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CHARACTERIZATION OF BARK RICH-IN-TANNINS EXTRACTS FROM DECIDUOUS TREES WITH EMPHASIS ON THEIR ANTIOXIDANT ACTIVITY

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Abstract. The barks of deciduous trees spread over a wide area of Northern Europe, were sequentially extracted using solvents of increasing polarity. The highest yields of hydrophilic extractives were found for ash tree and goat willow (23.6% and 23.8%, respectively). The highest total contents of polyphenols were found for black alder and goat willow bark (0.18 GAE·g^{-1} and 0.12 GAE·g^{-1}, respectively). Hydrophilic extract from the both grey and black alder barks contained high amount (up to 7 % on bark dry mass) of condensed tannins (CT) or oligomeric proanthocyanidins (OPC). In tests with free radicals (ABTS$^{\bullet+}$, DPPH$^{\bullet}$), the high radical scavenging capacities of the hydrophilic extracts enriched with OPC were demonstrated. The antioxidant efficiency of the hydrophilic extracts and purified OPC from alder barks was tested by their influence on thermo-oxidative destruction of model polyurethane (PU) films. The hydrophilic extracts enriched with tannins have good potential as a technical antioxidant for polyurethanes, with the most prominent activity for the hydrophilic extract from black alder and goat willow bark.

1. Introduction

Up till now wood bark from logging and wood mechanical processing is used as cheap fuels. Unique compositional characteristics of bark biomass and possibility of numerous value added products (individual compounds and mixtures of synergetic activity) obtaining promote researches aimed at installation of bark utilization in integrated biorefinery schemes of wood processing. The barks of deciduous tree species are known to contain bioactive chemicals, including the OPC, which belong to the group of polymeric polyphenols. Accumulation of polyphenols in bark is the results of plants evolutions as their response on biotic and abiotic stresses. The high concentration of OPC in trees bark makes it a prospective raw material not only for medicine and veterinary but also for industrial applications. The bark of deciduous trees species growing in the Northern Eirope, in particular, in Latvia, is scarcely explored as a source for obtaining of valuable extractives products [1]. Polymeric polyphenols pool of bark is considered to be beneficial for various technical applications, such as adhesive and binder, components of bio-composites, biocides for agricultural needs etc. Currently the commercialized products based on rich-in-tannins extracts from bark are manufactured on the basis of tree species growing in Southern countries. CT quebracho and mimosa are produced commercially from quebracho tree (*Schinopsis lorentzii*) and black wattle (*Acacia mearnsii*) barks and are often used as a raw material for the industrial purposes. The antioxidant properties of OPC make their application very attractive. Demand for natural antioxidants is increasing, primarily due to their higher safety in comparison with synthetic compounds. Bark extracts are a natural resource that could replace synthetic antioxidants as butylatedhydroxyanisole (BHA) and butylatedhydroxytoluene (BHT), which are suspected of


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being responsible for liver damage and carcinogenesis in organisms of laboratory animals [2]. As an antioxidant, PAC has two advantages: natural origin (much lower toxicity and biodegrability) and oligomeric structure. The interest in high-molecular weight antioxidants is connected with the possibility to condition the long-term stability to polymeric composite materials [3]. At present, oligomeric and polymeric antioxidant are topics of great interest for researchers in many fields, such as pharmaceuticals, cosmetics, food and polymer composites production.

The aim of the present work was screening of some widely spread in Latvia and Northern Europe deciduous tree species: grey alder (*Alnus incana*), black alder (*Alnus glutinosa*), ash tree (*Fraxinus excelsior*), and goat willow (*Salix caprea*) as potential sources for obtaining of hydrophilic extracts enriched with proanthocyanidins and their testing as a polymeric antioxidant for polyurethane polymer compositions.

2. Materials and methods

Hydrophilic extracts enriched with OPC were isolated from bark using sequentially extraction with Accelerated Solvent Extractor (ASE 350, Dionex) at 90° C (1500 psi) for 20 min using solvents of increasing polarity: hexane, ethyl acetate and finally, aqueous ethanol (1:1, v/v). The total amount of extractives was determined gravimetrically. The yields of the fractions are shown as % of dry bark. Total polyphenols (TP) content was determined using Folin-Ciocalteu procedure [4]. OPC content was measured by buthanol-HCl method [5] using procyanidin dimer B2 as a reference compound. Py-GC/MS/FID analysis was used for characterization of extracts on the presence of carbohydrates. OPC were purified from non-tannin phenolics using Sephadex LH-20. TOF-MS (QSTAR Elite System Hybrid Quadrupole-TOF-MS) were used for characterization of OPC. The extracts enriched with OPC were tested for their antioxidant activities using $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging assay. Free radical scavenging activity was expressed as IC_{50} (the concentration of antioxidant, $mg \cdot L^{-1}$, required for a 50% inhibition of the free radical). The lower is the IC_{50} value, the higher is the radical scavenging activity of the compounds. Trolox (a water-soluble derivative of vitamin E) was tested as a reference antioxidant. Effect of extracts and purified OPC on the thermo-oxidative destruction of PU elastomer (control and containing 2.0% extract) was studied in the temperature range of 20 to 200°C by TGA and DTA (thermogravimetry and differential thermal analysis) in an air atmosphere (flow rate of 50 mL min^{-1}) using a Perkin Elmer, STA 600 TGA/DTA system at a heating rate of 10°C min^{-1}. The commercial technical antioxidant Irganox was used in this trial as the reference antioxidant. The model PU elastomer films were obtained as described in Arshanitsa *et al.* [3].

3. Results and discussion

The highest yield of hydrophilic extractives were found for ash tree and goat willow (23.6 % and 23.8 %, respectively). The highest total contents of polyphenols (TP) were found for black alder and goat willow bark (0.18 $GAE \cdot g^{-1}$ and 0.12 $GAE \cdot g^{-1}$ of bark, respectively). Based on the Py-GC/MS/FID results, extracts from barks of all tree species contained polyphenols as the predominant component, although a certain amount of carbohydrates (15 – 28 % on extracts dry mass) are also present. Hydrophilic extracts from the both alder species contained high amount (up to 7% on bark dry mass) of OPC, whereas OPC content of extract from ash tree was negligible. The TOF–MS spectrum of proanthocyanidins from bark of alder species showed that the discharged compounds from deciduous trees barks are B-

type proanthocyanidines pentamers with catechins or epicatechins (288 Da) degree of polymerization being 5 (Figure 1).

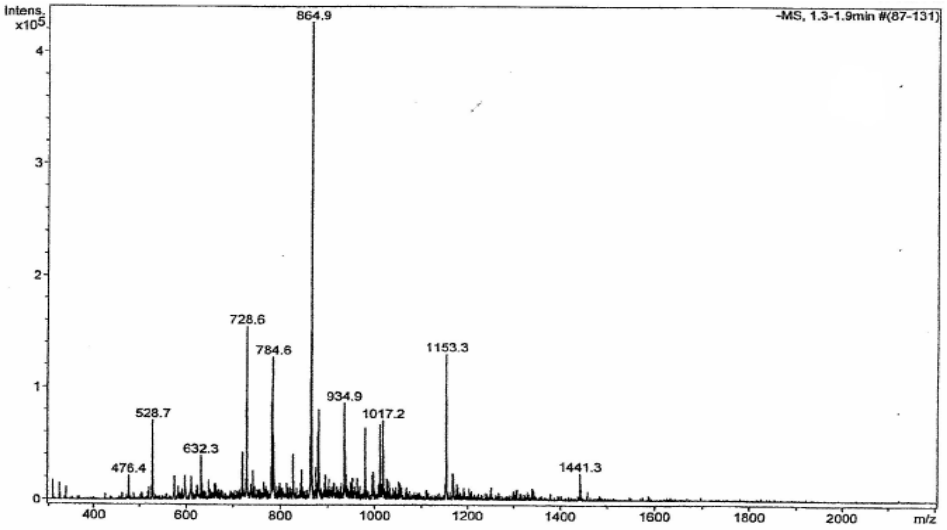

Figure 1. TOF –MS spectrum of OPC from grey alder bark

The assessment of antioxidant activity of deciduous tree barks extracts and OPC opens wider perspectives for rationalization of natural resources usage. The results of the tests on radical scavenging activity are presented in the Table 1. The comparison of the results of two tests ($DPPH^{\bullet}$ and $ABTS^{\bullet+}$) for hydrophilic extracts revealed the highest level of activity for the extract from bark of goat willow (IC_{50} of 1.8 $mg \cdot L^{-1}$). Among purified OPC, the highest antioxidative activity, which was higher than that for Trolox, showed OPC from the bark of grey alder. The data obtained (Table 1) on carbohydrates content in extracts (Py-GC/MC/FID) showed that with an increase of carbohydrate content the antioxidant activity reduced.

Table 1

Antioxidant activity of rich-in-tannins extracts and purified OPC from barks of deciduous trees

Sample	TP content, $GAE \cdot g^{-1}$ *(Folin-Ciocalteu method)*	OPC content % on o.d. extract *(buthanol-HCl method)*	Carbohydrates content % on o.d. extract *(Py-GC/MC/FID)*	IC_{50}, $mg \cdot L^{-1}$	
				$ABTS^{+}\bullet$	DPPH•
Goat willow	0.7	17.7	15.1	1.8	3.8
Black alder (BA)	0.7	25.9	24.3	3.0	6.1
Grey alder (GA)	0.6	25.2	28.3	3.8	7.5
Ash tree	0.2	0	n.d	8.7	33.6
OPC from GA	-	>90.0	<0.01	1.4	3.0
OPC from BA	-	>90.0	<0.01	3.3	6.9
Trolox	-	-	-	4.0	4.7

In order to evaluate an antioxidant activity of bark products under study they were introduced in compositions of model PU films in the amount close to the usually used in practice for technical antioxidants applications. The TGA data (Table 2) clearly showed the antioxidant effect of extracts and OPC revealed in increase in starting (T_{start}) and maximal rate (T_{max}) temperatures of PU thermo-oxidative destruction. The DTA data also confirmed the increase in thermo-oxidative stability of model PU films: the exothermal maximum connected with PU thermooxidation accompanied by volatiles release shifted towards the high-temperature region by 15-30°C.

The TGA data clearly revealed that the antioxidant effects of the hydrophilic extracts from grey alder and goat willow barks were on the same level as those for purified OPC and higher than that for commercial reference antioxidant Irganox used usually for PU systems.

Table 2

Thermal properties of rich-in-tannins extracts and purified OPC from bark of deciduous trees

Sample	T_{start}, °C	T_{max}, °C
PU without antioxidant	285	323
PU with 2 % GA extract	310	342
PU with 2 % BA extract	300	342
PU with 2 % GW extract	316	350
PU with 2% OPC from GA	319	341
PU with 2% OPC from BA	310	342
PU ar 2% Irganox	293	323

The research results have shown that the rich-in-tannins extracts from alder and goat willow barks are promising agents for stabilization of PU systems that enhance the high limit of PU exploitation temperatures.

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Reference

[1] Telysheva G, Dizbite T, Bikovens O, Ponomarenko J, Janceva S and Krasilnikova J 2011 Structure and antioxidant activity of diarylheptanoids extracted from grey alde (Alnus incana) and potential of biorefinery-based bark processing of European trees *Holzforshung* **65 (4)** 623-629

[2] Thompson D and Moldeus P 1988 Cytotoxicity of butylated hydroxyanisole and butylated hydroxytoluene in isolated rat hepatocytes *Biochem. Pharmacol.* **37 (11**) 2201-2207

[3] Arshanitsa A, Ponomarenko J, Dizhbite T, Andersone A et al 2013 Fractionation of technical lignins as a tool for improvement of their antioxidant properties *J. Anal. Appl. Pyrol.* **103** (SI) 78-85

[4] Waterhouse AL 2000 Determination of total phenolics *Food science* **2** 567

[5] Schofield P., Mbugua D M, Pell A N 2011 Analysis of condensed tannins: a review *Animalfeed sciece and tehnology* **91** 21-40.