *S. alba*, even though these species morphologically derived from different sections, Hastatae, Daphnella and Salix, respectively (Skvortsov, 1999). However, the corresponding clustering according to the leaf phytochemicals has resulted in totally different grouping of species (Julkunen-Tiitto, 1989a). Concomitantly, the chemotaxonomical classification of the specific *Salix* species and *S. pyrolifolia* is still uncertain.

It is known, that in the Salicaceae species the composition of phytochemicals varies highly among species, between geno- and ecotypes,

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and even within an individual as the phytochemical content in a plant depends on its the developmental phase as well as the environmental factors under which it is growing (e.g. Förster et al., 2008, 2010; Ikonen, 2002; Ikonen et al., 2002; Julkunen-Tiitto and Virjamo, 2017; Nyman and Julkunen-Tiitto, 2005; Paunonen et al., 2009; Randriamanana et al., 2015a,b). Furthermore, recent studies with several *Salix* species report remarkable plant part-specific differences in the concentrations of phenolic compounds between genders, and among clones and hybrids (Hallgren et al., 2003; Nissinen et al., 2016; Nybakken and Julkunen-Tiitto, 2013; Nybakken et al., 2012; Randriamanana et al., 2015b; Sivadasan et al., 2015; Torp et al., 2013; Sulima et al., 2017). More detailed characterization of the phytochemicals from different-aged individuals and various types of tissues is necessary also for improvement on the chemotaxonomical identification of the species, especially those that are highly active in hybridization.

S. pyrolifolia is a small tree, endemic to eastern taiga and especially to the southern parts of Siberia, where it grows mainly in chalky water meadows (e.g. Skvortsov, 1999). Because *S. pyrolifolia* is rare and protected in Finland, and all individuals are found only in two places of northern Finland (Hämet-Ahti et al., 1998), the previous phytochemical analyses have been made only for salicylates and some other low molecular-weight phenolic glucosides (Julkunen-Tiitto, 1989a). Therefore, the aim of this study was to further enlighten overall phenolic composition of *S. pyrolifolia* by expanding the phytochemical analyses to include various phenolic compound groups in addition to salicylate glucosides (other simple phenolic glucosides, phenolic acids, flavonoids, tannins) in different plant parts (vegetative buds, bark and wood of current- and previous-year growth). The main focus was on the effect of ageing on the phytochemical composition of the winter-dormant plant parts, and for this reason, the juvenile and adult twigs of different-aged *S. pyrolifolia* tree were analysed.

2. Results and discussion

2.1. Phytochemicals in winter-dormant S. pyrolifolia

In this study, the main phenolic compounds of clonal *S. pyrolifolia* tree were analysed from the different plant parts of winter-dormant twigs. The phenolic profile of the twigs was mainly composed of the salicylate glucosides and other *Salix*-specific, simple phenolic glucosides but also, several types of flavonoid glycosides and phenolic acids were detected (Table 1, Fig. 1). These low molecular-weight (LMW) compounds comprised about 20% of the dry weight in twigs (Fig. 2). In addition, all the plant parts contained high molecular-weight condensed tannins (proanthocyanidins), which comprised about 10% of the dry wt in twigs (Fig. 2). This high content of phytochemicals may be suggested to be partly attributable to the high resistance that *S. pyrolifolia* shows to microbes and herbivores (e.g. Boeckler et al., 2011; Riitta Julkunen-Tiitto, pers. observation).

Salicylates (salicinoids), the derivatives of salicyl alcohol with β-D-glucopyranose moieties, as well as some other simple glucosides derived from cinnamic acid, are typical compounds for *Salix* species. In here, the winter-dormant twigs of *S. pyrolifolia* were composed of four different types of salicylate glucosides: salicin, HCH-derivative (hydroxycyclohexenone) of salicin, salicortin and disalicortin (Table 1). Salicin (glucoside of salicyl alcohol) is the most widespread and simplest glucoside in *Salix* species, which is detected in fairly low amounts in leaf tissues but may occur in quantities in bark (Heiska et al., 2007; Julkunen-Tiitto, 1989a, 1986; Pobłocka-Olech et al., 2007), in buds (Julkunen-Tiitto, 1989a; Sivadasan et al., 2015) and also, is found in seeds (Randriamanana et al., 2015b). Additionally, most *Salix* species contain high amounts of salicortin, the HCH-ester of salicin, while only a few species are rich in its derivatives, acetylsalicortin or tremulacin (e.g. Boeckler et al., 2011; Meier et al., 1988; Julkunen-Tiitto, 1989a). In winter-dormant *S. pyrolifolia*, salicortin is the most abundant individual compound, and especially in bark, its concentration is extremely high, comprising over 10% of the dry wt (Table 2). Another HCH-derivative of salicin with the UV-spectrum similar to salicortin but much longer retention time, and which molecular structure is suggested to consist of salicin with the HCH moiety attached to glucose (Fig. 1) due to the accurate molecular mass of salicin + HCH based on the QTOF-analysis, is preliminary characterised here for the first time from the twigs of *Salix* species (Table 1).

The non-salicylate-based, simple phenolic glucosides in *S. pyrolifolia* were salireposide, triandrin (the glucoside of coumaroyl alcohol), picein (*p*-hydroxyacetophenone glucoside) and its derivative. These compounds are generally regarded as the twig-specific components of *Salix* species (e.g. Julkunen-Tiitto, 1989a; Meier et al., 1988). The concentrations of both picein and triandrin fluctuated among the sampled twigs and in some samples, triandrin was found only in trace quantities (Tables 2 and 3). The high within-species and intra-plant variation in the concentrations of these simple phenolic glucosides is a commonly reported phenomenon in *Salix* species (e.g. Heiska et al., 2007; Ikonen, 2002; Julkunen-Tiitto, 1989b; Nybakken and Julkunen-Tiitto, 2013; Nyman and Julkunen-Tiitto, 2005; Sulima et al., 2017). For the most part, the composition of both salicylate glucosides and simple phenolic glucosides in this study was in accordance with previous analyses from *S. pyrolifolia* (Julkunen-Tiitto, 1989a,b). Here, however, we did not find acetylsalicin (fragilin) or acetylsalicortin in the samples, but on the other hand, we identified the HCH-derivative of salicin, disalicortin, triandrin and salireposide as new components in *S. pyrolifolia* twigs. Salireposide has so far been detected in only a few *Salix* species: *S. myrsinifolia, S. petiolaris, S. purpurea* and *S. rosmarinifolia* (e.g. Boeckler et al., 2011; Julkunen-Tiitto, 1989a; Meier et al., 1988; Nybakken and Julkunen-Tiitto, 2013).

The flavonoid composition among *Salix* species is found to be varied and anthocyanins, flavonols, flavones, flavanones and chalcones are the different groups of flavonoids determined in the genus *Salix* (e.g. Bridle et al., 1970; Jarrett and Williams, 1967; Julkunen-Tiitto and Sorsa, 2001; Krauze-Baranowska et al., 2013; Nyman and Julkunen-Tiitto, 2000, 2005). In winter-dormant *S. pyrolifolia* twigs, the flavonoids were mainly composed of different derivatives of naringenin (flavanones) and isosalipurposide (phloridzin, chalcone 2′-*O*-glucoside), but also, the derivatives of quercetin and kaempferol (flavonols), the derivatives of luteolin (flavone), a dihydroflavonol ampelopsin, and a flavan-3-ol catechin were identified (Table 1). Two new isosalipurposide methoxyderivatives (monomethyl- and dimethylisosalipurposide) were also identified in *S. pyrolifolia*.

Generally, the concentration of flavonoids in *S. pyrolifolia* was relatively high (Fig. 2, Table 2), and similar levels, over 1% of the dry wt in bark, have previously been reported only in *S. acutifolia, S. daphnoides, S. purpurea* and *S. hastata* (Krauze-Baranowska et al., 2013; Meier et al., 1992). In these species, the main flavonoid glucosides in twigs were also the same as in *S. pyrolifilia*: naringenin 7-*O*-glucoside (prunin), naringenin 5-*O*-glucoside (salipurposide), a derivative of naringenin 5-*O*-glucoside and isosalipurposide (Jarrett and Williams, 1967; Kammerer et al., 2005; Krauze-Baranowska et al., 2013; Meier, 1988; Pobłocka-Olech et al., 2007; Sulima et al., 2017). Ampelopsin (dihydromyricetin) has previously been reported to be the major leaf component in *S. phylicifolia*, and it is also found from the leaves of *S. lapponum* (Nyman and Julkunen-Tiitto, 2000; Tegelberg et al., 2003), in the bark of *S. daphnoides*, *S. purpurea* and *S. pentandra* (Förster et al., 2008), and in the wood of *S. caprea* (Pohjamo et al., 2003).

The phenolic acid composition in winter-dormant *S. pyrolifolia* twigs consisted mainly the derivatives of *p*-hydroxycinnamic acids (*p*-coumaric acids) and chlorogenic acids (caffeoylquinic acids, CQA), and several of them were not able to get identified in our analytical conditions (Table 1). Similarly, the bark of *S. myrsinifolia* and *S. purpurea* has reported to contain different kinds of *p*-hydroxycinnamic and chlorogenic acid derivatives but also, the derivatives of benzoic acids have been found from the bark of *S. purpurea* (Nybakken and Julkunen-