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Phenol Compounds in the Species of *Salix* L. Genus in the World Flora

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Abstract

Review and analysis of the modern data on the content of phenol compounds in the plants of the *Salix* L. genus were carried out. The works aimed at the search for cheotaxonomic markers and their application in systematics are presented. It is demonstrated that the qualitative composition of phenol compounds is an invariable, stable sign for the taxons of the *Salix* genus. Phenol glycosides may be used to identify morphologically similar species and hybrid forms. Special attention is paid to the species that have been the subjects of investigations in different countries: *S. babylonica* L., *S. alba* L., *S. raddeana* Laksch. ex Nasarow, *S. caprea* L. and *S. acutifolia* Willd.

Keywords: *Salix* L., phenol compounds, flavonoids, phenol glycosides, chemotaxonomy

INTRODUCTION

The *Salix* L. genus is one of the numerous genera of Salicaceae Mirb. family, it includes 552 species of the world flora [1]. The number of species indicated for Russia and adjacent territories is 158 [2], for the Asian part of Russia – 103 species [3].

There are data on the antipyretic, disinfecting, astringent activity of osier bark both in folk and in traditional medicine [4, 5]. Research into the hemostatic, diuretic, antimicrobial, antiinflammatory and antitumour activity of the extractive substances of the plants of *Salix* genus are carried out [6–10]. Osier bark is one of the components of antirheumatic and antifebrile medicinal preparations in many countries [4]. The curative properties of osier are due to the presence of phenol compounds in the raw material [11, 12]. The information on the chemical composition of the species of *Salix* genus and their pharmacological activity was published by Zuzuk with co-authors [4], Bontsevich [13], Khiteva [14], Frolova with co-authors [15].

The *Salix* genus is represented by three subgenera: *Salix*, *Vetrix* Dumort. and *Chamaetia* (Dumort.) Nasarow, according to the traditional classification based on the morphological and ecological-geographical characteristics of the representatives of the genus [16, 17]. The systematic position of the species, ecological-morphological characterization are presented according to the literature and electronic sources [1, 3, 16, 17]. Cycles of studies in different countries have been dedicated to separate species during the two recent decades: *S. babylonica* L., *S. alba* L., *S. raddeana* Laksch. ex Nasarow, *S. caprea* L. and *S. acutifolia* Willd. The data on the pharmacological activity were described in detail in the indicated works.

The goal of the present work was to make a review of research and analysis of modern literature data on the composition of phenol compounds in the plants of *Salix* genus, and evalua-

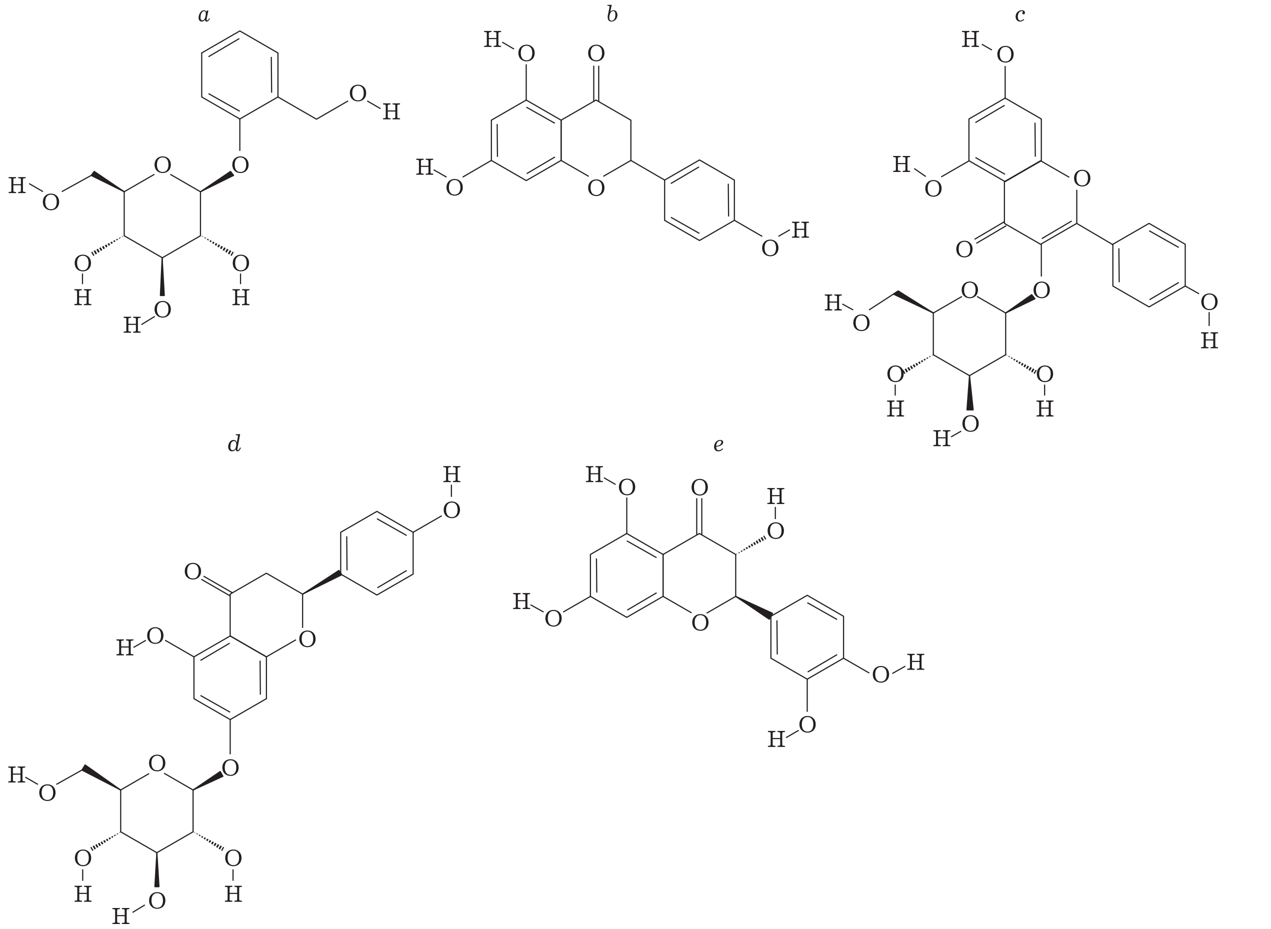

Fig. 1. The structural formulas of the main phenol compounds of *Salix* L. genus: *a* – salycine, *b* – naringenin , *c* – astragaline, *d* – prunine, *e* – taxifolin.

tion of the possibility to use them as chemotaxonomic markers.

PHENOL COMPOUNDS

The structural formulas of the main phenol compounds present in the plants of the *Salix* L. genus are presented in Fig. 1 [18].

Salix babylonica

S. babylonica L. (= *S. matsudana* Koidz.) is weeping willow belonging to the Subalbae Koidz. subsection of *Salix* subgenus. It is a tree up to 15 m high. The species is widespread in culture almost in all regions with moderate warmth over the globe. The origin is in arid and semi-arid regions of Central and Northern China.

Within several recent years, works dealing with the studies of composition and dynamics of phenol compounds in *S. babylonica* plants were published in China [19–25]. It was established that total flavonoid content is higher in leaves than in branches and in blossom clusters. It reached its maximum (6.044 %) in May, and minimum (1.875 %) in July [19]. The compounds isolated from the methanol extract of the leaves of *S. babylonica* include 7-O-β-*D*-glucuronid of chrysoeriole, 7-O-β-*D*-galactopyranoside of luteolin, 7-O-glucoside of kaempferol, 7-O-galactoside of apigenin, 4'-O-glucoside of luteolin, salycine, trichocarpine, and a new phenol glycoside 2-O-acetyltrichocarpine (benzyl ester of 2-O-β-*D*-glucoside of gentisic acid) [20, 21]. In addition, the compounds discovered in the leaves of S. babylonica for the first time include β-*D*-glucopyranuronide of apigenine, 7-O-β-O-glucopyranuronide of luteolin and 7-O-β-*D*-glucopyranuronide of chrysoeriole [22]. Later a new flavonoid named matsudon was isolated, along with five known flavonoids, such as luteolin, isoquercetin, 7-methoxyflavon, 7-O-glucoside of luteolin, 4',7-dihydroxyflavon, and two phenol glycosides – leonuriside and piceoside. Their structures were determined on the basis of extensive studies by means of 1D- and 2D-NMR, mass spectrometric analysis and comparison with literature data [23]. The content of phenol compounds in the abscissed leaves of the plants of this species was studied [24]. It was shown that an increase in the time of ultrasonic treatment during the extraction of flavonoids with a 60 % aqueous solution of ethanol promotes an increase in the yield of flavonoids from 3.83 to 11.23 %. It should be stressed that the content of phenol compounds in the abscissed leaves of the species of Salix genus growing in India was studied previously. It was discovered that the maximal amount of flavonols is characteristic of dead leaves [25].

Salix alba

S. alba L. is white willow belonging to the *Salix* section of *Salix* subgenus. It is a large tree up to 30 m high. The species is widespread in culture. The natural habitat occupies the major part of Europe; there are data on its occurrence in northern Africa, West and Middle Siberia, Middle Asia, Kazakhstan.

White willow is one of the best studied species, it is used in scientific and practical medicine [4]. The extract of the bark is known to contain eryodistiol, 5,7-dihydroxychromen-4-one and naringenine. These data were obtained using high-rate chromatography, mass spectrometry and NMR spectroscopy [26]. Investigation of water extracts from the bark of white willow and other species is carried out at the Pyatigorsk State Pharmaceutical Academy. It was revealed that the content of extractives is the highest in the extract prepared by the authors according to the XI edition of the State Pharmacopeia published in 1987 [27]. This extract of the bark of white willow possessed high antiinflammatory activity.

Salix raddeana

S. raddeana Laksch. ex Nasarow is Radde willow belonging to section *Vetrix* Dumort. of the *Vetrix* subgenus. It is a high bush or a tree up to 6–8 m high. The species is widespread in forests, forest-steppe and lower mountainous belts in Eastern Siberia, in the Far East, in the northern and north-eastern regions of Mongolia, China and Korea.

In China, several works dealt with this species [28–33]. For example, in 2004 high-performance liquid chromatography (HPLC) was applied to isolate raddeanalin (7-O-glucopyranoside of diosmetin) from S. raddeana and identify this compound; a procedure for more economical determination of flavons was proposed [28, 29].

Two new components for the genus were identified with the help of UV spectrophotometry and mass spectrometry: 7-O-β-L-arabinopyranosyl(1-6)β-*D*-glucopyranoside of luteolin and 7-O-β-*D*-xylopyranosyl(1-6)β-*D*-glucopyranoside of luteolin. For *S. raddeana*, also the following compounds were reported: 7-O-β-*D*-xylopyranosyl(1-6)β-*D*-glucopyranoside of diosmetine, rutin, astragalin and isoquercetin [30–33].

Salix caprea

S. caprea L. (= *S. hultenii* Flod.) is goat willow belonging to the *Vetrix* section of *Vetrix* subgenus. It is a tree up to 12–15 m high. It grows in forests, on clearings, at forest edges, near roads. The species is widespread over the territory of Eurasia.

The following phenol compounds were found in the wood and tree warts of goat willow: vanillic acid, catechine, gallocatechine, dihydrokaempferol, dihydromyricitin, naringenin and taxifolin. It was established that warts and branches contain higher concentrations of the substances than the stem of the same tree [34]. A procedure of an easier and rapid extraction of phenol compounds was developed for the blossom clusters of goat willow as example: an optimal version is the use of 50 % methanol, the ratio of the mass of raw material to the solvent mass should be 1 : 40, boiling time 3 h with a reflux condenser. The yield of flavonoids reached 26.82 mg/mL [35].

A comparative study of *S. caprea* and *S. repens* L. (*Vetrix* subgenus, *Incubaceae* Kerner section) species and their hybrids was carried out in 2003 in Sweden [36]. It was established that a high level of condensed tannines and low level of phenol glycosides is characteristic of *S. caprea*; for *S. repens*, quite contrary, the level of tannines is low and the level of phenol glycosides is high. Hybrids occupy an intermediate position in the content of phenol compounds. A similar investigation was also carried out for the species from North America, subgenus *Vetrix*: *S. sericea* Marshall (*Griseae* (Borrer) Barratt ex Hooker section), *S. eriocephala* Michx. (*Cordatae* Barratt ex Hooker section) and their F1 hybrids [37, 38].

The content of salicortin and 2'-cynnamoylsalicortin was determined in the leaves of *S. sericea*, and the low content of tannines int eh leaves was detected. In *S. eriocephala*, these phenol glycosides were not found, but a high content of condensed tanning agents was detected in leaves. These features are inherited by hybrids, for example, F1 hybrids exhibit an intermediate value for the content of condensed tanning agents. The content of phenol glycosides is often lower than in parent forms, but this parameter is unstable and may be controlled by the recessive alleles of genes. The genetic changes in the inheritance of phenol glycosides salicortin and 2'-cynnamoylsalicortin in *S. sericea* were studied. It was demonstrated that the parameters of plant growth exhibit almost no correlation with the concentrations of these compounds; in addition, the concentration of salicortin is much higher than the concentration of 2'-cynnamoylsalicortin, the degrees of inheritance also differ: 0.2 for salicortin and 0.59 for 2'-cynnamoylsalicortin. The authors of [37, 38] concluded that phenol glycosides and condensed tanning substances in S. sericea, S. eriocephala and in their F1 hybrids form one of the genetically stable features.

Salix acutifolia

S. acutifolia Willd. is acutifoliate willow belonging to the *Daphnella* Seringeex Duby section of *Vetrix* subgenus. It is a high bush or a tree up to 6 m in height. It grows only on open non-weedy sand. The species is widespread in Europe.

Along with white willow, this is one of the best studied species used in medical science and practice. A number of works dealing with the studies of the chemical composition of bark and leaves in the plants of this species were published [39–42]. The highest content of the sum of flavonoids was detected in *S. acutifolia* at the beginning of fruiting; by the end of vegetation it decreased nearly by a factor of 2. In comparison with acutifoliate willow, flavonoid content in the leaves of *S. triandra* L. f. concolor A. K. Skvortsov and *S. triandra* f. discolor A. K. Skvortsov is maximal at the beginning of vegetation. Flavonoid content is higher in the young leaves of male individuals is higher than in female ones. During the phase of shoot lignification, a trend for tannide accumulation was observed; a negative correlation was detected between their content in the bark of branches and the content of flavonoids in leaves [39]. In the bark of acutifoliate willow growing in the Samara Region, 14 phenol compounds were identified by means of column chromatography, UV, IR, NMR and mass spectrometry (among these compounds, 9 were identified for the first time in this species). These compounds are: (+)-catechine, naringenine, prunine, (-)-salipurposide, isosalipurposide, 6''-cumarylisosalipurposide, isosalipurpol, triandrin, syringin, saligenin and the products of its decomposition, acylsalingenin, salicin, salicortin and tremulacin [7, 40]. The content of salicin determined quantitatively by means of thin layer chromatography was found to vary from 3 to 10 % in the model populations of the natural growth of *S. acutifolia* in the basin of middle Volga [41]. A number of phenol metabolites were revealed by means of high-performance liquid chromatography and mass spectrometry (HPLC-MS) in S. acu- tifolia plants: salicortin, salicin, tremuloidin and a new compound – glycoside of benzoic acid named acutifoliside [42].

Other species of Salix genus

As mentioned above, investigation of the chemical composition of the extracts of bark, leaves, blossom clusters of the plants of willow genus was carried out mainly for pharmacological purposes to reveal different kinds of biological activity of the extracts and then to obtain new medicinal preparations. There are only a few works aimed at the search for chemotaxonomic markers and their application in the systematics of the *Salix* genus. For example, the flavonoid composition of 14 species of *Salix* genus growing in the arboretum of the CSBG SB RAS was studied in [43]. Various combinations of two flavons (apigenin and luteolin) and three flavons (kaempferol, quercetin and myricetin) were discovered. The plants of the same species were detected to have the same qualitative composition. The similarity of the flavonoid composition of some species was also determined, for example *S. purpurea* L. and *S. gracilistyla* Miq., *S. ledebouriana* Trautv. and *S. dasyclados* Wimm., however, it was stressed that the composition of glycosides is individual for each species.

Forster *et al.* [44] analyzed the data for the following species: *S. daphnoides* Vill., *S. pentandra* L. and *S. purpurea* growing at the territory of Poland and Germany. In the opinion of the authors, these species had clear differences in the composition of phenol glycosides. The major glycoside of *S. daphnoides* and *S. purpurea* was salicortin, the major compound of *S. pentandra* was 2'-O-acetylsalicortin. In general, *S. daphnoides* exhibited the highest content of salicylates and phenol glycosides. The authors indicated that the content of phenol compounds in willow bark decreased during the vegetation period from March to July 2007. The investigation showed that studying the qualitative and quantitative composition of phenol glycosides it is necessary to take into account the species, a specimen and the time when the material was collected during the season [44].

A group of authors from Poland and Belgium [45–47] investigated seven species of willows growing at the territory of Poland under natural conditions and in artificial growth: *S. alba* (clone 1100), *S. daphnoides* (clone 1095), *S. acutifolia*, *S. herbacea* L., *S. viminalis* L., *S. purpurea* and *S. triandra*. The differences in the composition of phenol compounds in all species were confirmed. In addition, the presence of 29-acetylsalicortin in *S. alba* (clone 1100) was discovered for the first time.

A series of works were carried out by Riitta Julkunen-Tiitto *et al.* [47–52], as well as by a number of other international projects [53–60] dealing with the chemotaxonomic studies of phenol glycosides in the leaves, bark, branches and blossom clusters of more than 60 willow species. It was confirmed that each species possesses specific glycoside composition. The traditional classification of the genus turned out to be incompatible with the classification based only on phenol glycosides. It was pointed out that simple phenol glycosides may be used to identify morphologically similar species and hybrid forms, in combination with other indices. Investigation of the regularities of intra- and interspecific chemical variability with respect to the content of tanniferous substances and the composition of 36 phenol components was carried out with the leaves of six species (120 samples). A multidimensional cluster analysis of the data revealed that the intraspecific variations are lower on average as the interspecific ones with respect to the chemical composition. The similarity in phenol compounds weakly correlated with the phylogeny of the studied species [51].

The authors of this work studied the composition and content of phenol compounds in the species of *Salix* genus with the help of HPLC. Apigenin was found in *S. recurvigemmis* A. K. Skvortsov and *S. saxatilis* Turcz. Ex Ledeb., luteolin – in *S. acutifolia*, *S. vestita* Pursh, *S. berberifolia* Pall., *S. myrtilloides* L., *S. saxatilis* and *S. pyrolifolia* Ledeb., *S. alba* x *S. blanda*, *S. alba* and *S. alba* var. *vitellina* (L.) Stokes. Kaempferol was identified in *S. recurvigemmis*, *S. alba* x *S. blanda* Andersson, *S. alba* and *S. alba* var. *vitellina*. Rutin was discovered only in *S. alba* and *S. alba* var. *vitellina*, quercetin – in *S. vestita*, *S. berberifolia*, *S. myrtilloides*, *S. nummularia* Andersson, *S. recurvigemmis*, *S. krylovii* E. L. Wolf, *S. sphenophylla* A. K. Skvortsov, *S. alba* x *S. blanda*, *S. alba* and *S. alba* var. *vitellina*, salicin – in *S. alba*, *S. alba* x *S. blanda* and *S. alba* var. *vitellina*. No substantial differences were detected in the composition of flavonoids between male and female plants of *S. alba*. During the vegetation period, flavonoid content in the leaves of *S. alba* var. *vitellina* varies from 0.85 to 4.60 % [61–65].

CONCLUSION

The analysis of literature data and the own studies provide evidence of the impossibility to use the quantitative content of phenol compounds as a chemotaxonomic marker for the *Salix genus*. The qualitative composition remains unchanged, genetically stable sign, so it may be used to identify the taxons. The taxons best studied on the world scale for the *Salix genus* are *S. babylonica*, *S. alba*, *S. raddeana*, *S. caprea* and *S. acutifolia*.

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