USE OF VARIOUS "GREEN" SOLVENTS FOR THE ISOLATION OF EXTRACTS CONTAINING BIOLOGICALLY ACTIVE CATECHOL MOIETIES FROM THE BARK OF ECONOMICALLY UTILIZED TREES

Maris Lauberts, M. Pals, J. Ponomarenko, K. Radovska.

Latvian State Institute of Wood Chemistry, Dzerbenes street 27, Riga, Latvia
Mob: +371 29172775; e-mail: maris.lauberts@kki.lv

Introduction

The amount of wood utilization from various trees species is large and leads to a high amount of residual materials, such as tree bark. Depending on the growing area, species, and other environmental factors, bark tissue accounts for about 10–20% of wood biomass. Thus, tree bark, as a residual material, especially from the production of pulp or wood-based materials, is available in large quantities and is mainly used for energy production. When considering the composition of bark biomass, it can be seen that bark biomass, has a high content of extractives and can be used for high-value products. Previous studies have shown that diarylheptanoids are major extractives found in the bark of alder and birch trees from the Betulaceae family, and proanthocyanidins are major extractives from the bark of pine trees, both bearing chemically and biologically active catechol moieties. In this project, the extraction of diarylheptanoids and proanthocyanidins from these economic trees bark be undertaken to transform underutilized woodworking and processing residues into high value-added products for application in the pharmaceutical and cosmetic industries. The original solution of this project includes the use of glycerol and water for the extraction of tree bark and the optimization of the composition of glycerol extracts for their high efficiency in high value products.

Experimental

Debarking
Collecting
Drying

Grinding

Temperature:
40/85/150 °C
Pressure: 100 atm.
Time/Number of cycle:
(4x5min.)x1/2/3

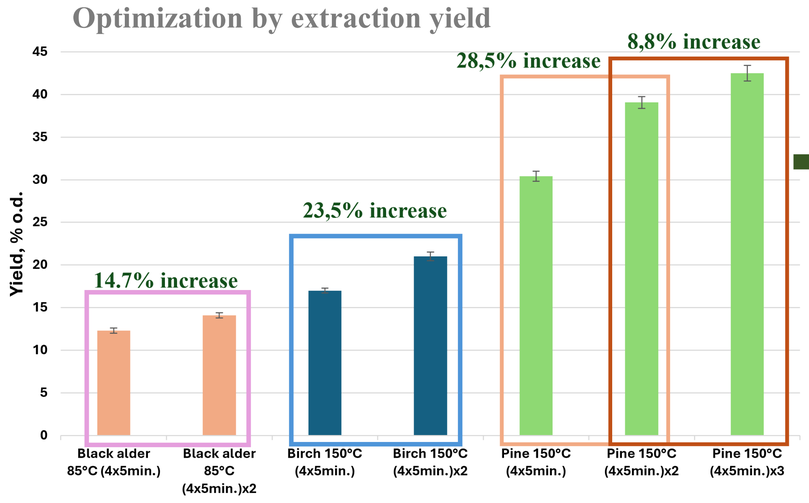
Optimization

Green solvents

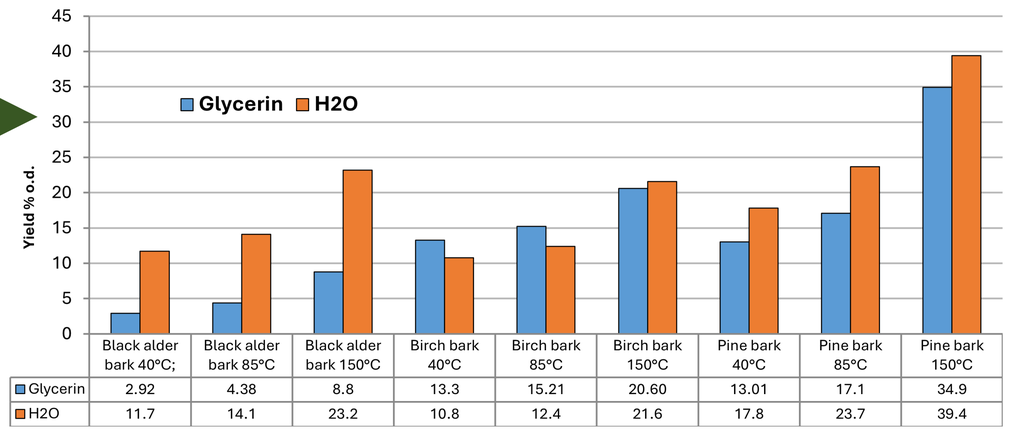
Characterisation

Results

Optimization by extraction yield

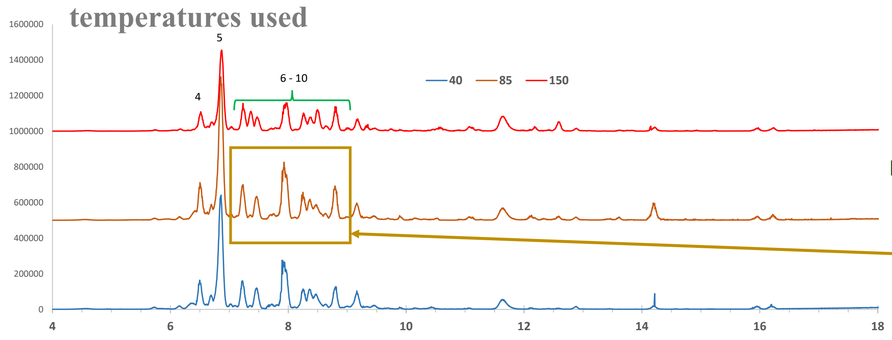


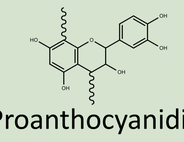
Comparison of optimized extraction parameters with respect to extractive yield.



	Black alder bark 40°C;	Black alder bark 85°C	Black alder bark 150°C	Birch bark 40°C	Birch bark 85°C	Birch bark 150°C	Pine bark 40°C	Pine bark 85°C	Pine bark 150°C
Glycerin	2.92	4.38	8.8	13.3	15.21	20.60	13.01	17.1	34.9
H2O	11.7	14.1	23.2	10.8	12.4	21.6	17.8	23.7	39.4

UHPLC chromatograms of pine bark aqueous extracts at the temperatures used



Nr	RT	[M-H]-	Identification
4	6.618	577.137	B2 Proanthocyanidin
5	6.969	289.057	Epicatechin
6	7.332	315.11	
7	7.568	495.183	
8	8.065	315.11	
9	8.595	315.11	Proanthocyanidin oligomers
10	11.729	723.514	

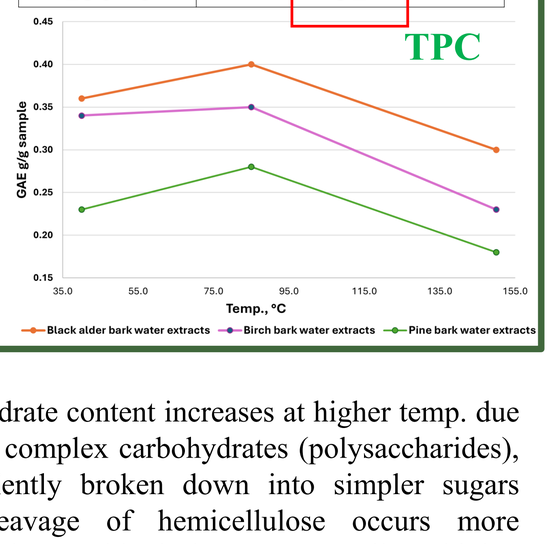
The UHPLC peak intensities [6-10] also show the highest PAC content in extracts obtained at 85 °C and are consistent with the PAC results determined spectrophotometrically.

The antioxidant activity of the obtained extracts was assessed using the test with free radical DPPH•, where free radical scavenging activity was expressed as the IC_{50} (the concentration required for 50 % inhibition of the free radical). The lower is IC_{50} value, the higher is the antioxidant activity. The DPPH of the obtained bark extracts was determined, and the results show a positive correlation between the results of total phenolic compounds (TPC) and proanthocyanidins (PAC).

Pine bark DPPH; IC_{50}, mg/L		
	H_2O extr.	Glycer. Extr.
40˚C	17	33
85˚C	14	25
150˚C	26	52

PAC content in pine bark H_2O extract; %	
40 ˚C	31
85 ˚C	50
150 ˚C	32

TPC



The obtained extracts as a raw material for the production of further products: The lower the indicators, the cleaner the product from a microbiological point of view. The microbiological purity of the raw materials, hygiene during the processing process, as well as the surrounding environment and conditions (temperature, humidity, container) during storage are important. Escherichia coli: Not detected – 0 CFU / 1g. This result indicates that there are no signs of fecal contamination in the material, which is a positive indicator in terms of hygiene and safety. * Yeasts and molds: 4.5 × 10² CFU / 1g The presence of yeasts and molds is low to moderate, indicating a slight microbiological contamination. These microorganisms may be of environmental origin and depend on the storage conditions. Mesophilic aerobic and facultative anaerobic microorganisms (MAFAM): 6.3 × 10² CFU / 1g This is a general indicator of the number of microorganisms, indicating the microbiological purity of the material. The amount detected is relatively low and indicates the appropriate hygienic condition of the material.

Microbiological purity of black alder bark water extract

Parameter	Results, CFU / 1g
Escherichia coli	0
Yeasts and molds	4.5 x 10^2
Mesophilic aerobic and facultative anaerobic microorganisms(MAFAM)	6.3 x 10^2

Total and individual carbohydrate content in the obtained pine bark extracts

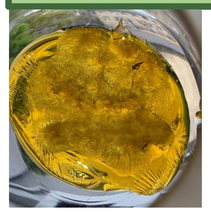
	Rha	Fuc	Ara	Xyl	Man	Gal	Glc	Total
Pine bark H_2O extr. 40˚C	0.95	0.01	1.21	0.08	0.74	1.45	15.37	19.81
Pine bark H_2O extr. 85˚C	0.93	0.01	1.49	0.58	0.62	1.44	14.87	19.94
Pine bark H_2O extr. 150˚C	1.59	0.01	9.64	1.09	1.50	2.76	16.70	33.29
Pine Glyc ekstr. 40˚C	0.1	0.0	0.0	0.2	0.1	0.5	4.4	5.3
Pine Glyc ekstr. 85˚C	0.3	0.0	0.0	0.1	0.1	0.4	3.3	4.2
Pine Glyc ekstr. 150˚C	0.2	0.0	3.2	0.3	0.3	0.7	2.7	7.5

In the extraction of bark with water, the carbohydrate content increases at higher temp. due to the increased efficiency of hydrolysis, where complex carbohydrates (polysaccharides), celluloses and hemicelluloses are more efficiently broken down into simpler sugars (monosaccharides and disaccharides).The cleavage of hemicellulose occurs more efficiently. Hemicellulose is less stable than cellulose and hydrolyzes more easily at higher temp. Therefore, hemicellulose is initially broken down, releasing pentoses (e.g. xylose) and hexoses (e.g. glucose, mannose).The high temp. acts on the lignocellulose complex in the bark, which helps to break down this rigid matrix. This makes cellulose and hemicellulose more accessible for hydrolysis and releases the sugars contained in them.

BUT USING GLYCERIN AS A SOLVENT IS A WHOLE DIFFERENT SCENE

CONCLUSIONS

This study shows how, using various green solvents such as water and glycerin, it is possible to convert various wood barks, which are waste products in very large quantities around the world, into valuable products. Positive correlations between different test/analysis methods are clearly visible, confirming the results of previous tests/analysis, e.g., increased TPC and PAC content in the samples shows the best antioxidant activity, which is additionally confirmed by UHPLC results of higher relative content of individual compounds in the respective samples. The DPPH• radical scavenging activity of phenolic extractives was measured, and low values of IC_{50} will be considered as indicators for their further testing as biologically active substance. Based on the good antioxidant activity of the extract, the best extract was subjected to microbiological evaluation to ensure its suitability for further processing, and the results obtained showed that this extract meets the standards for use as a raw material, for example, in healthcare and as an active ingredient in cosmetic products.



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