# CHANGES IN THE COMPOSITION OF FORESTRY WASTE – WATER EXTRACTS FROM DIFFERENT TREE BARKS DEPENDING ON EXTRACTION CONDITIONS

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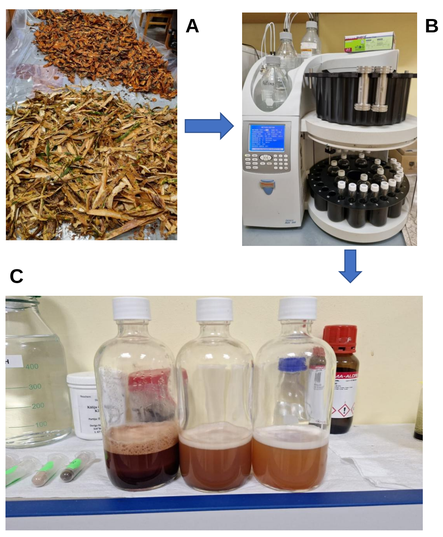
## Introduction

Forestry residues, such as bark, represent an abundant and potentially sustainable source of biomass that could serve as a raw material for producing value-added products. Research was conducted on the composition of biologically active extracts rich in polyphenols varying conditions of accelerated solvent extraction to enhance the value of plant biomass processing by-products specifically tree bark.
The effectiveness of water as "green" solvent for extraction from black alder (*Alnus glutinosa*), pine (*Pinus sylvestris L.)* and birch (*Betula pendula*) bark has been assessed to determine the antioxidant potential of the resulting extracts. This work outlines the impact of the extraction parameters on the targeted polyphenol compounds, their composition, and antioxidant activity.

## Methods




The bark of Black alder, Pine and Birch was **air-dried**. Then the samples **were ground** and **extraction** was carried out **with Dionex ASE-350** Accelerated Solvent Extractor for the isolation of biologically active compounds.
The ASE extraction was performed at a pressure of 105 bar under a nitrogen atmosphere, with **key parameters** such as temperature – **40 ºC**, **85 ºC** and **150 ºC**, and **number of static cycles** – 4 static cycles of 5 minutes, repeated 1, 2 and 3 times.

**Fig. 1.** The scheme of the bark extract preparation process; (A) Pine and Black alder bark drying process; (B) Dionex ASE-350 Accelerated Solvent Extractor; (C) Pine bark extract depending on the number of static cycles: extract after the first 4×5-minute cycle; after the second 4×5-minute cycle and after the third 4×5-minute cycle

**Total phenolic compounds (TPC)** were determined using the **Folin-Ciocalteu spectrophotometric method,** optimized for analysis in a 96-well microplate. A 25% ethanol was used as the solvent. For the **identification of phenolic compounds**, the **UHPLC method** was used.
**Antioxidant** content was assessed using **DPPH method** with DMSO as solvent.
**Carbohydrate content** was measured by the alditol acetate gas chromatographic method.

**Fig. 2.** Bark extracts TPC analysis using optimized Folin-Ciocalteu method on 96-well microplate

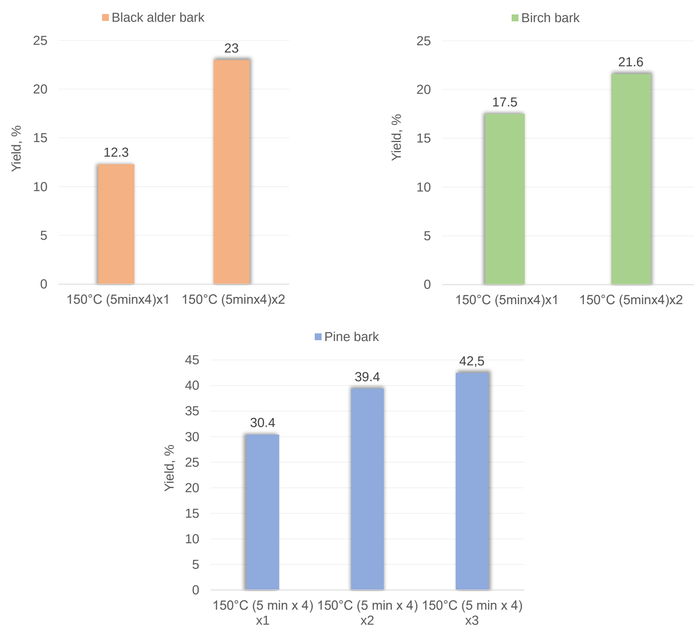



**Fig. 3.** Correlation between the number of extraction cycles and the yield of the extracted substance

The extraction of Black alder, Birch and Pine bark was optimized based on the number of extraction cycles. **The maximum yield** of extractives was obtained by performing a **(4x5 min) cycle twice**. Performing the cycle a third time increase in yield was insignificant. For Pine bark, the extractive yields were 30.4% after one cycle, 39.4% after two cycles, and 42.5% after three cycles. Therefore, two extraction cycles were chosen for further analysis.

## Results

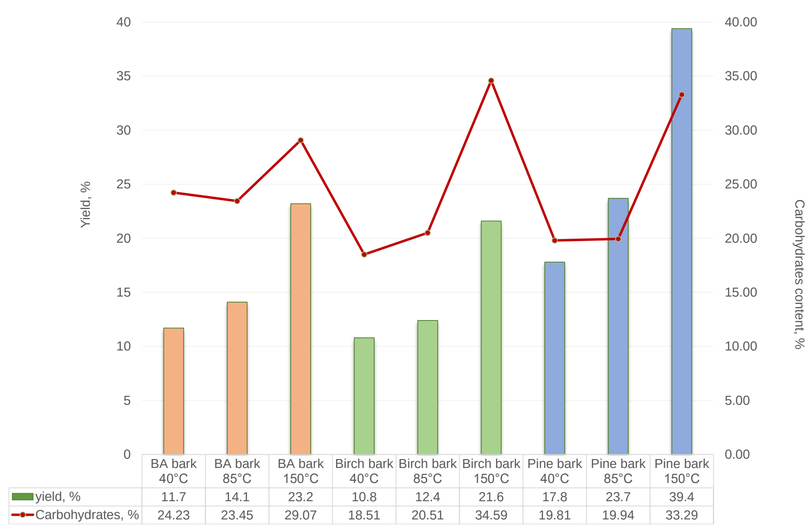



**Fig. 3.** Correlation between yield (%) from Black alder (BA), Birch and Pine bark samples at extraction temperatures of 40 ºC, 85 ºC and 150 ºC and carbohydrate total content in extracts

**Table. 1.**

| Bark | Ashes, % | Moisture, % |
|---|---|---|
| Birch | 2.4 | 10.5 |
| BA | 3.5 | 6.1 |
| Pine | 2.6 | 7.8 |

**The maximum yields of extractives - 23.2 %**, **21.6 %** and **39.4 %** for Black alder (BA), Birch and Pine bark, respectively - were obtained at **150 ºC**. Lowering the extraction temperature resulted in a significant decrease in both extractive yield and carbohydrate content.

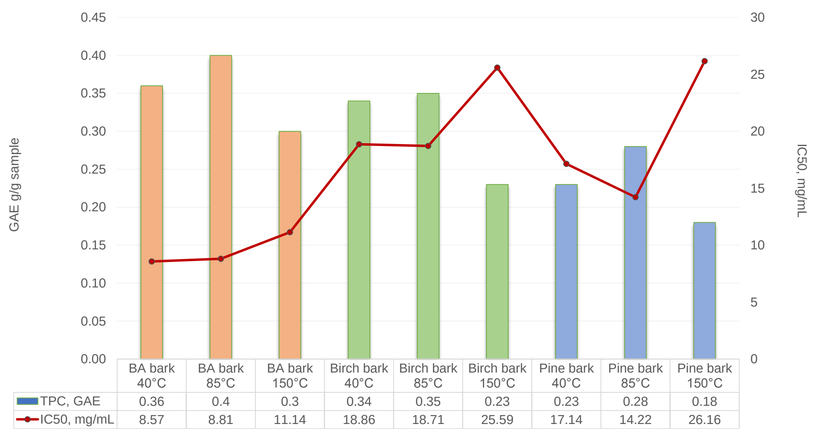



**Fig. 4.** Correlation between TPC (GAE) and antioxidant $IC_{50}$ (mg/mL) in Black alder, Birch and Pine bark extracts at temperatures of 40 ºC, 85 ºC and 150 ºC

TPC concentration showed a direct correlation with antioxidant activity – **an increase in TPC concentration** corresponded to an **increase in antioxidant activity**. The highest TPC concentration and antioxidant activity were observed at **85 ºC**. At higher temperature, an increase in carbohydrate content led to a significant reduction in both TPC concentration and antioxidant activity.

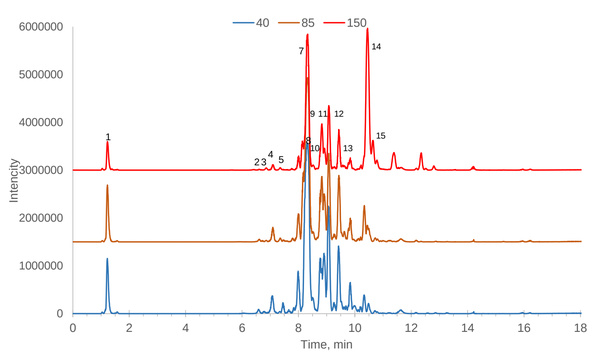



**Fig. 5.** Black alder poliphenolic compound identification chromatogram

**Table. 2.**

| Nr | RT | [M-H]- | Identification |
|---|---|---|---|
| 1 | 1.21 | 341.097 | Myzodendrone |
| 2 | 6.47 | 191.06 | Quinic acid |
| 3 | 6.92 | 761.14 | 3-O-galloyl- (epi)gallocatechin-(epi)gallocatechin |
| 4 | 7.33 | 409.105 | Epi)catechin- (epi)catechin |
| 7 | 8.20 | 477.174 | Oregonin |
| 8 | 8.42 | 477.174 | Oregonin |
| 9 | 8.66 | 479.155 | Hydroxyoregonin |
| 10 | 8.79 | 493.206 | Rubranol C |
| 11 | 8.96 | 327.13 | Hirsutenone |
| 12 | 9.32 | 461.18 | Aceroside VII |
| 13 | 9.74 | 493.21 | Rubranoside A |
| 14 | 10.22 | 327.111 | Rhododendrin |
| 15 | 10.33 | 461.22 | 1- (4-hydroxyphenyl)-7- (3.4-dihydroxyphenyl) heptan-3-one-5-O-pentoside |

**At higher temperatures**, changes in peak intensities were observed — most compounds showed a **decrease in intensity**, except for Rhododendrin (14. peak). The appearance of new peaks may also indicate the **thermal degradation** of certain compounds.

The extraction temperature significantly influenced both the yield and composition of bioactive compounds from Birch, Black alder and Pine bark. While 150 °C yielded the highest amount of extractives - 23.2%, 21.6%, and 39.4% respectively—the optimal temperature for extracting phenolic compounds and maximizing antioxidant activity was 85 °C. At this temperature, lower carbohydrate content resulted in the highest total phenolic compound concentrations (0.40, 0.35, and 0.28 GAE g/g sample), leading to higher antioxidant properties of samples.

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