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A CHEMOTAXONOMIC SURVEY OF PHENOLICS IN LEAVES OF NORTHERN SALICACEAE SPECIES

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Key Word Index—*Salix*; *Populus*; Salicaceae; willows; phenolics; condensed tannins; phenolic glycosides; chemotaxonomy.

Abstract—The leaves of 15 Salicaceae species were screened for total phenolics, condensed tannins, catechins and phenolic glycosides. The ability to produce phenolics varies widely between willow species. All species contain condensed tannins and phenolic glycosides, although some species showed only trace amounts. Five of the 15 species lack catechins. It was possible to distinguish each willow species on the basis of its leaf phenolic glycosides.

INTRODUCTION

Morphological identification of Salicaceae species is rendered difficult due to the complex but ready hybridization that occurs in nature; thus attempts have been made to use secondary phenolics for chemotaxonomic purposes [1, 2]. Phenolic glycosides, condensed tannins, (+)-catechins and phenolic acids are all present [2–6]. This paper reports the results of a series of analyses on the distribution of these constituents in the leaves of 15 Salicaceae species growing wild or under cultivation in Eastern Finland.

RESULTS AND DISCUSSION

Willow leaves contain comparatively high levels of phenolic constituents (Table 1). *Salix phylicifolia* has the highest total phenolics, with more than 15% dry wt. In most species the total phenolic content is more than 5%; *S. triandra*, *S. myrsinifolia*, *S. fragilis* and *P. tremula* exhibit the lowest concentrations. The low yield of total phenolics in the leaves of old *S. fragilis* and *P. tremula* trees is particularly striking, since the concentration of polymeric phenolics usually increases as plants age and also during the growing season [7, 8].

All the species examined, except the laboratory cultivated *S. triandra*, contained condensed tannins (Table 1). Very great differences existed between the willow species in tannin content but the differences were not necessarily positively correlated with the amounts of total phenolics. The extract of *S. cinerea* leaves showed by far the most intense colour production with both the butanol and vanillin tests. Moderately high tannin-containing species were *S. lapponum*, *S. viminalis*, *S. aurita* and *S. caprea*, while *S. fragilis* and *S. myrsinifolia* extracts produced only a very faint red colour. However, the tannin content seemed to vary slightly among different stands of *S. caprea* and *S. myrsinifolia*. The amounts of condensed tannin in Finnish willows differ slightly from those reported for English willows by Jaggi and Haslam [3], who found that leaves of *S. myrsinifolia*, *S. fragilis* and *S. pentandra* did not contain proanthocyanidins. Binns *et al.* [4] have not detected these tannins in *S. myrsinifolia* leaves either. However, they have shown proanthocyanidins to be present in *S. fragilis*, *S. pentandra* and in *S. triandra*. According to my analyses field-cultivated one-year-old *S. triandra* leaves contained also a moderate condensed tannin concentration [R. Julkunen-Tiitto, unpublished results]. Thus the leaves of different willow species vary greatly in their ability to accumulate condensed tannins. Bate-Smith [9] has claimed that the ability to form proanthocyanidin is a primitive characteristic. On the basis of this statement, *S. myrsinifolia* is more advanced than other willows.

The Salicaceae are characterized by their ability to synthesize low molecular phenolic glycosides, e.g. glycosides of salicyl alcohol and its derivatives [2, 10]. The leaves of all the species screened in this study by GC yielded phenolic glycosides, although the yield in some species remained very low (Tables 1 and 2). The Finnish willow species may be classified into three groups on the basis of the total glycoside content of their leaves. There are three species with large amounts: *S. myrsinifolia*, the cultivated *S.* cv. Aquatica and the introduced *S.* × *dasyclados*, which is closely related to *S.* cv. Aquatica [11]. These three species have mainly salicin and salicortin. The species containing moderate glycoside amounts are *S. triandra*, *S. fragilis*, *S. pentandra* and a *Populus* sp. In the third group of species, including *S. caprea*, *S. lapponum*, *S. phylicifolia*, *S. viminalis*, *S. cinerea*, *S. aurita* and *S. alba*, only very low amounts of glycosides were detected, although the number of different constituents was high. *S. alba*, *S. fragilis*, *S. cinerea* and *P. balsamifera* were quite old plants, which may partly explain the low amounts in their leaves, because, in contrast to the polymeric phenols, the content of phenolic glycosides is known to decrease during ageing [7, 12].

The present results mostly agree with previous investigations on European willows by Jaggi and Haslam [3] and Thieme [2, 12], but are qualitatively at variance with the results of Binns *et al.* [4]. These authors [4] detected most of these glycosides in all the nineteen species

Table 1. The screening of phenolics in leaves of Salicaceae species by colorimetric methods*

Species	Date	The crude extract FC ± s.e.	The crude extract BuOH–HCl ± s.e.	The crude extract Van–HCl ± s.e.	Aqueous fraction FC ± s.e.	Ethanol fraction Van–HCl ± s.e.
Wild species						
Salix pentandra†	June	70.00	35.50	49.47	1.922	0
S. lapponum	June	66.82 ± 3.05	66.39 ± 5.38	89.70 ± 3.67	0.567 ± 0.150	4.939 ± 0.186
S. cinerea†	August	66.87	160.12	143.26	0.598	3.015
S. aurita†	June	52.73	66.70	42.85	0.374	0
S. phylicifolia	June	152.24 ± 2.19	39.14 ± 0.71	13.12 ± 0.92	0.658 ± 0.073	1.823 ± 0.088
S. phylicifolia × *S. myrsinifolia*	July	115.28 ± 0.45	24.07 ± 2.17	22.93 ± 1.91	3.297 ± 0.138	0.959 ± 0.096
S. caprea						
Stand 1	July	71.17 ± 4.10	46.28 ± 2.07	71.26 ± 0.93	0.683 ± 0.065	3.865 ± 0.054
Stand 1	August	79.68 ± 2.42	71.40 ± 3.96	65.23 ± 1.10	0.465 ± 0.069	4.610 ± 0.380
Stand 2†	August	33.50	24.33	39.55	0.370	0.357
Stand 3	August	62.02 ± 0.12	66.84 ± 0.64	75.48 ± 2.67	0.887 ± 0.013	3.330 ± 0.846
S. myrsinifolia						
Stand 1†	June	34.99	0	+	8.180	0
Stand 2	July	44.26 ± 1.22	+	+	10.561 ± 0.120	0
Stand 3	July	56.93 ± 1.48	18.71 ± 1.20	+	10.043 ± 0.347	0
Stand 1	August	39.47 ± 1.29	43.50 ± 6.71	+	7.361 ± 0.810	0
Stand 4	August	50.72 ± 3.86	8.72 ± 0.27	0	10.458 ± 0.600	0
Populus tremula†	June	35.87	28.50	13.88	1.789	0
Cultivated species						
S. cv. Aquatica	August	55.04 ± 1.67	43.30 ± 1.60	40.45 ± 0.09	1.603 ± 0.091	1.876 ± 0.067
S. viminalis	August	80.48 ± 0.13	97.34 ± 0.47	86.60 ± 0.10	0.566 ± 0.170	3.770 ± 0.100
S. triandra (Labor. cult.)		16.96 ± 0.80	0	0	2.197 ± 0.160	0
Ornamental trees						
S. fragilis†	August	29.61	6.37	5.13	2.129	1.061
S. alba†	August	60.53	39.67	60.52	0.601	0.654
P. balsamifera	June	57.44 ± 1.77	41.31 ± 5.43	58.65 ± 5.12	1.740 ± 0.250	1.781 ± 0.100

*Folin–Ciocalteu reaction (FC) has been used for total phenolics, butanol–HCl hydrolysis (BuOH–HCl) for proanthocyanidins and the vanillin–HCl method (Van–HCl) for condensed tannins. Results are the mean values of two † or three composite sample runs (mg equivalents/g dry weight). Standard compounds used for total phenolics, proanthocyanidins and condensed tannins were phenol, anthocyanidin and (+)-catechin, respectively. +, Positive result, not calculated.

Table 2. The distribution of phenolic glycosides in leaves of Salicaceae species*

Species	Date	Salicin	Fragilin	Picein	Salidroside	Vimalin	Triandrin	Tremuloidin	Salicortin	Total ± s.e.
Wild species										
Salix pentandra†	June	0.452	0.424	0	0	0	0	0	0.264	1.140
S. lapponum	June	0.160	0	0.067	0.232	0	0	0	0	0.459 ± 0.044
S. cinerea†	August	0.075	0.011	0.036	0	0.155	+	0	0	0.277
S. aurita†	June	0.029	0	0.395	0	0	0	0	0.262	0.686
S. phylicifolia	June	0.063	0.062	0.077	0.184	0	0.236	0	0.052	0.673 ± 0.025
S. phylicifolia × *S. myrsinifolia*	July	1.490	0.050	0	0	0	0	0	5.423	6.963 ± 0.330
S. caprea										
Stand 1	July	0.376	0.076	0	0	0	0.088	0	0.341	0.881 ± 0.049
Stand 1	August	0.296	0.101	0.091	0	0	0.084	0	0.219	0.791 ± 0.137
Stand 2†	August	0.091	0.028	0	0	0	0.040	0	0.320	0.490
Stand 3	August	0.182	0.067	0	0	0.174	0.165	0	0.796	1.385 ± 0.399
S. myrsinifolia										
Stand 1†	June	3.549	0.229	0	0	0	0	0	28.280	32.060
Stand 2	July	16.340	0	0	0	0	0	0	49.810	66.150 ± 3.540
Stand 3	July	6.440	0.150	0	0	0	0	0	25.207	31.780 ± 3.700
Stand 1	August	1.485	0	0	0	0	0	0	25.900	27.390 ± 0.850
Stand 4	August	3.383	0.110	0	0	0	0	0	45.653	49.145 ± 0.630
Populus tremula†	June	0.584	0.064	0	0.072	0	0	0	5.465	6.190
Cultivated species										
S. cv. Aquatica	August	1.956	0	0	0	0	0	0	10.057	12.873 ± 0.329
S. viminalis	August	0.040	0.050	0.474	0	0	0.099	0	0.637	1.305 ± 0.264
S. triandra (Labor. cult.)		0	0.018	0	4.171	0	0	0	0	4.189 ± 0.109
Ornamental trees										
S. fragilis	August	0.375	0.160	0	0	0	0	0	0.664	1.199 ± 0.057
S. alba	August	0.093	0	0.088	0	0	0.098	0.202	0.273	0.754 ± 0.012
Populus balsamifera	June	1.184	0.073	0.090	0	0	0	0	1.178	2.520 ± 0.340

*Results are the mean values of two† or three sample runs (mg/g dry weight). +, Positive result, not calculated.

examined, including *S. caprea*, *S. alba*, *S. cinerea* and *S. phylicifolia*. However, Thieme [2, 12] and Jaggi and Haslam [3] have reported that the leaves of *S. caprea*, *S. alba*, *S. cinerea*, *S. aurita* and *S. phylicifolia*, which according to my analysis contain trace amounts of glycosides, are totally devoid of salicin and its derivatives.

On the basis of my results, the distribution of glycosides in willow leaves appears to be relatively species-specific. Salicin and salicortin were the most common glycosides. Salicortin was found in 12 of 15 species, and it forms the main component in most species. A rather similar trend has been found in the leaves of Central European willow species [2, 12], except that *S. myrsinifolia*, yielded salicin as the main constituent instead of salicortin (see Table 2).

Salidroside, which is unique to the bark of Finnish willows [7], is also rare in the leaves, occurring mainly in *S. triandra* and in two low glycoside species (Table 2). Binns *et al.* [4] have reported different results; they detected salidroside in all the 19 species they investigated. Besides salidroside, Finnish *S. triandra* (field and laboratory cultivated) produced a trace amount of salicin and fragilin (Table 2) [11]. Salicin and its derivatives were not found in Central European *S. triandra* leaves [2, 12] and the production of salidroside seems to be much lower there than in cultivated Finnish *S. triandra*.

Tremuloidin and vimalin are quite rare, being detected only in a few species (Table 2). Populin and grandidentatin were not found in any species in this study. Thieme [2, 12] has reported populin in *S. fragilis* leaves, but grandidentatin was not detected in the leaves of any willow species screened. Binns *et al.* [4] found grandidentatin in the autumn leaves of English *S. pentandra*, *S. phylicifolia* and *S. alba*, among other species. They could not, however, detect any qualitative differences among the Salicaceae species they examined but this was probably because they used lead acetate purification, which may have adsorbed some of the minor components [13, 14].

Although physiologically comparable leaves were used throughout the present analysis, there was moderate intraspecific variation in the secondary phenolic content among the different stands of *S. myrsinifolia* and *S. caprea* (Tables 1 and 2). Within these species the relative amounts of the main glycosides (salicin and salicortin) and the presence of trace glycosides varied slightly, but, nevertheless, the differences between the species are still clear. The quantitative variations within the *S. myrsinifolia* and *S. caprea* stands studied probably reflect environmental factors rather than genetic variations [15].

The three phylogenetically primitive Pleiandreae species, *S. fragilis*, *S. triandra* and *S. pentandra* are considered to be closely related [16]. However, the glycoside composition of *S. triandra* is very different from that of *S. pentandra* and *S. fragilis* (Table 2). The latter two species are identical, containing, in addition, similar unknown components [R. Julkunen-Tiitto, unpublished results].

The Diandrae species, *S. myrsinifolia* and *S. phylicifolia*, hybridize relatively easily. In morphology the hybrid analysed, resembles *S. phylicifolia*, and similarly on the basis of total phenolics. However, in its glycosidic composition it was similar to *S. myrsinifolia* (Tables 1 and 2). The same trend was found in the hybrid of *S. myrsinifolia* × *S. phylicifolia* originating from Umeå, Sweden [unpublished results]. In this case, at least, the identification of the hybrid is possible on the basis of phenolic glycoside composition and total phenolic concentration.

Central European willows have been classified chemically on the basis of their leaf constituents into three groups: (1) no phenolic glycosides and much proanthocyanidin; (2) no proanthocyanidin and much salidroside; and (3) a predominance of salicin and salicortin [17]. Finnish willows cannot be classified so precisely. According to the present data both phenolic glycosides and proanthocyanidins occur in almost all species, although sometimes only in trace amounts. In most cases, however, a negative correlation between the amounts of phenolic glycosides and proanthocyanidins was found according to Hegnauer's [17] classification. Although the separation of willow species into three chemical groups is not so clear, nevertheless the phenolic constituents are still very useful for identifying and separating these taxa.

EXPERIMENTAL

Materials. In this study eight wild willows (*S. myrsinifolia* Salisb., *S. pentandra* L., *S. phylicifolia* L., *S. phylicifoliax*, *S. myrsinifolia*, *S. cinerea* L., *S. lapponum* L., *S. aurita* L. and *S. caprea* L.), two introduced, field-cultivated willows (*S.* cv. Aquatica and *S. viminalis* L.), one laboratory-cultivated willow (*S. triandra* L.) and two ornamental willows (*S. alba* L. and *S. fragilis* L.) were analysed. The aspen species included in the study were wild *Populus tremula* L. and an introduced ornamental tree of *P. balsamifera* L. In most cases leaf samples were taken from young shrubs or trees. The samples of the ornamental species, *S. cinerea* and *P. tremula*, however, originated from older, full sized individuals. The composite leaf samples were obtained from 40 cm long distal parts of the shrubs, the age of the leaves being at most 4 weeks. Samples were taken from five or six different clones, except for *S. fragilis*, *S. alba*, *S. aurita*, *P. tremula* and *P. balsamifera* where only one clone was used. The time of sampling was 9–10 a.m. The leaves were dried immediately at 48° in a well-ventilated oven, milled and stored in plastic containers in a cold room to 4° until used.

Methods. The detailed analytical methods have been described in a previous paper [18]. Aq. Me_2CO extracted samples were concd, refluxed and filtered giving a crude extract. From this extract total phenolics were determined by the Folin–Ciocalteu phenol test and condensed tannins by the vanillin–HCl test or by hydrolysing with BuOH–HCl. The crude extract was exhaustively extracted with EtOAc, concd, suspended in distilled H_2O and purified with a polyamide column eluting with distilled H_2O and aq. EtOH. Freeze-dried eluates were dissolved in MeOH, silylated and analysed by capillary GC for phenolic glycosides. Authentic glycosides were, in elution order: salicin, fragilin, picein, salidroside, vimalin, triandrin, tremuloidin, populin, salicortin and grandidentatin. From MeOH extracts, the total phenolics and catechins were also analysed by colour reactions. Two or three subsample runs were used.

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