

Chemical characterization of proanthocyanidins isolated from bark of seven plantation cultivars of *Salix* spp.

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Willows - fast growing, easily cultivable trees from the genus *Salix* are widespread in cold and moderate temperate regions of the Northern Hemisphere. Willows are known as a source of such important compounds as phenolic glycosides, namely, salicin, and their esters (tremulacin or salicortin), as well as proanthocyanidins (PACs). PACs are considered as valuable compounds prospective for usage in different areas. Chemical composition of PACs varies depending on plant species from which they were obtained, conditions of their growth and method of extraction.

This study focus on characterization of PACs from different plantation *Salix* spp. (*Klara*, *Lisa*, *Gudron*, *Tora*, *Sven*, *Inger*, *Stina*) growing in Latvia. Content of PACs was determined by vanillin and acid butanol assays. The liquid chromatography coupled with high resolution massspectrometry was used to analyse individual polar monomeric, oligomeric and polymeric compounds. Antioxidant properties were assessed in assays with radical cation of 2,2'-azino-bis(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS^{•+}) and free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH[•]).

The willow bark was deoiled and then extracted with 70 % ethanol water solution using accelerated solvent extraction. The solvent was evaporated in vacuo and the extracts were freeze-dried. The highest content of hydrophilic extractives was obtained from *Salix Gudrun* (24% o.v.d. bark), the lowest from *Lisa* (13% o.v.d. bark)

Content of proanthocyanidins in willow bark extracts is very high compared to the known PACs sources. This value determined using butanol assay varied from 12,7% (*Salix Inger*), to 23,4 % (*Salix Tora*). Vanillin assay show that PACs content in dry extract achieved 14,6% (*Salix Inger*) up to 26,6% (*Salix Klara*). It is proofed that partly the difference between two assays is conditioned by the presence of different amount of monomeric (epicatechin or galocatechin), dimeric and oligomeric PACs in extracts.

The antioxidant activity of the obtained extracts were assessed using the tests with free radicals DPPH[•] and ABTS^{•+} (Fig.1.) where the value was expressed as the IC₅₀ (the concentration required for 50 % inhibition of the free radical). The lower is IC₅₀ value, the higher is the antioxidant activity. IC₅₀ values in ABTS^{•+} test varied from 1,6 to 3,2 mg/L. Highest values were observed for *Salix Klara* and *Salix Gudron*: 2,7 and 3,2 mg/L, respectively. Lowest value was observed for sample *Salix Sven* with 1,6 mg/L. Similar tendencies were observed in the results obtained from DPPH[•] tests, highest values for samples *Salix Klara* and *Salix Gudron*: 10,6 and 13,8 mg/L, respectively. Lowest value was observed for *Salix Lisa* with 8,9 mg/L. Comparison with synthetic antioxidant – water soluble E vitamin analogue - Trolox (4,0 and 4,7 mg/L for ABTS^{•+} and DPPH[•] tests respectively), showed that willow extracts could be used as antioxidants.

Fig. 1. PAC content in extracts of different *Salix* cultivars and their antioxidant activity.

Qualitative composition of willow extracts determined using UPLC-MS/MS showed the presence of the following PAC monomers – epicatechin, catechin and galocatechin, as well as dimers: ((E)C-GC and (E)C-(E)C), trimers: ((E)C-(E)C-(E)C and (E)C-(E)C-GC), and tetramers: ((E)C-(E)C-(E)C-(E)C, (E)C-(E)C-(E)C-GC and ((E)C-(E)C-GC-GC). Both A type and B type linkages between monomeric units were estimated. Such variety in composition open the different opportunity for usage of these extracts. For example, A type proanthocyanidin oligomers are suspected to be useful in urinary tract infection treatment [1]. B type oligomers as well as monomers are being studied as molecular modulators of skeletal muscle growth and differentiation [2].

Acquired results allows to conclude, that extracts obtained from investigated willow barks are a valuable commodity with high antioxidant activity and high total proanthocyanidin content. These extracts can be used in wide variety of ways, including health care, food ingredients, agriculture and as ingredients in composite materials etc.

References

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