RIGA TECHNICAL UNIVERSITY

Faculty of Material Science and Applied Chemistry
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RESEARCH AND MODIFICATION OF NATURAL POLYPHENOLS AND PROANTHOCYANIDINS EXTRACTED FROM THE DECIDUOUS TREE BARK GROWING IN LATVIA FOR PRODUCTION OF ECOLOGICAL FUNCTIONAL PRODUCTS AND MATERIALS

Summary of the Doctoral Thesis

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DECLARATION OF ACADEMIC INTEGRITY

I hereby declare that the Doctoral Thesis submitted for the review to Riga Technical University for the promotion to the scientific degree of Doctor of Engineering Sciences is my own and does not contain any unacknowledged material from any source. I confirm that this Thesis has not been submitted to any other university for the promotion to other scientific degree.

Ms Sarmīte Janceva ....
Date: ....

The doctoral Thesis is written in Latvian and includes an introduction, a literature review, an experimental work, results and discussion, conclusions, six annexes, a bibliography containing 298 literature sources, 104 illustrations, and 26 tables, totalling to 171 pages.

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TABLE OF CONTENTS

ABBREVIATIONS

$ABTS^{+\bullet}$	2.2-azino-bis (3-ethylbenzothiazoline)-6-sulfonic acid cationic radical
AT	Ash tree
BA	Black alder
CHIMAR	Innovative research and development company in Greece
$DPPH^{\bullet}$	2.2-diphenyl-1-picrylhydrazyl radical
EtOH	Ethanol
FTIS	Fourier transform infrared spectroscopy
GA	Grey alder
GAE	Gallic acid equivalent
GC	Gas chromatography
GW	Goat willow
HHC	Highest heat of combustion
IP	Induction period
LHC	Lowest heat of combustion
MeKA	Forest and Wood Products Research and Development Institute
MS	Mass spectrometry
MW	Microwaves
NMR	Nuclear magnetic resonance
ORAC	Oxygen radical absorbance capacity
PAC	Proanthocyanidins
PACE	Proanthocyanidins-containing extract
PF	Protection factor
RTU	Riga Technical University
LSIWC	Latvian State Institute of Wood Chemistry
TBHQ	Tertiary butyl hydroquinone
TE	Trolox equivalent

GENERAL DESCRIPTION

Materiality of the Topic

In relation with an increasing demand for renewables by limiting the use of fossil raw materials, a number of studies on the use of renewables for generating innovative and valuable products increases in the world. Wholesome use of raw materials, biorefinery, is one of the conditions for sustainable use of forest resources; therefore, evaluating the opportunities for rational and efficient use of wood processing by-products such as bark is required. The market of biorefinery products made of deciduous tree bark is currently underdeveloped, thus there are various opportunities to research the production of bioproducts from mechanical wood processing waste, including bark, which accounts for about 15 % of sawing by-products. Currently, the deciduous tree bark growing in Latvia is used as a renewable source of energy not using its potential as a source for high-quality products. Thanks to the unique chemical composition of bark, a variety of products with added value can be made of bark, including individual compounds or compound groups with synergistic biological activity, and raw materials for producing different materials. Various biologically active polyphenol compounds are concentrated in the bark, including proanthocyanidins. Natural oligomeric proanthocyanidins (PAC) have become a popular subject for intense fundamental and applied research in recent years, which is related to its unique structure of macromolecules and their functionality that provides wide opportunities for the use of proanthocyanidins in various industries such as pharmaceuticals, food industry, production of composite materials, etc.

Despite a large number of studies that are devoted to studying the antioxidant properties of low molecular weight plant polyphenols, the interest in safe and natural antioxidants continues to grow, which can be explained by the increasing demand for various ecologically safe additives that would improve the consumer-desired properties of lipid-containing products and the thermal oxidation stability of composite materials.

Proanthocyanidins-containing extracts (PACEs) are among the super potential materials for producing ecological binders as an alternative to phenol formaldehyde (PF) resins, which would enable both substituting phenol or resorcinol and reducing the formaldehyde emission from the finished composite material. Despite a large number of studies that are devoted to production of ecological binders, the results thereof are not fully evaluated. Researches on the use of PACE derived from the deciduous tree bark growing in Latvia in the production of ecological binders have not been carried out yet. The PACE-based binders could replace toxic PF resins thanks to the low price of PACE and environmental considerations.

The research carried out within the framework of the Doctoral Thesis was focused on further extension of the scientific base for bark biorefining in order to develop a flexible technological scheme that would enable combining its clusters depending on regional conditions (the need for a particular product, the presence of raw material base, the logistics of raw materials and product delivery, and so on) in the local markets.

Overall Goal of the Thesis

The overall goal of the Doctoral Thesis is to assess the potential of widespread deciduous tree bark growing in Latvia as a source of proanthocyanidins, to determine optimal proanthocyanidins-containing extracts (PACEs) and the extraction modes of proanthocyanidins (PAC) they contain, to characterize the chemical composition of PACEs, the structure and properties of PAC, and to explore the opportunities for using PACEs and PAC for the production of ecologically wholesome natural products in the context of biorefining.

The following objectives are set to achieve the overall goal of the Thesis:

- ❖ assess the potential of the bark of deciduous trees – grey alder, black alder, ash tree, and goat willow – growing in Latvia as a source of high-quality raw material in the context of bark biorefining;
- ❖ identify the optimal proanthocyanidins extracts (PACEs) and the extraction modes of proanthocyanidins (PAC) they contain by means of one-stage extraction process, and offer the technological solutions for those processes;
- ❖ characterize the chemical composition and structure of the deciduous bark extracted PACEs and PAC;
- ❖ research the antioxidant activity of PACEs and PAC, assess their efficiency in various chemical and biological systems, and assess the opportunities of their practical use in the production of ecological products;
- ❖ study the opportunities of using the isolated PACEs in the production of ecological adhesives, and evaluate the efficiency of resulting adhesives in the production of particleboard and plywood;
- ❖ evaluate the options for subsequent use of lignocellulosic residues.

Theses Proposed for Discussion

- ❖ The deciduous tree bark growing in Latvia is rich in polyphenols and is a suitable raw material for the extraction of proanthocyanidins-containing extracts (PACEs) and proanthocyanidins (PAC).
- ❖ Identified optimal extraction modes provide higher PAC transiting from the bark to extracts.
- ❖ Single-cycle PACE extraction taking into account the products' yield and power consumption is more cost-effective than three-cycle extraction.
- ❖ PAC and PACEs with their functional composition and high antioxidant activity are ecological, wholesome natural products and an alternative to synthetic antioxidants.
- ❖ PACE is a suitable raw material for the production of low-viscosity polyether polyols and ecological adhesives.
- ❖ After extraction, the deciduous bark can be used as a raw material for the production of nanofiller, increasing the mechanical strength of coniferous wood pulp paper, or as a solid biofuel due to its increased calorific value.

Scientific Novelty of the Doctoral Thesis

- ❖ The bark of grey alder, black alder, ash tree, and goat willow growing in Latvia is studied for the first time as a potential raw material for the production of high-quality products, proanthocyanidins-containing extracts (PACEs) and proanthocyanidins (PAC).
- ❖ The chemical composition and structure of the proanthocyanidins isolated from grey alder and black alder are determined.
- ❖ The PACEs and PAC antioxidant activity as well as their potential use in food industry, cosmetics, medicine, polymer composite production by replacing synthetic compounds with natural substances are proven.
- ❖ The opportunity to partly replace oil-based phenols in the synthesis of phenol-formaldehyde resin with the PAC-containing extract from grey alder bark in the production of plywood by increasing the mechanical strength of plywood and reducing the emissions of formaldehyde is demonstrated.
- ❖ The usefulness of the production and conversion of produced PAC as well as the introduction of a new cluster in the use of lignocellulosic residues in the scheme of deciduous bark biorefining is proven.

The Practical Significance of the Thesis

- ❖ The opportunity to extract PAC-containing products from the bark of the most common foliage trees in Latvia is demonstrated, which will contribute significantly to the development of bio-economy that is important for the Latvian economy and complies with the strategic objectives of the European Union.
- ❖ The optimum parameters of single-stage extraction are determined and the technological recommendations for the extraction of the PAC-containing extracts from the deciduous tree bark and further conversion thereof into proanthocyanidins are provided.
- ❖ The used modes of sequential extraction expand a range of extracted natural substances and ensure a maximum efficiency of proanthocyanidins extraction, although its use is disadvantageous from the economic point of view if compared with single-stage extraction.
- ❖ It is proven that the PAC-containing extracts and PAC are potential antioxidants for foods, cosmetics, and composites, having become an alternative to synthetic, environmentally unsafe antioxidants.
- ❖ The effect of PAC on the decreasing level of pyruvate in plasma displays the huge potential of these biologically active compounds in healthcare.
- ❖ New environmentally friendly PACE adhesives are offered for the production of wood composites with reduced emissions of formaldehyde.
- ❖ The option to use deciduous bark after PAC extraction as a solid biofuel of higher calorific value if compared with the initial bark and as a raw material for obtaining nanoparticle wood pulp by improving the mechanical properties of coniferous wood pulp paper is shown.

Approbation of the Thesis

The results of the present Doctoral Thesis are discussed and reflected in 13 scientific publications and 16 international scientific conferences. One patent of the Republic of Latvia is registered. The list of the author's publications is at the end of the Thesis.

1. SUMMARY OF LITERATURE REVIEW

Proanthocyanidins are macromolecular compounds with the structure of polyphenols, which may be considered as the products of flavan-3-ol monomer (+) – catechin and/or (–) epicatechin condensation. Proanthocyanidins are common in various plant products with hydroxyl group layout in 3’, 4’ B ring, as well as in mixtures with prodelfinides and other proanthocyanidin agents. Depending on the source of proanthocyanidins, the major part of proanthocyanidins consists of 4–10 units of flavanols. The chemical structural formula of proanthocyanidin and its monomer – catechin – is given in Fig. 1.

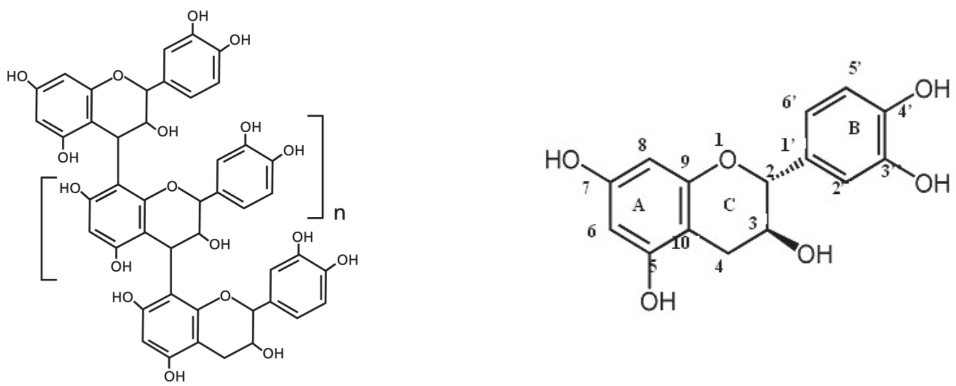


Fig. 1. Chemical structural formula of proanthocyanidin and its monomer – catechin.

Plant evolution resulted in the accumulation of proanthocyanidins and other polyphenols in bark. Proanthocyanidins are active metabolites in cellular metabolism and play an important role in various physiological processes of plant lifetime. These compounds are necessary for plants, because they protect the plants against microbial attacks, pathogens, mechanical damage, and infections increasing the resistance of trees to rotting and degradation [1], [2]. The presence of proanthocyanidins and other polyphenols provides colour, aroma, and taste of plants, protecting them from herbivores and making their taste unpleasant for them. The accumulation of proanthocyanidins and their allocation depend on genetic factors, climatic and ecological conditions.

From the medical point of view, proanthocyanidins and other similar polyphenols are potent antioxidants that protect a human body from free radicals that cause a variety of pathologies and diseases (such as ischemia, asthma, arthritis, inflammation, or even epilepsy). Proanthocyanidins also exhibit biological activity such as anti-inflammatory, anticancer, fungicide, antibacterial, and immunomodulating activity [3]. Due to a large number of OH groups, proanthocyanidins have explicit ability to form complexes, chelates or salts with proteins, alkaloids, heavy metals, and to eliminate them from the body or to facilitate their biological activity [4], [5]. The main difference of proanthocyanidins from other polyphenols is that they form a key part of the bioflavonoids consumed by a human. The production of preventive, sanitary, veterinary medicines as an area for using the biological activity and antioxidant properties of proanthocyanidins is of equal importance [6], [7].

Pycnogenol® is one of the commercial proanthocyanidins-containing extracts used in medicine. The drug is an extract obtained from the bark of coastal pine containing 70 % to

75 % of proanthocyanidins [8]. Research was carried out for more than 40 years to ensure safety, efficacy, and quality of Pycnogenol®, while more than 370 scientific articles were published, which confirmed the absence of Pycnogenol® toxicity. Nowadays, the Pycnogenol® extract is available in more than 700 food supplements, vitamins, cosmetics, and other health products around the world [9]. The product is a potent natural antioxidant. Clinical studies have shown that Pycnogenol® expands arterial blood vessels, reduces blood glucose levels, improves blood circulation, strengthens the immune system, and neutralizes damaging effects of free radicals on the cells of body. Pycnogenol® can improve renal function in patients with metabolic syndrome [10], [11].

Today, there are proanthocyanidin products made commercially with high proanthocyanidins content of the bark of oak, eucalyptus, and the Australian acacia in the world. For example, one can obtain about 180 kg of extract with high PAC content from 1 tonne of dry acacia bark (70 % to 80 % of extracts). Proanthocyanidins are also found in the wood, roots, and leaves of trees and shrubs, but in lesser concentrations than in the bark.

The ability of proanthocyanidins to form complexes with proteins, including blood proteins, justifies the use of proanthocyanidins in medicine, for instance, astringents and antimicrobial agents for treating inflammation in mouth cavity, gums, respiratory organs, and other inflammation, as well as burns and for stopping local bleeding. In the treatment of burns, proanthocyanidins solution precipitates proteins by forming a protective film on the burn site (wound) and protecting it from infection [12], [13]. Thanks for antioxidant properties, bitter taste, and interaction with proteins, proanthocyanidins-containing extracts are used for improving the wine flavour, clarification, and stabilization [14], [15].

The ability of proanthocyanidins to form insoluble compounds with alkaloid salts and heavy metals inhibits oral poisoning with morphine, cocaine, atropine, nicotine, lead salts, copper, cobalt, radionuclides, and other harmful substances. Proanthocyanidins with reduction characteristics are suitable as adsorbents for purifying the water from heavy metals (lead, cadmium, and copper) and for producing precious metals. Proanthocyanidins in the form of hydrogel are patented as the adsorbents of metal elements capable of adsorbing actinides, such as uranium, thorium, or other metals like cobalt, lead, chromium, mercury, etc. [16], [17]. Due to antioxidant properties of polyphenol, proanthocyanidins in the form of additives are used as inhibitors of metal corrosion (for instance, *Docker Nittron*). The proanthocyanidins-based composition provides anticorrosive protection by interacting with rust, converting iron oxides into inactive compounds of corrosion, and practically minimizing the rate of corrosion [18], [19].

One considers the production of ecological adhesives mainly for producing wood composite materials as one of the most promising areas of industrial application of proanthocyanidins-containing extracts.

Following the unique structure and functionality of proanthocyanidin macromolecules ensuring opportunities for widespread use of proanthocyanidins across different sectors, it would be rational to use deciduous bark as wood processing residue for extracting the proanthocyanidins or extracts containing the latter.

2. EXPERIMENTAL WORK

The subject of the present Doctoral Thesis is the bark of the deciduous trees grey alder (*Alnus incana*), ash tree (*Fraxinus excelsior*), goat willow (*Salix caprea*), and black alder (*Alnus glutinosa*) growing in Latvia to produce high-quality natural products, proanthocyanidins-containing extracts (PACEs) and the proanthocyanidins (PAC) obtained from them as an alternative to synthetic products. In collaboration with the institute "Silava", the specimen of the bark were collected in the region of Ogre, the forests of Ogresgals, quarters No. 197 and No. 198.

The structure of the Doctoral Thesis is subject to the previously mentioned objectives. The Thesis is structured in four chapters. In the first chapter, the potential of widespread deciduous tree bark growing in Latvia as a source of high-quality products (PACE and individual compounds like PAC) is assessed. There are optimal extraction modes of PACE and PAC determined in the second chapter. The third chapter includes the chemical characterization of PACE and PAC isolated from the deciduous bark and the evaluation of the opportunities for practical application of those products using PACE and PAC for the production of ecologically wholesome natural products in the context of biorefining. The fourth chapter, according to the concept of bark biorefining, assesses the opportunities for using the residue of deciduous bark or so-called lignocellulosic residue and includes a flexible technological scheme for a wholesome use of the bark.

For isolating PACE, the deciduous bark with the humidity of 7 % and the particle size of 0.5 mm to 2 mm was used. The bark with finer particle fraction (<0.05 mm) burdened PACE diffusion from the bark volume, therefore this fraction, accounting for 18 % of the total amount of the bark, was separated and used for obtaining other products according to the concept of biorefining.

To determine the optimal extraction modes of PACE, the following parameters were chosen as variables: extractant, hydromodule, extraction temperature and time, and the number of extraction cycles.

The need for sequential extraction of the deciduous bark depends on a species of trees, the regional needs for the biologically active compounds, such as triterpenoids, low molecular weight polyphenols, and a special purpose of the product. Sequential extraction of the deciduous bark was carried out with solvents according to their polarity in ascending order, starting from hexane, ethyl acetate, and finally with 40 % EtOH.

In order to evaluate the potential of the deciduous bark for extracting the PACE and individual compounds, proanthocyanidins, the main components, chemical composition and structure of the extracts were determined by means of the Folin-Ciocalteu, Porter, vanillin, GC and UHPLC-ESI-MS/MS analysis and equipment.

Proanthocyanidins were isolated from the extracts with high content of these compounds. The following analyses were used for characterising the proanthocyanidins: FTIS, GC, ^{13}C-NMR, and TOF-MS.

Antioxidant activity of proanthocyanidins (PAC) and proanthocyanidins-containing extracts (PACEs) was characterised using the $DPPH^{\bullet}$ (2.2-diphenyl-1-picrylhydrazyl radical), $ABTS^{+\bullet}$ (2.2-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid cationic radical), and oxygen radical absorbance capacity (ORAC) tests. The radical absorbance capacity of PACE and PAC in $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ tests is expressed as IC_{50} value, which shows the required concentration of an antioxidant to achieve the inhibition of the free radicals of 50 %. In its turn, in the ORAC test, the antioxidant activity of proanthocyanidins is expressed in mmol $TE^{\bullet}\,g^{-1}$, which shows the reduced concentration of superoxide radical anion $O_2^{-\bullet}$.

In order to evaluate the effects of PAC and PACEs on the oxidation stability of lipid-containing products, the Oxipress method was used. The antioxidant activity of PAC and PACEs in the lipid oxidation test is measured as induction period (IP), in which the oxidant is able to prevent the oxidation of the substrate. Synthetic phenol, tert-butyl hydroquinone (TBHQ) and Trolox, analogue of vitamin E, were used as reference antioxidants in the research.

For evaluation of the potential of the natural antioxidants in the inhibition of polymer thermal oxidation, there were thermal oxidative destruction tests of polyurethane (PU) films by adding the antioxidant at a stage of PU prepolymerisation.

Based on the functional composition of PACE components, the PACE extracted from grey alder bark was used for the synthesis of polyether polyols and ecological adhesives for wood processing production. The testing of particleboard and plywood was carried out in collaboration with CHIMAR HELLAS and the relevant departments of the MeKA and the LSIWC. The bonding quality of plywood was determined according to the requirements of the Articles 5.1.3 and 5.1.1 of the standard LVS 314 (6), whereas the boding quality of particleboard was determined according to the requirements of the standard EN312 P2.

The emission of formaldehyde in plywood samples was defined pursuant to the requirements of the standard JIS A 1460 and EN 717-2:1994.

Following the concept of bark biorefinery, the perspective usage of lignocellulosic residue was exemplified on the bark of grey alder. Evaluation of the perspective usage of lignocellulosic residue as solid biofuel was made based on combustion heat calculations using the elemental analysis data. In cooperation with the Pulp Laboratory of the LSIWC, lignocellulosic residue was tested as a filler for improving the mechanical properties of paper.

3. RESULTS AND DISCUSSION

The Doctoral Thesis focuses on the biologically active compound proanthocyanidins-containing extract (PACE) and the obtaining of the proanthocyanidins (PAC) by using the bark of the deciduous trees growing in Latvia. These studies have been conducted based on the insufficient exploration of deciduous tree bark for obtaining valuable products.

3.1. Assessment of the potential of deciduous tree bark for obtaining PACEs and PAC

For the research on the bark of deciduous trees growing in Latvia in order to obtain PACEs and PAC, ethanol and water were used as extractants based on their selectivity with respect to the PAC, chemical inertness, small toxicity, and low cost. For determining the most efficient extractants and their concentration for obtaining PACEs and PAC in laboratory conditions, a single-stage extraction was carried out under recommended extraction parameters using the data from the bibliography [20]–[23] and the results of previous experiments [23] that included: bark and extractant weight ratio 1 : 8 (hydromodule – 8), extraction temperature 80 °C, and time 30 minutes (Table 1).

Table 1

Effect of the concentration of extractant on the efficiency of PACE and PAC extraction (single-stage extraction, 30 min, 80 °C, hydromodule 8)

Extractant	Grey alder bark	Black alder bark	Goat willow bark	Ash tree bark
Extract yield from bark, %				
96 % EtOH	10.3	10.2	11.3	8.9
80 % EtOH	11.1	10.7	14.1	11.4
60 % EtOH	12.9	13.6	15.4	15.4
40 % EtOH	15.3	14.7	15.8	16.2
20 % EtOH	13.5	13.8	13.6	16.4
H_2O	12.6	11.7	11.2	14.8
PAC content in the extract / PAC yield from bark, %				
96 % EtOH	15.9 / 1.6	15.4 / 1.6	9.2 / 1.0	<0.01 / <0.01
80 % EtOH	17.4 / 1.9	17.1 / 1.8	11.4 / 1.6	<0.01 / <0.01
60 % EtOH	18.5 / 2.4	18.2 / 2.5	13.7 / 2.1	<0.01 / <0.01
40 % EtOH	21.9 / 3.4	21.6 / 3.2	14.1 / 2.2	<0.01 / <0.01
20 % EtOH	20.7 / 2.8	21.2 / 2.9	12.6 / 1.7	<0.01 / <0.01
H_2O	17.4 / 2.2	16.8 / 2.0	9.8 / 1.1	<0.01 / <0.01

The obtained results show that 40 % EtOH (w/w) ensures the highest extract and PAC yield from deciduous tree bark. Despite the high extract yield from the ash tree bark, while carrying out a quantitative analysis of proanthocyanidins, it was found that the extract did not contain proanthocyanidins as the target products.

3.2. Assessment of deciduous tree bark for obtaining biologically active compounds using sequential extraction

Depending on the species of tree, regional needs for other biologically active natural compounds such as triterpenoids and low molecular weight polyphenols, as well as product targets, sequential extraction can be used instead of a single-stage extraction of deciduous bark, including hexane and ethyl acetate (solvents permitted in food production) to 40 % ethyl alcohol into the extraction system. Under these conditions, the methodology becomes more promising for obtaining several high-value compounds, which confirms deciduous bark as high-quality raw material for obtaining natural products. In the result of sequential extraction of deciduous bark, three extracts were obtained: (1) extract of lipophilic compounds; (2) extract of low molecular weight polyphenol; and (3) extract of proanthocyanidins (PACE) (Fig. 2).

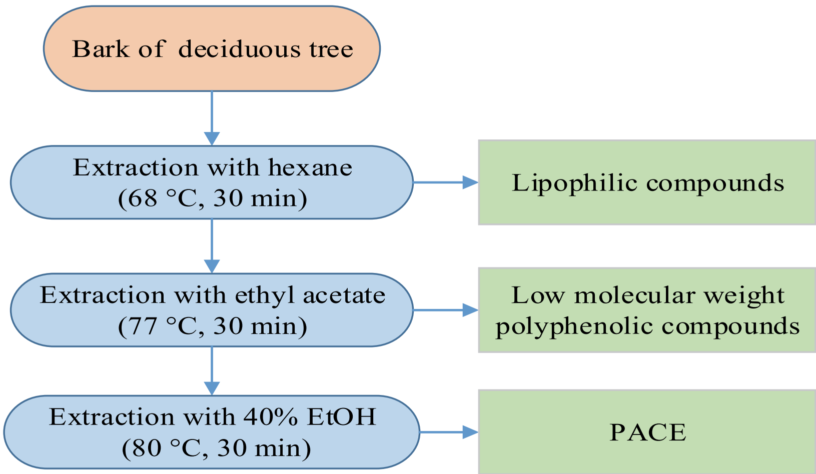


Fig. 2. Flowchart of sequential extraction of deciduous bark.

The yield of extracts at individual extraction stages depending on the species is given in Fig. 3.

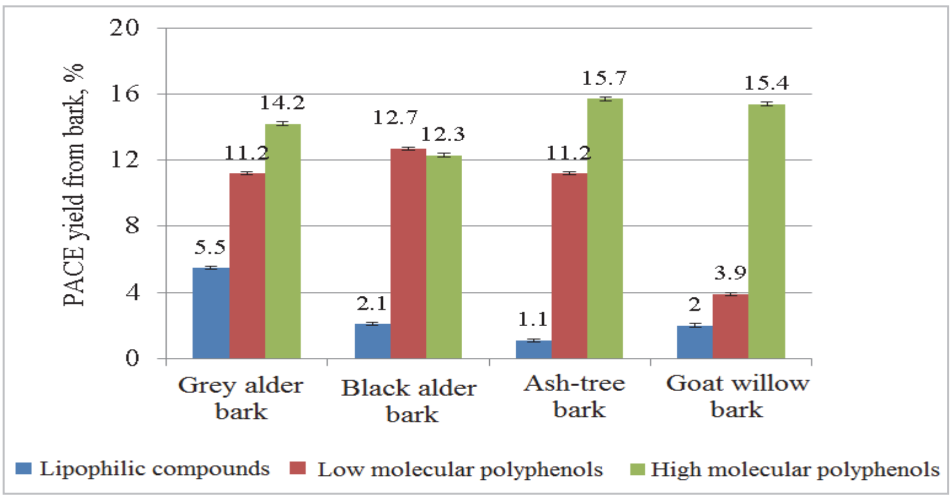


Fig. 3. The yield of extracts from deciduous tree bark at individual extraction stages.

The extraction of deciduous tree bark with a nonpolar solvent shows that the bark of ash tree, black alder, and goat willow contains only 1 % to 2 % of lipophilic compounds, which is significantly less than in the bark of grey alder (5.5 %). The yield of hydrophilic extracts accounting for the largest part of the total extracts, for instance, is about 82 % in the bark of grey alder; thus, deciduous bark cannot be considered as a potential raw material for obtaining lipophilic compounds. Approximately a half (42 % to 50 %, except the bark of goat willow) of hydrophilic extracts consist of ethyl acetate-extractable substances, which enables to conclude that deciduous bark is a valuable raw material for obtaining moderately polar compounds such as diarylheptanoid, including oregonin.

Having summarised the obtained results, it is clear that the bark of alders is the most preferable raw material for obtaining both low molecular weight (oregonin) and high molecular weight polyphenols (proanthocyanidins), whereas the bark of goat willow growing in Latvia is a potential source for obtaining low molecular weight polyphenols.

The yield of extracts obtained with 40 % EtOH from deciduous tree bark ranges between 12 % and 15 % of absolutely dry bark. In order to evaluate the potential of deciduous bark for obtaining proanthocyanidins and the extracts containing the latter, the extracts obtained with 40 % EtOH were subject to the analysis of their chemical composition. The chemical composition of hydrophilic extracts summed up in Table 2 shows that the total content of polyphenols in the extracts ranges between 16 % and 51 % (0.16 to 0.51 GAE$^{\bullet}$ g^{-1}). A part of polyphenols in the extracts is in the form of O-glycosides composed of aglycone and carbohydrate residue, which mainly consist of xylose and glucose. Among the compounds identifiable in the extracts of deciduous bark, there were polyphenols such as catechin (proanthocyanidin monomer), epigallocatechin, catechin dimer, trimer or even tetramer as well as hirsatunonol, oregonin, hydroxyoregonin, other polyphenols, and their glycosides found.

Table 2

Description of the chemical composition of hydrophilic extracts

Raw material	Oregonin content in ethyl acetate extract [29]	Polyphenols content in 40 % EtOH extract	Catechin content in 40 % EtOH extract	PAC content in 40 % EtOH extract	Carbohydrate content in 40 % EtOH extract
	% per absolutely dry extract				
Goat willow bark	<0.01	51.1	35.2	16.6	5.5
Grey alder bark	72.2	39.2	22.6	24.6	37.1
Black alder bark	75.0	46.1	23.6	24.4	25.8
Ash tree bark	<0.01	16.1	4.3	<0.01	52.8

The results show that by including ethyl acetate into the extraction scheme, biologically active compound, oregonin, can be obtained additionally from the bark of alder. Despite the high polyphenols content in the extract of goat willow bark (51 % of absolutely dry extract or 0.51 GAE$^{\bullet}$ g^{-1}), it contains less proanthocyanidins, that is, 16.6 % of absolutely dry extract. Catechin dominates in the extract of goat willow bark. The low content of polyphenols in the extract of ash tree bark, namely, 16 %, points to the low potential of this bark for obtaining biologically active compounds. The presence of oregonin and proanthocyanidins has not been determined in the hydrophilic extract of ash tree. Summarizing the results, a conclusion can be drawn that alder bark is the most appropriate raw material for obtaining PACE and individual compounds, PAC, out of the studied four kinds of deciduous tree bark that enables obtaining 120 g to 140 g of extract from 1 kg of bark with proanthocyanidins content of 24.4 % to 24.6 %. If sequential extraction (hexane, ethyl acetate, and alcohol) with the separation of the first two mentioned extracts is carried out, the content of proanthocyanidins slightly increases in the extract compared to the yield obtained by means of single-stage extraction (Table 3).

Table 3

Effect of extraction method on the content of proanthocyanidins in the extract

Raw material	Single-stage extraction		Sequential extraction	
	PACE yield from bark, %	PAC content in extract, %	PACE yield from bark, %	PAC content in extract, %
Grey alder bark	15.3	21.9	14.2	24.6
Black alder bark	14.7	21.6	12.3	24.4
Goat willow bark	15.8	14.1	15.4	16.6
Ash tree bark	16.2	<0.01	15.7	<0.01

The content of proanthocyanidins in bark depends not only on the species of tree but also on the site where the trees are growing and on the year when the bark is harvested. In view of this, throughout the process of developing the Doctoral Thesis, deciduous tree bark was harvested every autumn and the yield of their extracts by means of sequential extraction, as well as the content of proanthocyanidins in the obtained extract ranging from 16 % to 46 % for all these six years were determined based as per absolutely dry extract. The screening of PAC content in the extracts of deciduous bark showed that the content of PAC in alder bark extract was higher starting from 2012 if compared to 2011, which shows the high potential of alder as a raw material for obtaining PACE and proanthocyanidins (Fig. 4).

Fig. 4. The content of proanthocyanidins in the extracts of deciduous tree bark.

The high content of proanthocyanidins in the extract was observed also in 2009 and 2010, while conducting studies under the national research programme "New products and innovative forest management, production technologies for forest wood and non-wood products by rationally using forest resources and substantially increasing the added value of production". Given that goat willow has naturally afforested agricultural lands and this tree species is known as a high-quality raw material to obtain salicin in North America, much attention has been paid to studying goat willow bark in Latvia starting from 2011. Using high-resolution UHPLC-ESI-MS/MS mass spectrometry, it was found that goat willow bark from the trees growing in Latvia is rich not in salicin but in such polyphenols as catechins and catechin dimer (procyanidin B2). Catechin content in the extract of goat willow bark varies from 35 % to 40 %, which justifies the high total content of polyphenols (50 % to 60 %) in the extract.

3.3. Determining the optimum PACE and PAC extraction modes

For more efficient extraction of PACE and PAC from bark, the optimum PACE extraction modes were defined repeatedly and the optimum PACE extraction parameters mentioned above and used from the bibliography [20]–[21] were checked. The impact of the modifications of the following extraction parameters on the efficiency of PACEs and PAC extraction was investigated in order to develop a technology for PACE and PAC extraction:

1) extractant concentration,
2) bark and extractant weight ratio – hydromodule,
3) extraction temperature,
4) extraction time.

The efficiency of PACE extraction was monitored as per the yield of PACE and the content of PAC in the extract. Based on the previously conducted screening of PAC in the bark of various deciduous species (Fig. 5), the bark of grey alder was chosen as a target object of this research, because it is potentially full-fledged and most available raw material. The comparison of the chemical composition of the PACEs obtained by means of single-stage and sequential extraction, mainly PAC content in the extract (Table 3), revealed no significant changes thereof. In addition, sequential extraction (Fig. 2) raises the cost of PACE as a product, therefore single-stage extraction was used for extracting PACE from grey alder bark due to economic reasons.

In order to ensure that exactly 40 % ethanol-water solution is the most appropriate extractant for an efficient extraction of PACE from the bark, the extraction of grey alder bark was conducted once again for 30 minutes at 80 °C by changing the percentage of the extractive. The results obtained suggest that 40 % of EtOH provides the highest yield of extract from the bark, which reaches 15.3 % with proanthocyanidins content of 22 % calculated as per absolutely dry extract.

The previously carried out experiments aimed at determining the choice of hydromodule illustrated that the bark and extractant weight ratio of 1:4 (hydromodule – 4) is not sufficient because the extractive is not enough to soak the bark particles completely; thus, PACE extraction decreases from biomass solution. Having changed the hydromodule from 6 to 8, no specific changes in the PACE yield were observed. In its turn, increasing the hydromodule from 8 to 14, the PACE yield slightly decreased but power consumption increased from 0.38 kWh to 0.49 kWh, which is related to the increasing volume of the liquid phase in the extraction system. The impact of the hydromodule on the efficiency of PACE extraction is shown in Table 4. Optimum PACE extraction is reached at the bark and extractive weight ratio 1:8.

Table 4

Impact of hydromodule on the efficiency of PACE extraction

Bark weight, g	Extractant (40 % EtOH) weight, g	PACE yield, % of absolutely dry bark	Proanthocyanidins content in extract, %	Power consumption, kWh
10.1	60	15.1	21.7	0.32
10.1	80	15.3	21.9	0.38
10.3	100	14.2	21.8	0.42
10.2	120	13.7	21.8	0.46
10.1	140	12.6	21.9	0.49

It is generally known that high temperature of extraction increases the efficiency thereof. To determine the optimum temperature of extraction, the extraction of grey alder bark was performed at the temperature of 20 °C, 40 °C, 60 °C, 80 °C, 100 °C, and 120 °C with 40 % EtOH at the determined optimum hydromodule (1 : 8) for 30 minutes. The results showed that the highest PACE yield is achieved if the temperature of the extraction is 80 °C. Having increased the temperature of extraction to 120 °C and performed the extraction in an accelerated solvent extraction device (ASE 300), the content of proanthocyanidins in the extract decreased suggesting the thermal destruction of proanthocyanidins at an elevated temperature (Fig. 5).

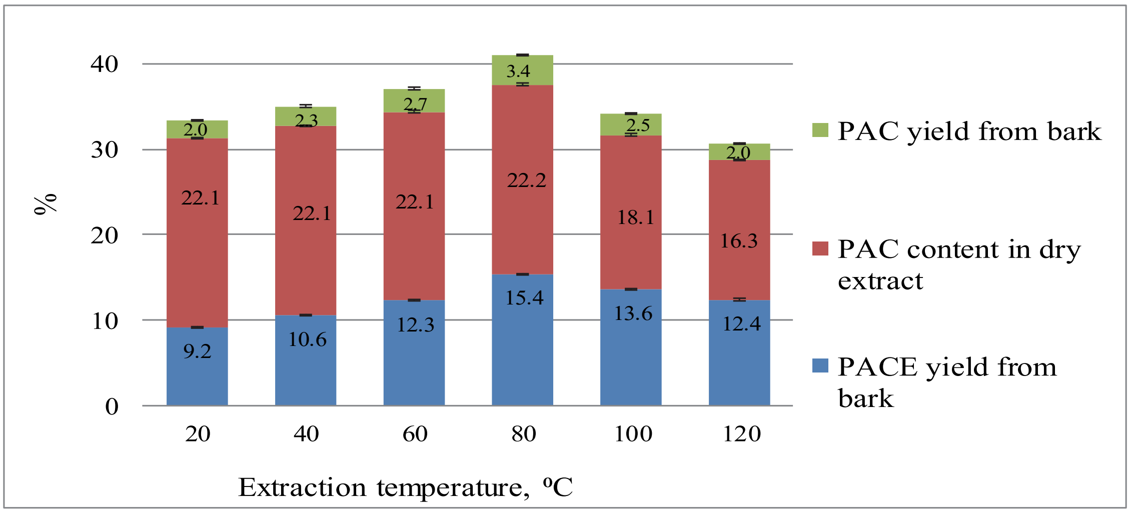


Fig. 5. Impact of the temperature of extraction on the efficiency of PACE and PAC extraction.

The efficiency of PACE extraction depending on the time of extraction is demonstrated in Fig. 6. For optimising the time of extraction, the extraction of grey alder bark was carried out at the determined optimum temperature of extraction of 80 °C, optimum hydromodule 8, using 40 % of EtOH as an extractant. The time of extraction was changed from 5 to 60 minutes by defining the maximum yield of extract instead of the content of proanthocyanidins in the extract. The results obtained show that the optimum time of extraction for extracting the PACE was 30 minutes by obtaining 153 mg of the extract with the content of proanthocyanidins in the extract being 22.0 %.

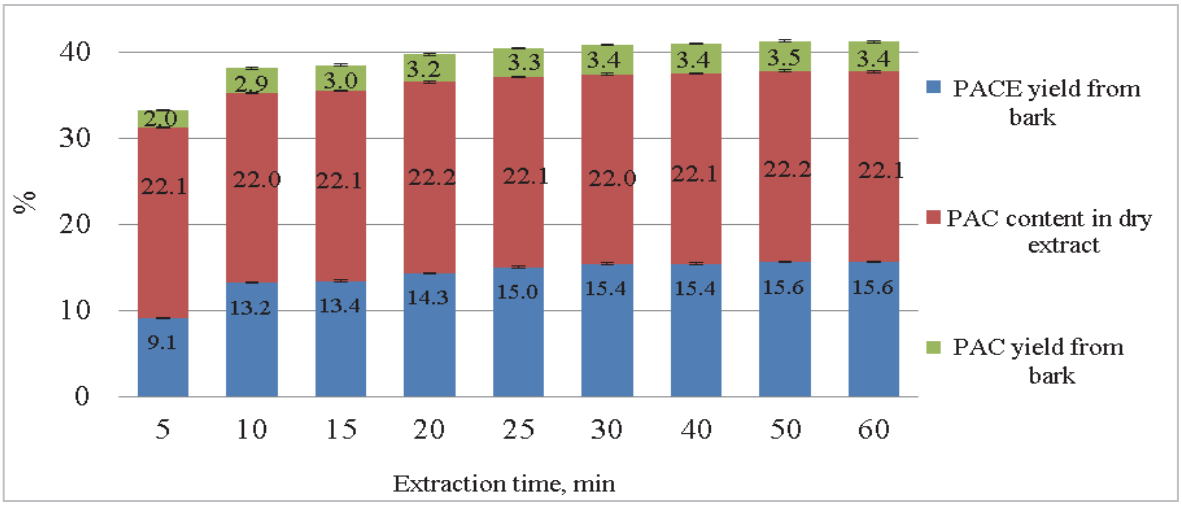


Fig. 6. Impact of the time of extraction on the efficiency of PACE and PAC extraction.

Having repeated single-stage extraction of grey alder bark three times (three-cycle extraction) when each cycle lasted 30 minutes (40 % EtOH; 80 °C; hydromodule 8; 3 min × 30 min), it was found that the highest PACE yield was achieved during the first cycle of extraction, when it constituted 87 % of the total extracted amount of PACE. During the second and third cycle, PACE yield was 9 % and 4 % respectively by calculating as per absolutely dry bark. Having compared the contents of proanthocyanidins in the extract, it is seen that the content of proanthocyanidins slightly increases with each subsequent cycle of extraction and the purity of PAC increases 1.1 times (Fig. 7).

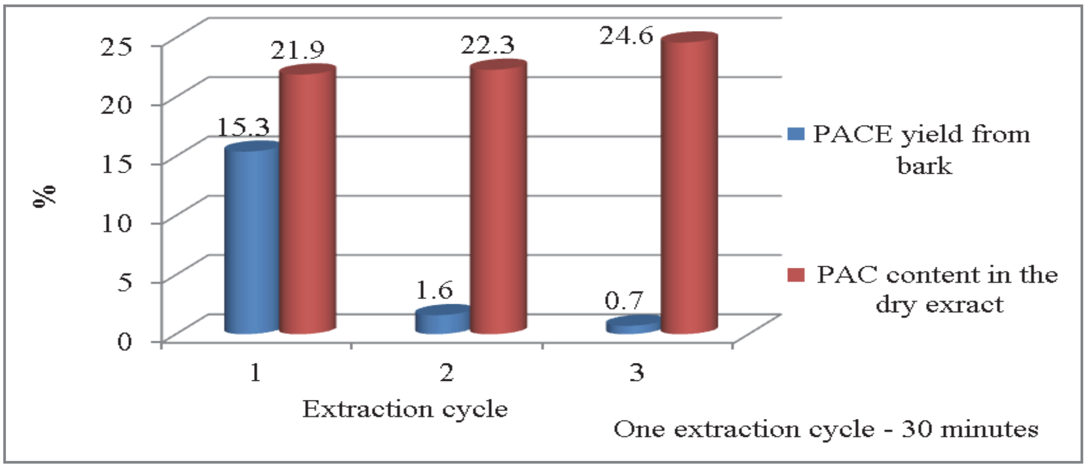


Fig. 7. Distribution of obtained PACE per fractions by carrying out three-cycle extraction of grey alder bark.

Calculated as per absolutely dry bark, the PAC yield from bark constituted 0.4 % and 0.2 % respectively during the second and third cycle of extraction. Given that in the event of three cycles of bark extraction, power consumption increases three times, which is unprofitable from the point of view of PACE extraction, while the increase in the extracted PACE and PAC is small, it is rational to use one cycle of extraction for 30 minutes for the extraction of PACE and PAC from deciduous bark. Taking into account the results of the previous experiments (Tables 1–4, and Figs 3–8), the following optimum extraction parameters were set for efficient extraction of PACE and PAC from grey alder bark:

1) extractant – 40 % EtOH;
2) hydromodule – 8;
3) temperature – 80 °C;
4) time of extraction – 30 min.

The mentioned parameters provide the highest yield of hydrophilic extracts (15.3 % as per absolutely dry bark) with the content of proanthocyanidins being 22 %. Under such conditions, 86.5 % of proanthocyanidins transfer to the first fraction of hydrophilic extracts from grey alder bark containing proanthocyanidins (Fig. 8).

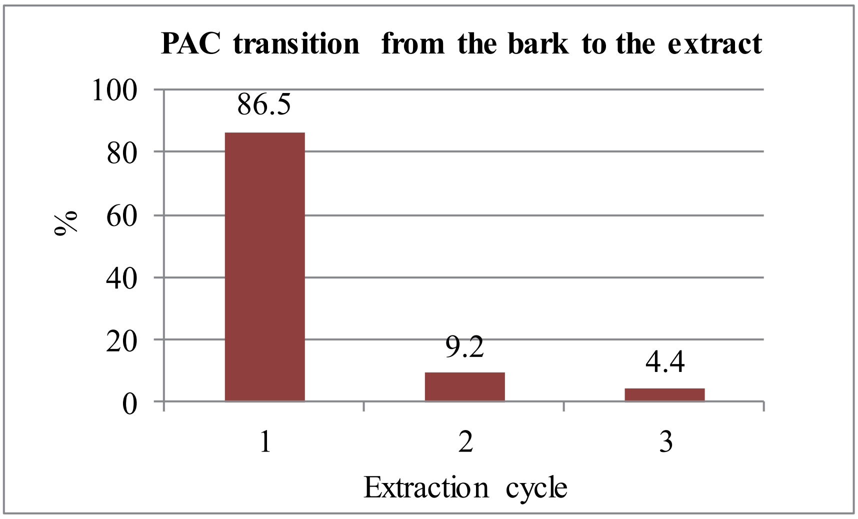
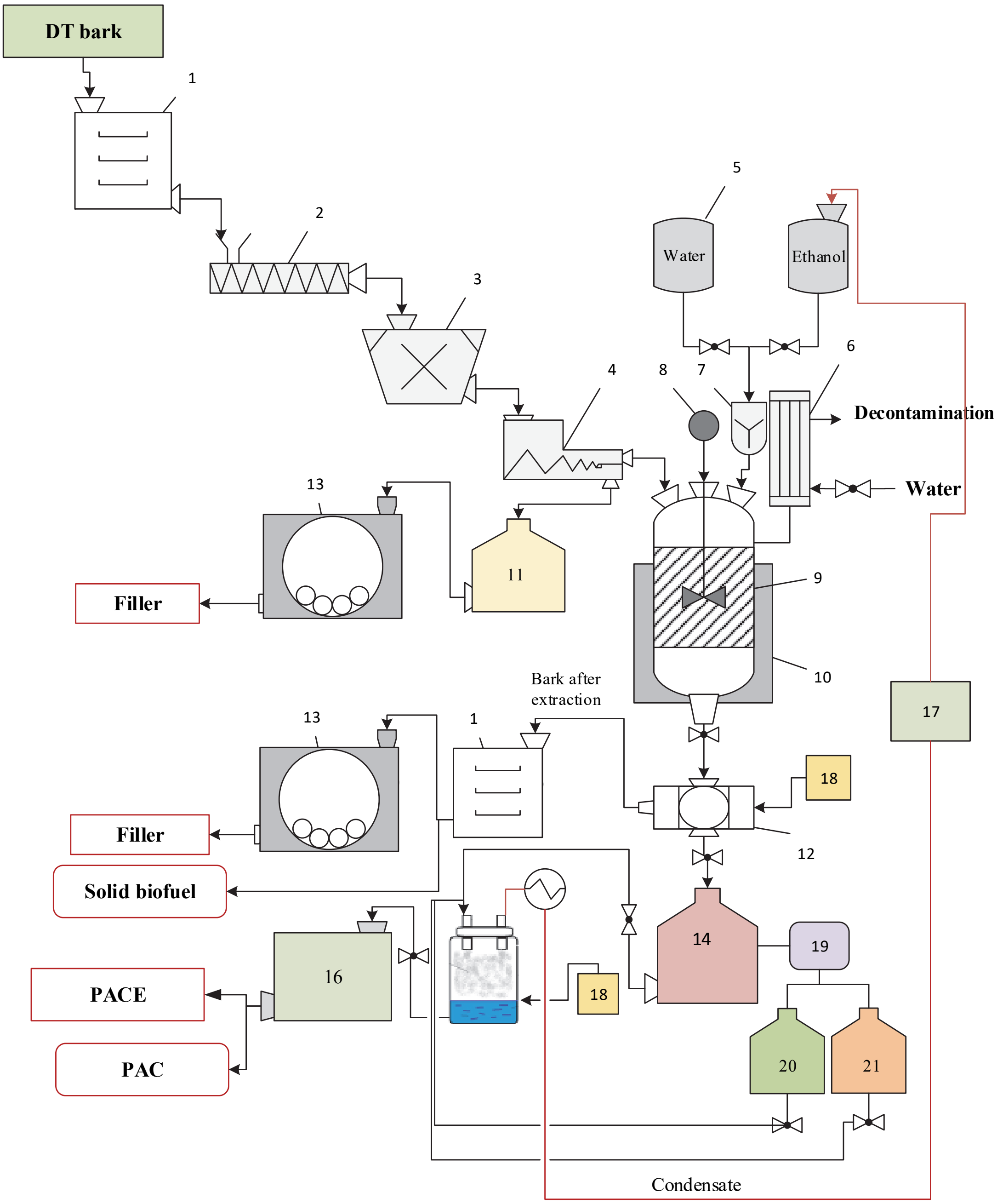


Fig. 8. Transition of proanthocyanidins from grey alder bark to the extract.

Based on the obtained experimental data, a technological scheme for extracting PACE and PAC from deciduous bark was developed, which is approximated for use under industrial conditions (Fig. 9). The technological scheme provides non-waste processing of deciduous bark, resulting in a large variety of natural products, including proanthocyanidins and others for further use thereof. After the extraction, deciduous bark (lignocellulosic residue) can be used as a raw material for obtaining a filler in order to improve the mechanical properties of softwood pulp paper or as solid biofuel with an increased calorific value.

3.4. Technological scheme for obtaining PACE and PAC

A tendency to take full advantage of the wood suggests the effective use of wood waste, including deciduous bark; therefore, the development and use of new technologies and products in the various sectors of national economy by replacing synthetic products with much more full-fledged natural products are of crucial importance. In woodworking plants, there are approximately 0.5 million cubic metres of bark formed in log processing, which are mainly used for heat generation at local boiler houses or taken away for disposal in landfills, which involves additional costs for forwarding and landfill maintenance. In view of this, the most rational way to utilise industrial wood waste is using bark for producing high-quality environmentally safe natural products. The recommended technological scheme for wholesome bark processing is shown in Fig. 9.



1 – drier; 2 – bark conveyor; 3 – crusher; 4 – sieving mesh; 5 – solvent tank; 6 – capacitor; 7 – extractant tank; 8 – mixer; 9 – reactor; 10 – electric heater; 11 – bark reservoir (particle size <0.5 mm); 12 – filter; 13 – ball mill; 14 – container with the extract; 15 – evaporator; 16 – lyophilisation device; 17 – recovery device; 18 – vacuum; 19 – proanthocyanidin isolation column.

Fig. 9. Technological scheme for obtaining PACE and PAC.

After bark is peeled off from logs, deciduous bark is dried (1) and when reaching the moisture content of <10 %, is fed into a cutter by means of a conveyor (2). After bark is crushed, it is delivered to a sieving mesh (3), where the finest fraction of bark is deselected (<0.5 mm), which is transported from a reservoir (11) to a ball mill (13), where it is milled to a size of nanoparticles for producing a filler. Remaining fraction of bark with a particle size of 0.5 mm to –2 mm is fed into an extractor (9) for extracting the proanthocyanidins-containing extract. An electrical heater (10) provides the optimal temperature of extraction. The extraction of deciduous bark results in two products: proanthocyanidins-containing extract, and residual deciduous bark (lignocellulosic residue). Lignocellulosic residue is fed into a dryer (1), then into a ball mill (13) for producing a filler or for producing heat when grinding process is omitted.

Depending on the purpose of final product, the extract of proanthocyanidins from the tank (14) is fed into a lyophilic dryer (16) for obtaining dry extract or, in the latter case, for the isolation of individual compounds (proanthocyanidins) into a column (19) followed by distillation (15) and lyophilic drying for producing dry proanthocyanidins. In both cases, distillation-separated condensate is fed into a recovery device (19) and re-used as an extractant for obtaining the extracts of proanthocyanidins.

By virtue of six-year PAC screening in deciduous bark (Table 5), the obtained results show that PAC extraction from bark is an expensive and labour-consuming process. From an economic point of view, the industrial production of these compounds with the yield of <7.8 % (data during the research under the national research programme in 2009) from the bark of deciduous trees growing in Latvia is not rational, but one cannot say the same about obtaining the PACE.

Table 5

The yield of PACE and PAC from deciduous tree bark by using sequential extraction

Grey alder bark						
Bark harvesting year	2009	2010	2011	2012	2013	2014
PACE yield from bark, %	17.0	15.4	14.1	14.8	12.7	17.2
PAC yield from bark, %	7.8	6.5	3.5	5.2	5.3	7.2
Black alder bark						
Bark harvesting year	2009	2010	2011	2012	2013	2014
PACE yield from bark, %	16.2	15.1	12.3	14.2	13.7	16.8
PAC yield from bark, %	5.7	5.3	3.0	6.0	6.3	6.4
Goat willow bark						
Bark harvesting year	2009	2010	2011	2012	2013	2014
PACE yield from bark, %	–	–	15.4	18.1	14.4	12.6
PAC yield from bark, %	–	–	2.5	3.8	2.0	3.0

3.5. Assessment of opportunities for industrial production of PACE

There are several major items contributing to the costs for obtaining proanthocyanidins-containing extracts (PACEs): price of wood waste (bark), electricity expenses, labour costs, price of manufacturing equipment, which includes installation and depreciation costs. For producing 1 kg of PACE, 6.3 kg of bark with humidity content of 7 % and particle size of 0.5 mm to 2 mm is needed. The cost of deciduous bark is minimal because wood waste from local sawmills is mainly used as a raw material, for instance, black alder as a fast-growing tree is still required in furniture industry for both producing veneer and finishing materials. As for grey alder, goat willow and ash tree, which are not widely sought after in furniture industry, the purchase price of fresh bark is ~500 EUR per ton. The cost of electricity constitutes the largest share of total expenditure of lyophilisation of proanthocyanidins-containing extracts to obtain dry extracts in particular. Laboratory-scale recovery of ethyl alcohol reduces the costs of reagents and the finished product cost wherewith. The flowchart and material balance of the process for obtaining PACE from deciduous bark are provided in Fig. 10.

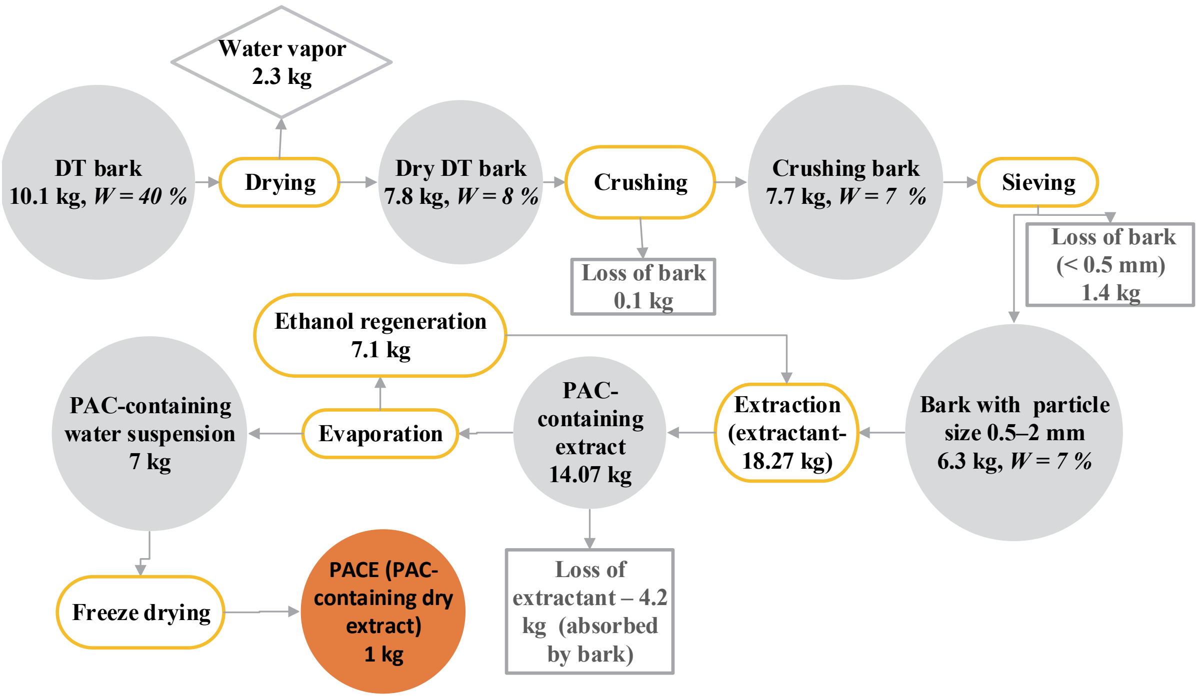


Fig. 10. Flowchart of PACE production with material balance of the process.

Whereas energy efficiency is among the prevailing characteristics for any technological process, the extraction in a microwave field (MW extraction) is an alternative to the previously discussed single-stage extraction for reducing the energy consumption required for obtaining PACE, which significantly reduces the warm-up time of the substrate compared to convective heating. It has been proved that the use of MV extraction and lower power consumption enable obtaining a higher PACE yield than in the event of convective heating. Comparing the PACE yields from grey alder bark and the energy consumption when producing this targeted product, it was found that the PACE yield in the first 5 minutes of MV extraction was 1.6 times higher and constituted 82 % of the total PACE yield from bark with power consumption of only 0.02 kWh (Fig. 11).

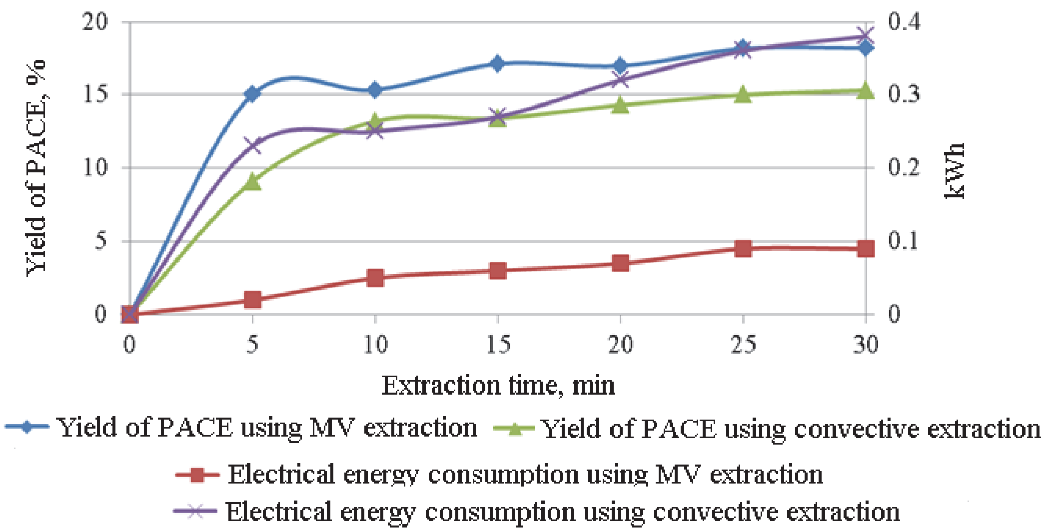


Fig. 11. PACE yield and power consumption when using MW extraction and convective heating.

Microwave extraction saves the consumed energy; thus, it significantly reduces the time and cost of producing the targeted products. The estimated price of product analogues containing similar polyphenols that can be obtained from bark varies between 1000 EUR/kg up to 8000 EUR/kg according to the results of the literature review depending on the type of the product and its content in the bark. *Picnogenola®* costs 60 EUR to 120 EUR per 100 mg depending on the content of proanthocyanidins in the dry extract [9]. Having calculated the prime cost of proanthocyanidins-containing extracts, the price of this product in laboratory scale except equipment costs (price of the equipment, maintenance, and depreciation) would be about 300 EUR/kg. The labour costs contribute the most or 70 % to the product price. When working seven hours a day for five days a week, one week is required to obtain 1 kg of PACE, i. e., 35 working hours. At such a load, labour costs would be 217 EUR. Automation of the process can significantly reduce the prime cost of the targeted products.

3.6. Description of the proanthocyanidins extracted from alder bark

The extraction of individual compounds, proanthocyanidins, was conducted from the extracts of grey alder and black alder bark based on their high content in extracts (Table 5). The proanthocyanidins extracted from alder bark extracts showed a high degree of purity (≈99 %) following the butanol-HCl method, compared to procyanidin B2 standard. In the case of grey alder bark, carbohydrate content in the extract decreased nine times.

For the determination of the molecular weight of proanthocyanidins, MALDI-TOF MS/MS was used. The resulting TOF-MS mass spectra (Figs 12 and 13) showed that the molecular weight of purified proanthocyanidins did not exceed 1441 Da, which suggests that proanthocyanidins are oligomer polyphenol compounds.

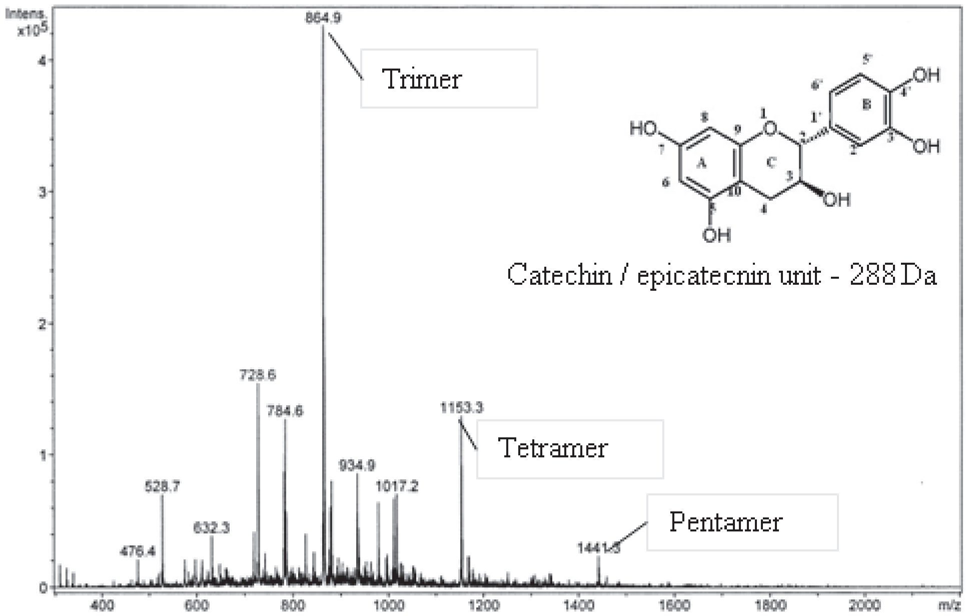


Fig. 12. TOF mass spectra of proanthocyanidins from grey alder bark.

Regular fragmentation by 288 Da corresponding to a catechin/epicatechin unit indicates that proanthocyanidins contain catechin/epicatechin trimers (865 Da), tetramers (1153 Da), and pentamers (1441 Da).

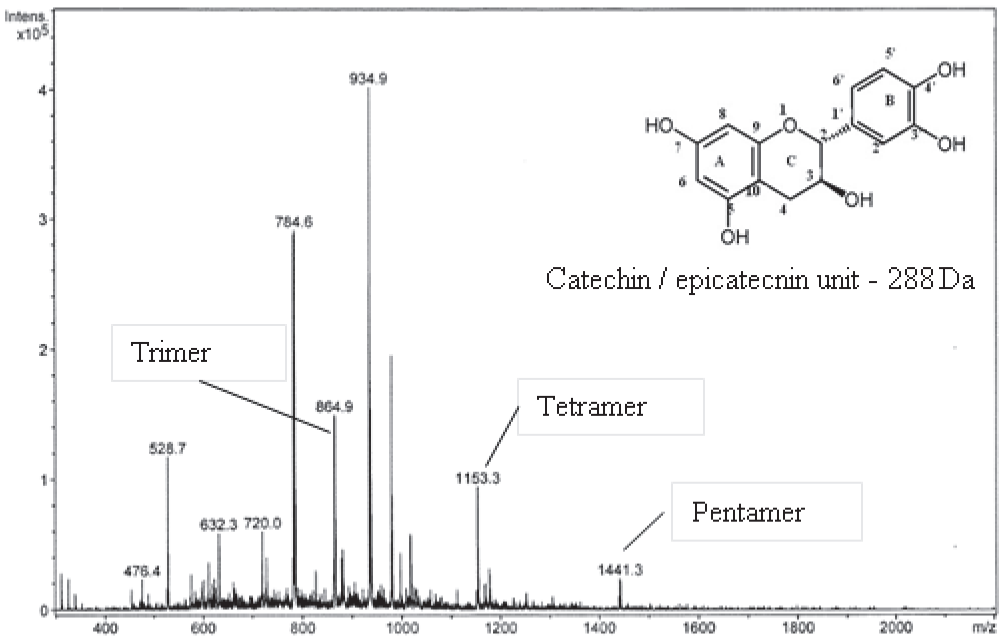


Fig. 13. TOF mass spectra of proanthocyanidins from black alder bark.

When taking ^{13}C-NMR spectra of purified proanthocyanidins, more detailed information on the substructures and functional groups of the compound under research was obtained, which enabled identifying the chemical structure of the tested proanthocyanidin (Figs 14 and 15).

The ^{13}C-NMR spectra signals were interpreted based on the literature data, which indicated that the tested substance formed procyanidin (catechin or epicatechin) units.

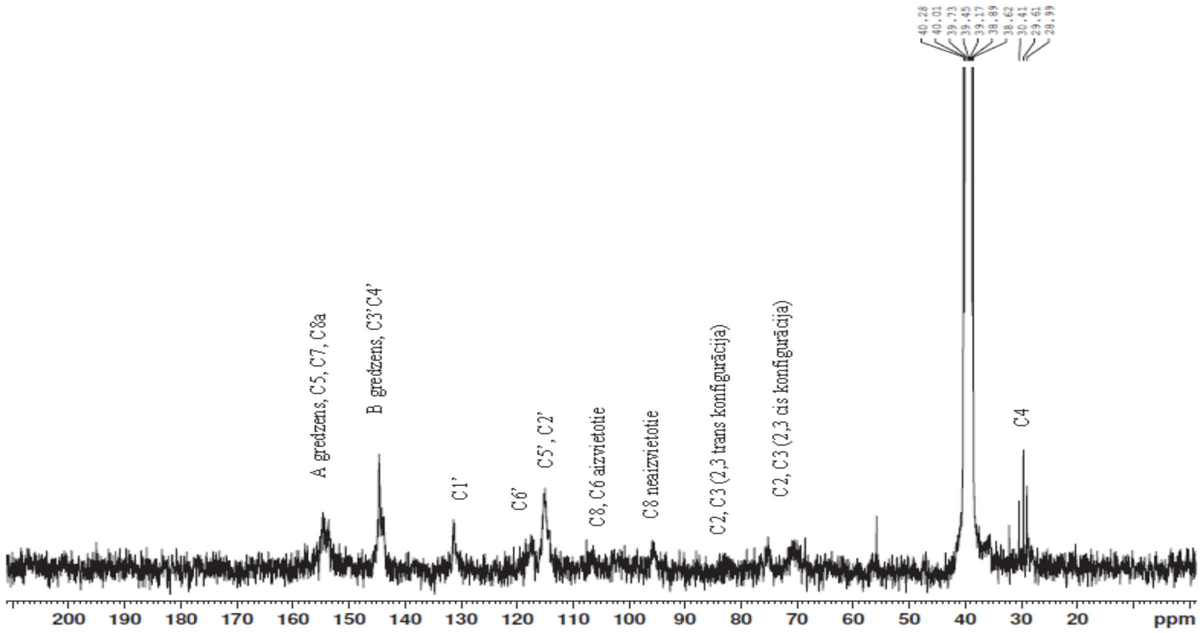


Fig. 14. The ^{13}C-NMR spectra of purified proanthocyanidin from grey alder bark.

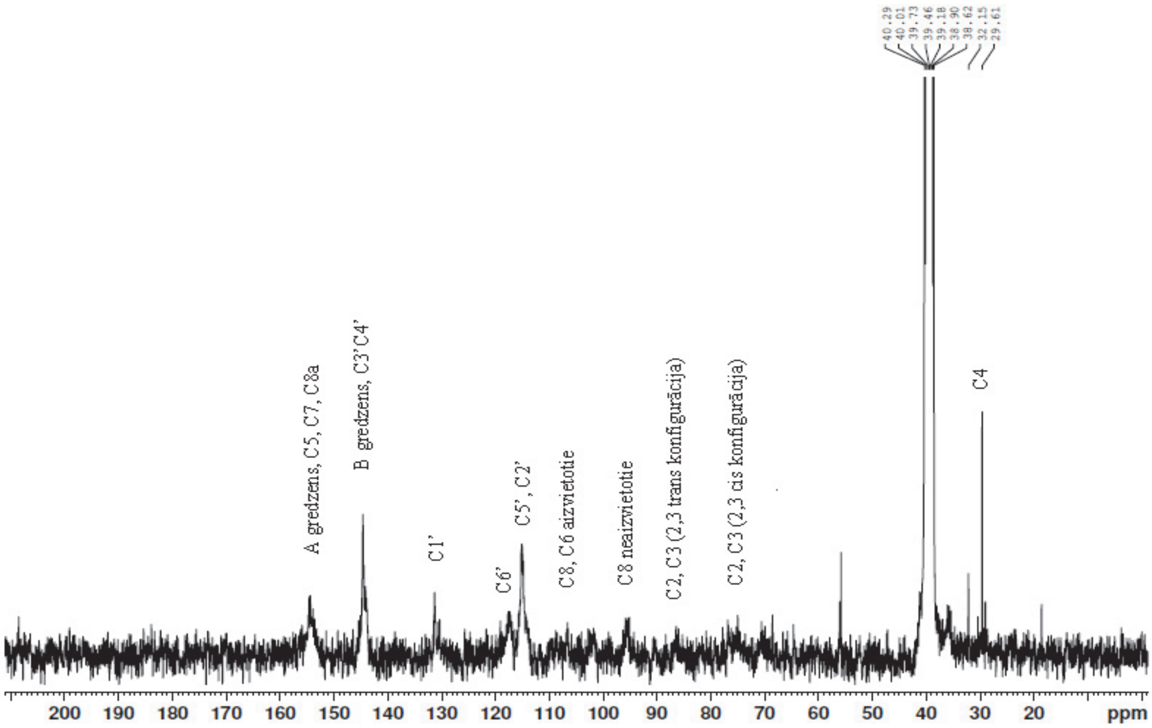


Fig. 15. The ^{13}C-NMR spectra of purified proanthocyanidin from black alder bark.

Combining the results of the TOF-MS and ^{13}C-NMR analysis displayed that extracted proanthocyanidins from the alder growing in Latvia were procyanidin of type B, where the units of catechin/epicatechin monomer in the oligomers with a degree of polymerization being 2–5 were related to bond C4-C8.

3.7. Antioxidant activity of the PACEs and PAC

When comparing the antioxidant activity of the PACEs from deciduous bark, a correlation between the polyphenol content in an extract and their antioxidant activity can be observed, i. e., when the content of polyphenol compound increases in the extract, radical deactivation activity increases in the $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ tests. Among the researched PACE, the extract of goat willow bark manifested the highest antioxidant activity compared to other extracts (Table 6).

Table 6

Antioxidant activity of PACEs in the $ABTS^{+\bullet}$ and $DPPH^{\bullet}$ tests

Sample	Total polyphenols in dry extract, $GAE^{\bullet}\,g^{-1}$	Carbohydrate content in dry extract, %	IC_{50}, $mg{\cdot}L^{-1}$	
			$ABTS^{+\bullet}$	$DPPH^{\bullet}$
PACE from grey alder bark	0.51	5.5	2.2	3.8
PACE from black alder bark	0.39	25.8	3.3	6.1
PACE from goat willow bark	0.46	37.1	4.3	7.5
Trolox	–	–	4.0	5.0

While analysing proanthocyanidins, if compared to Trolox, the purified proanthocyanidins from alder bark showed the highest antioxidant activity (Table 7). Compared with previously obtained results, it is evident that when proanthocyanidins are obtained from the extracts, antioxidant activity of these compounds increases.

Table 7

Antioxidant activity of proanthocyanidins

Sample	IC_{50}, $mg{\cdot}L^{-1}$		ORAC test, $(mmol\ TE){\cdot}g^{-1}$
	$ABTS^{+\bullet}$ test	$DPPH^{\bullet}$ test	
PAC from grey alder bark	1.4	3.0	5.3
PAC from black alder bark	1.1	2.4	4.8
Trolox	4.0	4.7	4.0

IC_{50} – concentration of antioxidant weight required for achieving 50 % inhibition of free radical;

$(mmol\ TE){\cdot}g^{-1}$ – Trolox equivalent mmoles (TE) per 1 gram of the antioxidant under study.

Oxygen radical absorption capacity (ORAC) is one of the most widely used methods that provide information about the antioxidant ability to protect a substratum (fluorescein in this particular case) from oxidation with oxygen peroxyl radicals. The test was carried out involving the proanthocyanidins isolated from the extracts of grey alder and black alder bark. Using a 0.0025 %-proanthocyanidins solution for testing, the induction period of fluorescein oxidation is ≈25 min. When compared to Trolox (4.0 $(mmol\ TE){\cdot}g^{-1}$), it is seen that PAC as antioxidants are more efficient (4.8 $(mmol\ TE){\cdot}g^{-1}$ to 5.3 $(mmol\ TE){\cdot}g^{-1}$) by protecting fluorescein against oxidation (Table 7).

The findings give evidence (Tables 6 and 7) that all proanthocyanidins-containing extracts and extracted proanthocyanidins have a high antioxidant activity, which confirms their perspective use for inhibiting the oxidation of various organic products.

3.8. The impact of PAC and PACEs on product oxidation process

Lipid-containing products are exposed to the air and oxygen during production and storage, which leads to a series of oxidation reactions that result in lower nutritional value and shelf life of the products. The main way to protect products from oxidation is use of antioxidants. Based on the results obtained (Tables 6 and 7) on the high antioxidant activity of proanthocyanidins and extracts containing them from deciduous bark by neutralizing free radicals, the opportunities to use PACEs and PAC have been studied as antioxidants for practical stabilisation of foods and cosmetics. Fig. 16 describes the impact of antioxidant (AO) concentration on the oxidation time of mayonnaise.

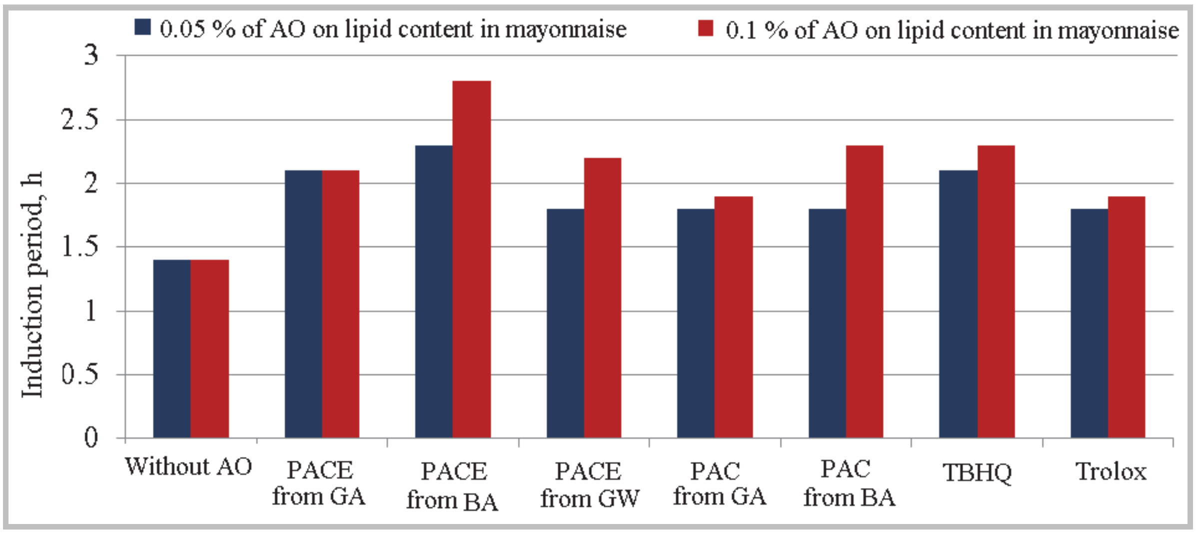


Fig. 16. PAC and PACE impact on mayonnaise oxidation time.

Adding 0.05 % to 0.1 % PACE to mayonnaise significantly improves the stability of the latter if compared to the initial sample. When comparing with synthetic antioxidants, PAC extract isolated from black alder bark is the most promising natural antioxidant. Adding 2 % of PACE from black alder bark into a cosmetic cream, its stability increases 2.4 times. Despite the high antioxidant activity of proanthocyanidins, the extracted proanthocyanidins manifested a lower protection factor in the oxidation test of both products than PACE. Most probably that the group of polyphenol compounds in the extract exhibit synergistic biological activity, which increases the efficiency of protection during the oxidation of the product.

One of the areas where PACE and PAC could be used as antioxidants-stabilizers is the synthesis of polyurethanes (PU) by adding 2 % of the mentioned antioxidants into a formula of polyurethanes at the prepolymerisation stage. According to the data summed up in Table 8, when antioxidants are added, the start temperature of polyurethane destruction increases and thermal oxidation is delayed compared to the destruction of non-stabilised PU, which already begins at 285 °C. Compared to synthetic antioxidant *Irganox 1010*, natural antioxidants hinder PU oxidation more efficiently by increasing their thermal stability. The most efficient thermal stabilizers of PU are proanthocyanidins extracted from grey alder bark.

Table 8

Antioxidant effect on thermal oxidation caused the destruction of PU films

Sample	T_{start}, °C	T_{max}, °C	Weight loss ($mg \cdot min^{-1}$) at 285 °C
PU without antioxidant	285	323	0.31
PU + Irganox 1010	293	323	0.27
PU + PACE from grey alder bark	310	342	0.16
PU + PAC from grey alder bark	319	341	0.08
PU + PACE from black alder bark	300	342	0.28
PU + PAC from black alder bark	316	342	0.10
PU + PACE from goat willow	310	341	0.19

3.9. Biological activity of PACEs and PAC in a biological system

In collaboration with RSU Department of Human Physiology and Biochemistry, *in vitro* tests of proanthocyanidins and proanthocyanidins-containing extracts were conducted to assess their potential use in healthcare. With regard to the amylase activity, the obtained results show that the added amount of PACE, 100 µL, stimulates the activity by increasing the amylolytic force by 15 % to 30 %. PAC and PACE reduce lipid absorption in the blood, inhibit pancreatic lipase activity, and reduce pyruvate concentration in plasma from 3 mg/dl to 2.2–2.5 mg/dl, which suggests reduction in blood sugar.

3.10. Use of PACE in the synthesis of polyether polyols

Studies on the potential use of PACE in the synthesis of polyether polyols were carried out in collaboration with the Lignin chemistry laboratory of LSIWC in Latvia, where the oxypropylation of PAC extracts from grey alder bark resulted in polyether polyols. Comparing the viscosity of other biomass used for polyol synthesis, the low viscosity of resulting polyols of 0.8 Pa·s facilitates the production of polyurethanes, which allows working with less pressure in atomising equipment. The comparison of the resulting polyol viscosity and the viscosity of other biomass found in the literature is shown in Table 9.

Table 9

Viscosity of a sample for the synthesis of polyether polyols

Sample	Viscosity, Pa·s
PACE from grey alder bark	0.8
Wheat straw organasolv lignin [24]	63.7
Date seed [25]	6.9
Organasolv lignin [24]	93.0

3.11. Use of PACE in production adhesives

Following the functional composition of PACE components, the PAC extracted from grey alder bark was investigated regarding the production of ecological binders for manufacturing of particleboard and plywood.

Partial replacement of phenol with PACE in the synthesis of phenol and formaldehyde resins

In cooperation with CHIMAR Laboratory (Greece), FF resins were synthesised by substituting 20 % of phenol with proanthocyanidins-containing extracts obtained from grey alder bark in 2012. A proportion of PACE (20 %) was taken based on the literature data [28] on the high reactivity of proanthocyanidins with formaldehyde. Carrying out sample testing, the applied PACE-FF resin as a binder for wood showed equivalent strength indicators, reaching the value of ≥1 MPa, which complies with the requirements under the Articles 5.1.3 and 5.1.1 of EN 314 (Table 10). In dry operating conditions (Class 1), the values of shear strength for the plywood glued by means of PACE-FF resin were higher compared to the control samples glued by means of FF resin.

Table 10

Plywood test results

Adhesive	Shear strength, MPa			Formaldehyde emission, $mg \cdot L^{-1}$
	Without pre-treatment	Pretreament required by Class 1	Pretreament required by Class 3	
PF	2.6	1.5	1.3	0.09
PACE-PF	2.1	1.6	1.1	0.05

Partly substituting phenol with PACE, the formaldehyde emission of plywood decreased 1.9 times compared to the control (FF).

Adhesives on PACE basis

For developing new and ecological adhesives for the production of wood materials, the PACE made of grey alder bark harvested in 2013 was modified by polyethylenimine (PEI)

widely used in pulp industry. The crosslinking reaction between PACE and PEI was demonstrated by thermogravimetric analysis (TGA).
The experimentally obtained PACE-PEI-based binder has been described as a viscous liquid (172 cP ± 10 cP), brown colour, with a dry matter content of 25 %, without a specific smell. To test the glue ability of the PACE-PEI binder obtained in the laboratory conditions, the samples of particleboard and plywood were produced.

Particleboard was made of pine chippings by impregnating them with PACE-PEI adhesive. During the experiment, the optimal concentration of PACE and PEI solutions was defined, which was 20 % and 50 % respectively. In its turn, the optimal weight ratio of PACE and PEI was 2 : 1, which provided easy mixing of the gel into the particle mass. Pressing modes of particleboard were as follows: temperature – 150 °C; time – 15 min; pressure – 2 MPa. The made particleboards were tested for use in dry conditions by determining the bending tensile strength and the modulus of elasticity of the samples according to the standard EN 310. The values of the bending strength and the modulus of elasticity of particleboard are given in Table 11.

Table 11

Particleboard test results

Amount of adhesive per dry chippings, %	Bending strength, MPa	Module of elasticity, MPa	Bending strength, MPa, EN 312-P2	Module of elasticity, MPa EN 312-P2
PACE from grey alder bark (40 % PACE aqueous solution)				
20	13.2	1305	11	1800
PACE (20 % PACE aqueous solution) modified by polyethyleneimine (PEI) – 2 : 1				
20	16.9	1904	11	1800

The resulting strength figures show that the particleboard impregnated with PACE-PEI binder complies with the requirements of the standard EN312-P2.

The particleboard impregnated with PACE 40 % aqueous solution as adhesive demonstrated promising results. Significant dispersion of the results occurred due to an uneven distribution of the binder in the particle mass. In general, the bending strength and the modulus of elasticity showed the potential use of binder in the production of particleboard for dry operating conditions. The thickness of produced particleboard was 10 mm, density was 1010 kg/m^3 to 1100 kg/m^3 (Fig. 17).

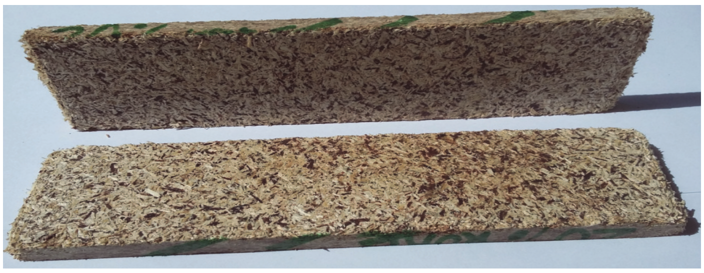


Fig. 17. The experimentally obtained plywood sample.

For producing plywood (Fig. 18), the average consumption of PACE-PEI adhesive was 170 g/cm^3 to 230 g/cm^3. The results of plywood strength test (Table 12) showed that the adhesive containing PACE-PEI up to 60 % was hopeful by reaching the value of ≥1 MPa, which corresponds to the requirements under the Articles 5.1.3 and 5.1.1 of the standard EN 314.

Table 12

PACE-PEI-FF-based plywood test results

PACE-PEI content in an adhesive	Shear strength, Mpa Pretreament required by Class 1		Shear strength, Mpa Pretreament required by Class 2	
	PACE with 25 % PAC content in PACE-PEI	PACE with 46 % PAC content in PACE-PEI	PACE with 25 % PAC content in PACE-PEI	PACE with 46 % content of PAC in PACE-PEI
80 %	0.9	1.0	broke apart	broke apart
60 %	1.2	1.5	0.9	1.3
40 %*	1.3	1.8	1.2	1.3
20 %	1.3	1.8	1.2	1.6
FF	2.3	2.3	1.7	1.7

* Formaldehyde emission of plywood is 0.027 mg/(m^2·h) (EN 717-2:1994), which is close to Class E0 according to EN 13986. Formaldehyde emission of the plywood of *Latvijas Finieris* meets the requirements of Class E1 according to EN 13986 [emission of <3.5 mg/(m^2·h)] [2].

When using the extracts with a higher content of proanthocyanidins, i. e., 46 % as per absolutely dry extract (PACE from grey alder bark harvested in 2013) for producing adhesives as opposed to 25 % (PACE from grey alder bark harvested in 2011), the values of plywood strength are relatively higher, which explains the high values of the strength of the samples obtained in the studies by Li K. and other co-authors when using adhesives based on purified proanthocyanidins and testing them for plywood production.

Formaldehyde emission of the obtained plywood was 0.027 mg/(m^2·h), which corresponds to Class E0 (EN717-2: 1994).

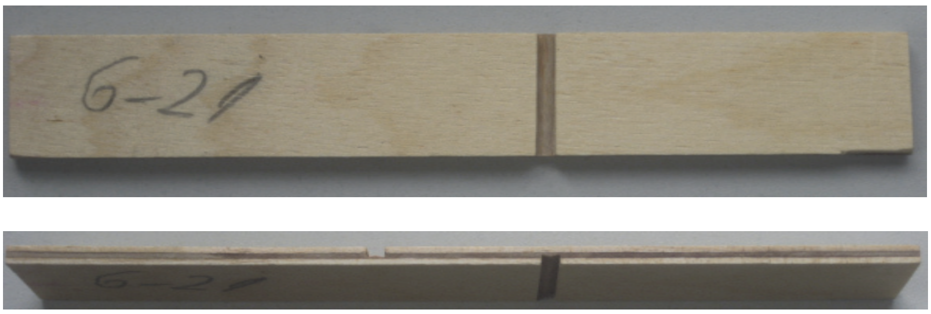


Fig. 18. Experimentally produced sample of plywood.

The values of plywood shear strength and the modulus of elasticity when changing the content of PACE-PEI in the adhesive were close and varied from 996 MPa to 1058 MPa. Due to the dispersion of the results, which is typical of natural adhesives, it is difficult to judge about the changes in plywood elasticity. Density of the made plywood ranged from 666 kg/m^3 to 736 kg/m^3.

The results show that PACE-PEI-based adhesives are potentially usable in the production of particleboard and plywood by partly replacing the commercial FF resin with the PACE-PEI binder.

3.12. Use of residue deciduous bark

Use of bark residue as a solid biofuel

Following the concept of biorefining, the perspective usage of grey alder bark residue as a solid biofuel is presented. The findings of elemental analysis demonstrate that deciduous bark residue after sequential extraction becomes richer in carbon. Based on the literature, it is known that the calorific value of the biomass containing more carbon is higher. The highest (LCH) and the lowest (HCH) combustion heat of sampled bark were calculated by means of regression equations [26], [27], including the obtained data of elemental analysis therein. Comparing the combustion heat of bark and bark residue after extraction, the combustion heat of bark residue is higher (Table 13).

Table 13

Parameters of grey alder bark and bark residue

Parameters	Grey alder bark	Grey alder bark residue
C content, % as per dry sample	50.0	51.6
H content, % as per dry sample	6.63	6.67
N content, % as per dry sample	1.19	1.38
HCH, MJ as per 1 kg of dry sample	20.2	21.0
LCH, MJ as per 1 kg of dry sample	18.8	19.5
Ash content, % as per dry sample	3.4	3.5

Use of bark residue for composite materials

In cooperation with the LSIWC Pulp laboratory, the grey alder bark crushed to nanoparticle size and its residue after extraction were examined as a filler for improving the mechanical properties of pulp composite materials. The casts of coniferous pulp with nanoparticle concentration of 5 % to 20 % as a filler were made. The studies showed that in the case of grey alder bark residue if the filler content was 20 %, tensile index in dry and wet condition increased by 44 %, while punch penetration index increased by 90 %. In the case of grey alder bark, tensile index of this cast in dry condition increased by 28 % and did not change in wet condition, but punch penetration index rose by 78 %.

3.13. Bark biorefinery cluster

Development of bark biorefining cluster allows converting deciduous bark into a raw material with added value. The developed sophisticated scheme for rational use of the bark includes the use of PAC-containing extracts and PAC alternatives for improving and preserving the quality of various products and goods, and the use of bark residue for additional energy production and modification of various material properties. The scheme enables combining its clusters depending on regional conditions, i. e., the need for a certain product, the existing raw material basis, logistics of raw material and product delivery, and so on. A simplified biorefining scheme of deciduous bark is shown in Fig. 19.

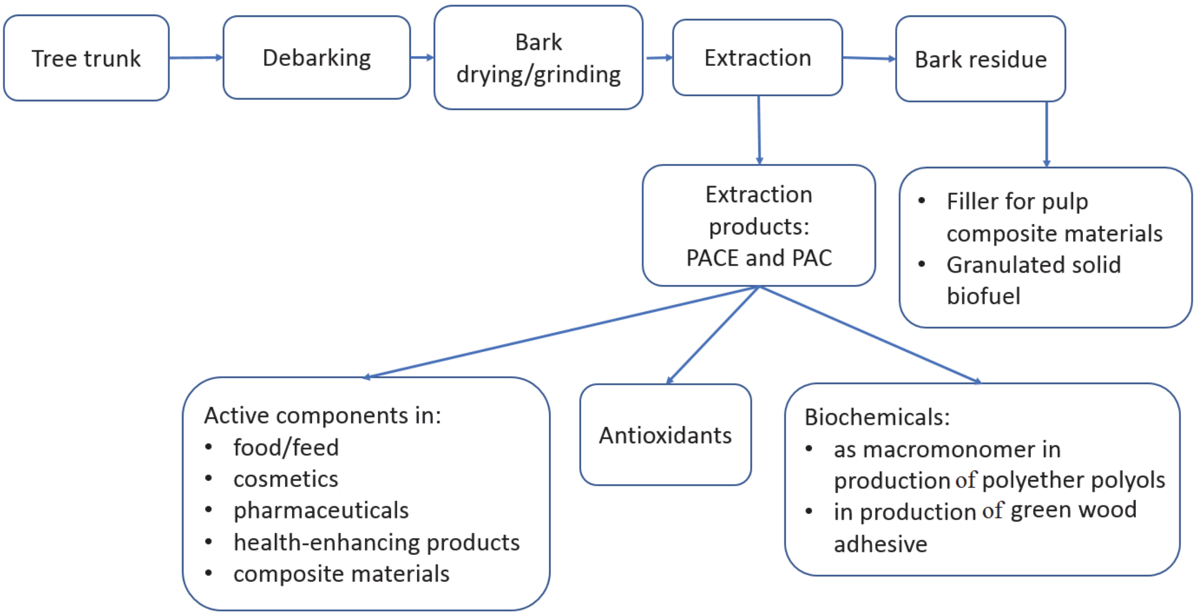


Fig. 19. Simplified biorefining scheme of deciduous bark.

CONCLUSIONS

1. It was found that among grey alder, black alder, ash tree, and goat willow bark, grey alder and black alder bark ensure the highest yield of proanthocyanidins-containing extracts (PACEs) and proanthocyanidins (PAC).
2. Goat willow bark is a potential source for obtaining catechin and catechin dimer.
3. The optimal modes for extracting PACE and PAC in their composition in a single-stage extraction from grey alder bark by using 40 % ethanol as an extractant are defined. To reduce power consumption, convective heat can be replaced with high-frequency heating.
4. When conducting sequential extraction with hexane, ethyl acetate, and 40 % ethanol, triterpenoids and low molecular weight polyphenols can be additionally obtained, as well as PAC yield can be slightly increased; however, power consumption significantly increases, which is not economically feasible.
5. The chemical composition and structure of PACEs and PAC from the bark of deciduous trees growing in Latvia were studied. In PACEs, prevailing components are carbohydrates, polyphenols, and their glycosides, whereas PAC consists of procyanidin units containing catechin/epicatechin monomer units in oligomers with a degree of polymerization of 2-5 related to bond C4-C8.
6. High antioxidant activity of PACEs and PAC was proved allowing the use of the extracts as an antioxidants or additives in healthcare and prevention and for stabilising the PU films, lipid-containing foods, and cosmetics.
7. The opportunities for using PACE in the production of adhesives for manufacturing particleboard and plywood were demonstrated by partially or totally substituting commercial FF resin and partially replacing oil-based phenols in the synthesis of commercial FF resin.
8. Deciduous bark residue after extraction can be used as a raw material for obtaining the filler of nanoparticle size by increasing the mechanical strength of coniferous pulp by 80 % or as a solid biofuel thanks to its increased calorific value.

LIST OF PUBLICATIONS

1. Telysheva G., Dizhbite T., Bikovens O., Ponomarenko J., **Janceva S.**, Krasilnikova J. Structure and antioxidant activity of diarylheptanoids extracted from bark of grey alder (*Alnus incana*) and potential of biorefinery-based bark processing of European trees. – Holzforschung, 2011, 65(4), pp. 623–629, http://dx.doi.org/10.1515/HF. 2011.096. WoS, Scopus.
2. **Janceva S.**, Dizhbite T., Telisheva G., Spulle U., Klavinsh L., Dzenis M. Tannins of deciduous trees bark as a potential source for obtaining ecologically safe wood adhesives. – In: Environment, Technology, Resources: Proceedings of the 8th International Scientific and Practical Conference, June 20–22, 2011, Rezekne (Latvia), Vol. I, RA Publishing House: Rēzekne, 2011, pp. 265–270. WoS, Scopus.
3. **Janceva S.**, Dizhbite T., Telysheva G., Arshanitsa A., Spulle U., Klavinsh L., Dzenis M. Isolation and testing of condensed tannins from alder bark for obtaining environment friendly adhesives and biofuel granules. – In: The third Nordic Wood Biorefinery Conference NWBC 2011, 22–24 March 2011, Stockholm, Sweden, pp. 351–352.
4. Arshanitsa A., Krumina L., **Janceva S.**, Telysheva G. Study of the reactivity of Kraft and organosolv lignins with 4,4'-diphenylmethane diisocyanate and characterization of the obtained products. – In: The third Nordic Wood Biorefinery Conference NWBC 2011, 22–24 March 2011, Stockholm, Sweden, pp. 348–350.
5. Teliševa G., Dižbite T., Krasiļņikova J., Ponomarenko J., **Janceva S.,** Bikovens O., Roze L., Lauberts M., Andersone A. Valorization of the bark of tree species spread in Latvia as a source for obtaining high value-added phytochemicals and bio-based material. Sustainable Use of Local Resources (Entrails of the Earth, Forest, Food and Transport) – New Products and Technologies (NatRes). National Research Programme, 2010–2013. Proceedings, 2013, Riga, KĶI, pp. 136–140.
6. **Janceva S.**, Papadopoulou E., Kulinsh L., Lauberts M., Dizhbite T., Telysheva G. Evaluation of grey alder bark tannin as phenol substitute in the synthesis of phenol-formaldehyde resins suitable for plywood. The 13th European Workshop on Lignocellulosics and Pulp, EWLP-2014, Seville, Spain, 24–27 June 2014, pp. 443–446.
7. **Janceva S.**, Dizhbite T., Vevere L., Telysheva G., Dzenis M. Characterization of bark extract from deciduous trees with emphasis on their antioxidant activity. IOP Conf. Series: Materials Science and Engineering, 111, Baltic Polymer Symposium 2015, Sigulda, 16–18 September 2015, pp. 1–4, doi: 10.1088/1757-899X/111/1/012013, WoS, Scopus.
8. **Janceva S.**, Dizhbite T., Telysheva G., Vevere L., Krasilnikova J., Dzenis M. Assessment of alder tree bark potential as a renewable source of proanthocyanidins in Latvia. – Materials Science and Applied, 2016, No. 33, pp. 26–30, doi: 10.1515/msac-2016-0005.

9. **Janceva S**., Lauberte L., Dizhbite T., Telysheva G., Krasilnikova J., Dzenis M. Proanthocyanidins rich extracts from bark of deciduous trees growing in Latvia as antioxidant additive for lipid based systems. – The 14th European Workshop on Lignocellulosics and Pulp, Autrans, France, 28–30 June 2016, pp. 413–416.
10. Laka M., Vikele L., Rozenberga L., **Janceva S**. Nanoparticle Fillers obtained from wood processing wastes for reinforcing of paper. AIP Conf. Proceedings, 2016, No. 1736, http://dx.doi.org/10.1063/1.4949687. WoS, Scopus.
11. **Janceva S**., Andzs M., Tupciauskas R., Telysheva G., Dizhbite T., Dzenis M. Condensed tannins rich grey alder bark extract potentials as a raw material for wood adhesive production. Proceedings of the 12th meeting of the Northern European Network for Wood Science and Engineering (WSE). Wood science and engineering. The 12th Annual Meeting of the Northern European Network for Wood Science and Engineering WSE, Riga, Latvia, 12–13 September 2016, pp. 58–62.
12. **Janceva S.**, Lauberte L., Dizhbite T., Krasilnikova J., Telysheva G., Dzenis M. Protective effects of proanthocyanidins extracts from bark of deciduous trees in lipid systems. Holzforschung, 2016, doi: 10.1515/hf-2016-0185. WoS, Scopus.
13. **Janceva S.**, Lauberts M., Lauberte L., Arsanica A., Dizbite T., Telysheva G. Bark of European deciduous trees as a potential source for production of proanthocyanidins-rich extract. ISCHP 2017 – International Scientific Conference on Hardwood Processing (6th Edition), Lahti, Finland, 25–27 September 2017 (submitted for publication).

Patent

Krasilnikova J., Zalomonson M., Telysheva G., Dizhbite T., **Janceva S**. Patent of the Republic of Latvia 15130 A. Remedy for decreasing pyruvate level in blood. Patent application date: 20.06.2016, http://www.lrpv.gov.lv/sites/default/files/ 20160620.pdf

BIBLIOGRAPHY

1. Kabir M. S. H. *et al.* Evaluation of total flavonols, total proanthocyanidins content and thrombolytic activity of methanol extracts of three Bangladeshi plants // International Journal of Pharmacy. – 2015, – No. 5(3), – pp. 747–751.
2. Борисова Г. Г., Ермошин А. А., Малева М. Г., Чукина Н. В. Основы биохимии вторичного обмена растений. Учебно методическое пособие. – Екатеринбург, Издательство Уральского университета, 2014, ISBN 978-5-7996-1296-2, с. 40–41.
3. Garcia D. E., Glasser W. G., Pizzi A., Paczkowskief S. P., Laborieef M.-P. Modification of condensed tannins: from polyphenol chemistry to materials engineering // New J. Chem. – 2016, – No. 40, – pp. 36–49.
4. Oligomeric Proanthocyanidins (OPCs) // Alternative Medicine Review. – 2003, – No. 8(4), – 442–450 pp.
5. Porter L. J. Structure and chemical properties of the condensed tannins // Chapter, Plant Polyphenols, Vol. 59 of the series “Basic Life Sciences”, – pp. 245–258, doi: 10.1007/978-1-4615-3476-1.
6. Fine A. M. Oligomeric proanthocyanidin complexes: history, structure, and phytopharmaceutical applications // Alternative Medicine Review. – 2000, – No. 5(2), – pp. 144–151.
7. Yamakoshi J., Saito M., Kataoka S., Kikuchi M. Safety evaluation of proanthocyanidin-rich extract from grape seeds // Food and Chemical Toxicology. – 2002, – No. 40, – pp. 599–607.
8. Oliff H. S., Barrett M. Scientific and clinical monograph for Pycnogenol®. American Botanical Council. // Internet – http://abc.herbalgram.org/site/DocServer/Pycnog_FullMono120809_LOW.pdf?docID=1741
9. Pycnogenol // Internet – http://www.pycnogenol.com/home/
10. Stuard S., Belcaro G., Cesarone M. R. *et al*. Kidney function in metabolic syndrome may be improved with Pycnogenol // Panminerva Med. – 2010, – No. 52(2 Suppl. 1), – pp. 27–32.
11. Belicaro G., Luzzi R., Dugall M., Ippolito E., Saggino A. Pycnogenol® improves cognitive function, attention, mental performance and specific professional skills in healthy professionals aged 35–55 // J. Neurosurg Sci. – 2014, – No. 58, – pp. 239–248.
12. Khanna S., Venojarvi M., Sashwati R., Sharma N., Trikha P. *et al*. Dermal wound healing properties of redox-active grape seed proanthocyanidins // Free Radical Biology & Medicine. – 2002, – No. 33(8), – pp. 1089–1096.
13. Mehansho H., Butler L. G., Carlson D. M., Dietary tannins and salivary proline-rich proteins: interactions, induction and defense mechanisms // Annual Review of Nutrition. – 1987, – No. 7, – pp. 423–440.
14. Vchovsek U., Vanzo A., Nemanic J. Effect of red wine maceration techniques on oligomeric and polymeric proanthocyanidins in wine, cv. Blaufränkisch // Vitis. – 2002, – No. 41(1), – pp. 47–51.

15. Genebra T., Santos R. R., Francisco R., Pinto-Marijuan M., Brossa R. *et al*. Proanthocyanidin accumulation and biosynthesis are modulated by the irrigation regime in Tempranillo seeds // Int. J. Mol. Sci. -2014, -No. 15(7), – pp. 11862–11877, doi: 10.3390/ijms150711862
16. Патент – 2072895. Способ получения сорбента ионов металлов (варианты) и способ выделения металлического элемента из его раствора. 1997 г.
17. Ogata T., Nakano Y. Mechanisms of gold recovery from aqueous solutions using a novel tannin gel adsorbent synthesized from natural condensed tannin // Water Research, – 2005, – No. 39(18), – pp. 4281–4286.
18. Docker / Internet – https://litoshop.ru/Neytralnyy-preobrazovatel-rzhavchiny-DOCKER-NITTRON-1l_1649t.html
19. Wan Nik W. B., Hajar H. M., Noor Idora M. S., Suriani M. J., Yabuki A. Effect of mangrove bark condensed tannins (*Rhizophora apiculata*) as corrosion inhibitor for mild steel in simulated splash zone // Journal of Scientific Research and Development. – 2015, – No. 2(13), – pp. 59–63.
20. Benmeziane F., Djamai R., Cadot Y., Seridi R. Optimization of extraction parameters of phenolic compounds from Algerian fresh table grapes (*Vitis Vinifera*) // International Food Research Journal, – 2014, – No. 21(3), – pp. 1061–1065.
21. Wengui D., Ohara S., Hashida K., Makino R. Condensed tannins from steamed Acacia mearnsii bark // Holzforschung, – 2005, – No. 59(3), – 289– 294 pp.
22. Chew K. K., Ng S. Y., Thoo Y. Y., Khoo M. Z., Wan Aida W. M., Ho C. W. Effect of ethanol concentration, extraction time and extraction temperature on the recovery of phenolic compounds and antioxidant capacity of *Centella asiatica* extracts // International food research journal, – 2011, No. 18, – pp. 571–578.
23. Janceva S. Baltalkšņa kondensēto tanīnu (proantocianidīnu) raksturošana un testēšana videi draudzīgo adhezīvu iegūšanai // RTU, – 2010, – 33.–35. lpp.
24. Nadji H., Bruzzese C. Oxypropylation of lignins and preparation of rigid polyuretane foams from the ensuing polyols // Macromolecular Material and Engineering. – 2005, – 290, – pp. 1009–1016.
25. Rodrigo Briones, Luis Serrano. Polyol production by chemical modification of date seeds // Industrial Crops and Products, – 2011, – 34, – pp. 1035–1040.
26. Bridgeman T., Jones J., Shield I., Williams P. Torrefaction of reed canary grass, wheat straw and willow to enhance solid fuel qualities and combustion properties // Fuel. – 2008, – No. 87, – pp. 844–856.
27. Friedl A. *et al*. Prediction of heating values of biomass fuel from elemental composition // Anal. Chem, – 2005, – 554, – pp. 191–198.
28. Papadopoulou E. The challenge of bio-adhesives for the wood composite industries / Internet – http://www.chimarhellas.com/wp-content/uploads/2009/03/paper_9th-pacific-rim_final-2_.pdf
29. Lauberts M., Telysheva G., Venskutonis P. R., Lauberte L., Dizhbite T., Kazernavičiute R., Pukalskas A. Diarylheptanoid rich extract of grey and black alder barks – an effective dietary antioxidant in mayonnaise // Chemical Papers, – 2016, doi: 10.1007/s11696-016-0017-3.