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Pilot-scale extraction of Norway spruce bark polyphenols with purification for tannin-enriched fractions

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ABSTRACT

Softwood bark represents an abundant and promising source of high-value bioactive polyphenols. However, it is currently used mainly for energy production, and its efficient extraction remains a major bottleneck for the industrial valorization of tannins and other polyphenols. The aim of this study was to demonstrate the pilot-scale extraction and purification of antibacterial polyphenols from industrially sourced Norway spruce (*Picea abies*) bark using pressurized hot water extraction and XAD7HP polymeric resin. Nine batches of winter- and summer-harvested bark were processed in a 300 L reactor at 58–122 °C for 40–80 min, with characterization of moisture, particle size, and bark-to-wood ratio to account for feedstock variability. The maximum overall extraction yield (140.4 mg/g) on a dry bark basis (DB) was obtained at 122 °C. Seasonal differences were evident, as winter bark consistently provided higher yields than summer bark. Resin purification significantly enriched the polyphenol content, increasing condensed tannins from 2.1 to 4.6 mg/g to 23.4–59.2 mg/g (DB) while markedly reducing carbohydrate contamination. The tannin-enriched fractions also exhibited strong antibacterial activity against bioluminescent *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) biosensor strains. These results confirm the technical feasibility of producing polyphenol-rich fractions from spruce bark at pilot scale, supporting their use in biobased industrial applications such as cosmetics, feed, and water treatment.

1. Introduction

Boreal forests are dominated by coniferous tree species, mainly Norway spruce (*Picea abies* [L.] Karst.) and Scots pine (*Pinus sylvestris* L.) (European Environment Agency EEA, 2006). In particular, spruce bark shows potential as a raw material for high-value biochemicals, as extractives concentrations up to 22–26% of dry mass have been reported (Miranda et al., 2012; Nurmi, 1992). In Finland alone, the forest industry consumed 13.3 Mm^3 of spruce logs in 2024 (Official Statistics of Finland OSF, 2024). Bark constitutes approximately 10–15% of the roundwood stem volume (Hakkila, 1967). Therefore, the theoretical annual spruce bark potential exceeds one million dry tonnes. However, a portion of the bark is lost during the supply chain. In addition, extractives are susceptible to microbial degradation and physicochemical changes, especially when the material is comminuted (Jyske et al., 2020; Routa et al., 2020). Currently, most bark is used for energy generation despite its potential as a feedstock for sustainable production of antioxidants and antimicrobial compounds (Co et al., 2012; Granato et al., 2022; Jyske et al., 2024; Pap et al., 2021; Tienaho et al., 2025). To realize this potential, scalable extraction techniques are required to isolate the valuable compounds from heterogeneous biomasses while maintaining economic feasibility. After extraction, the residual bark material still has potential for energy generation or can be further refined through pyrolysis, anaerobic digestion, or additional extraction steps (Kumar et al., 2024; Rasi et al., 2019).

Among the valuable extractives present in bark, tannins are of particular interest due to their wide range of applications. Traditionally used in leather tanning and wine industry, tannins have potential in wood adhesives, binding agents, pharmaceuticals and medical applications, fire-resistance and insulating foams, mining processes, and inhibitors of metal corrosion (Jyske et al., 2023; Lu et al., 2024; Pizzi, 2019; Pizzi et al., 2024; Raitanen et al., 2020; Varila et al., 2020).

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Additionally, cationized tannins have been demonstrated to be efficient as coagulants and flocculants in wastewater treatment (Beltrán-Heredia and Sánchez-Martín, 2009; Grenda et al., 2020; Sánchez-Martín et al., 2010). Currently, the primary sources of commercially used condensed tannins in Europe are black wattle (*Acacia mearnsii*) bark for mimosa tannin and quebracho from *Schinopsis balansae* and *S. lorentzii* (Bianchi, 2017; Holmbom, 2011; Pizzi et al., 2024). Both originate from, and are mainly cultivated in, the southern hemisphere. Replacing these exotic species with locally produced residual materials, such as spruce bark, could improve sustainability and reduce the environmental burdens associated with the raw material logistics (Brennan et al., 2020).

From a chemical perspective, condensed tannins (CTs), i.e., proanthocyanidins, are poly- and oligomers formed of flavan−3-ol units (Bianchi et al., 2015). Their properties are strongly influenced by structural features such as hydroxylation pattern and degree of polymerization (DP) (Arbenz and Avérous, 2015; Bianchi et al., 2015). CTs in Norway spruce bark are mostly procyanidins with minor amounts of prodelphinidins (Hammerbacher et al., 2014; Jyske et al., 2020; Matthews et al., 1997). (Epi)catechins are the main building blocks of CTs, with smaller contributions from (epi)gallocatechins. The average DP typically ranges from 3 to 8 (Holmbom, 2011), with differences between outer and inner bark (Jyske et al., 2020).

Despite the relatively high content of CTs in spruce bark (Lacoste et al., 2015; Tienaho et al., 2025), the extraction process remains the main bottleneck in efficient recovery and tannin valorization (De Hoyos-Martínez et al., 2019). Total tannin contents in Norway spruce bark have been reported around 15% of dry mass (Kemppainen et al., 2014, 2012), although values vary depending on analytical methods and reference standards. For example, differences between quebracho tannin and spruce bark tannins complicate the comparisons in the acid butanol assay. When purified sorghum tannin was used as a reference standard in pilot-scale spruce bark extractions, total tannin contents ranged from 11.5% to 25.1% of the dry extract, depending on processing conditions (Tienaho et al., 2025).

Efficient extraction requires both appropriate pretreatment and optimized extraction conditions (Bart, 2011; Pfennig et al., 2011). Pretreatment methods, such as particle size reduction, improve solvent penetration due to shortened diffusion pathways. However, no universal extraction protocol exists for all biomass types, and the process parameters, such as solvent composition, extraction temperature and time, the particle size of the solid material, liquid-to-solid ratio, and pH, must be optimized case by case (Arbenz and Avérous, 2015; De Monte et al., 2014; Pfennig et al., 2011).

In addition to pretreatment, extraction conditions themselves have a critical role in determining the process performance. Increasing extraction temperature and solvent-to-solid ratio generally enhances the extraction rates and yields (Seidel, 2012; Zhang et al., 2018). However, excessive temperatures may cause thermal degradation or denaturation of tannins (De Hoyos-Martínez et al., 2019). Higher temperatures tend to increase solvent losses and co-extraction of impurities (Seidel, 2012; Zhang et al., 2018; Tienaho et al., 2024). Similarly, high solvent-to-solid ratio leads to excessive solvent consumption and increased energy and time input required for concentration. Extraction efficiency improves with longer extraction times only until sufficient solvent-solid contact has enabled effective mass transfer (Pfennig et al., 2011). However, after reaching equilibrium, no further increase in extraction efficiency is gained (De Hoyos-Martínez et al., 2019; Zhang et al., 2018). Thus, achieving efficient extraction requires balancing these competing factors to maximize yield while preserving compound integrity.

Beyond extraction, the composition and purity of the obtained extracts are crucial when considering their suitability for various applications (Bianchi et al., 2015; Pfennig et al., 2011). Tannins are often bound to other biomolecules such as carbohydrates or proteins, and the hot water extracts from spruce bark typically contain co-extractives (Bianchi et al., 2015). Impurities hinder the successful development of tannin-based aromatic building blocks (Arbenz and Avérous, 2015). Common purification and fractionation processes include filtration, evaporation, and adsorption-based separations. For example, in comparison to evaporation, ultrafiltration was shown to halve the environmental burden (Ding et al., 2017). The use of purification and fractionation methods is product dependent. In cases where crude extract can be used, only concentration and drying are needed. However, if highly purified fractions are required, additional processing steps are needed. Purified CT fractions can be obtained using adsorbent materials or resin-based techniques (Buran et al., 2014; Zeller, 2019).

In this context, there remains a clear need for scalable and efficient processes for tannin recovery from industrial side streams. In the present study, we demonstrate a pilot-scale method for the efficient extraction and enrichment of condensed tannins from industrially sourced Norway spruce bark. Thus addressing the limitations of previous laboratory-scale studies with small and homogeneous feedstock batches. Pressurized hot water extraction was selected as a green and scalable technology that supports the cascading use of residual bark under industrially relevant conditions. The study was guided by the following research questions: (i) how efficiently can condensed tannins be recovered from industrial Norway spruce bark using pilot-scale pressurized hot water extraction; (ii) how do variations in feedstock, such as seasonal differences between winter- and summer-harvested bark, influence extraction yield and composition; and (iii) to what extent can downstream purification enhance tannin enrichment and functional properties. To address these questions, winter- and summer-harvested feedstock bark batches were analyzed in detail, and comprehensive chemical profiling was performed for the feedstock bark, crude extracts, purified fractions, and extraction residues. In this way, the study provides one of the most extensive pilot-scale evaluations of spruce bark polyphenol recovery to date. Furthermore, the tannin-enriched fractions were evaluated for antibacterial activity. Two widely applied model organisms were used, Gram-negative *Escherichia coli* and Gram-positive *Staphylococcus aureus*, thereby linking process performance with functional outcomes.

2. Materials and methods

2.1. Bark feedstock

The industrially sourced Norway spruce bark used in pilot-scale extractions originated from two sawmills located in Pietarsaari (63°7'N, 22°7'E) and Haapajärvi (63°7'N, 25°3'E) in Finland. In both cases, the bark feedstock originated from several logging sites within the same region. In summer, the logs were harvested at most two weeks prior to processing, whereas in winter they were stored for a maximum of four weeks. The first extraction series consisted of four batches (1–4) produced from a bark sample obtained from winter-harvested trees. The second series included five batches (5–9) produced from a bark sample obtained from sawlogs harvested in summer. The winter bark was sourced from the Pietarsaari mill, whereas the summer bark originated from the Haapajärvi mill. The top diameters of the logs used in these experimental series were 19.0 cm and 17.5–18.5 cm, respectively. The overall process scheme of the study is presented in Fig. 1.

Bark feedstock characteristics were analyzed to account for the feedstock variability and to enable comparison between winter- and summer-harvested material. Relevant ISO and SCAN standards were chosen to ensure comparability with industrial quality control procedures. **Particle size distributions** of the bark used in the extraction experiments were analyzed from 10-L samples following the standard method SCAN-CM 40:01. For the winter-harvested bark (batches 1–4), five samples were collected independently of the extraction batches, whereas for the summer-harvested bark (batches no. 5–9), three 10-L samples were collected from each extraction batch. **Wood content** in the winter-harvested bark were determined from twenty 1-L samples. For the summer-harvested bark, wood content was determined for each particle size fraction (excluding the fines fraction containing particles < 3 mm) from one randomly selected sample per extraction batch. Wood

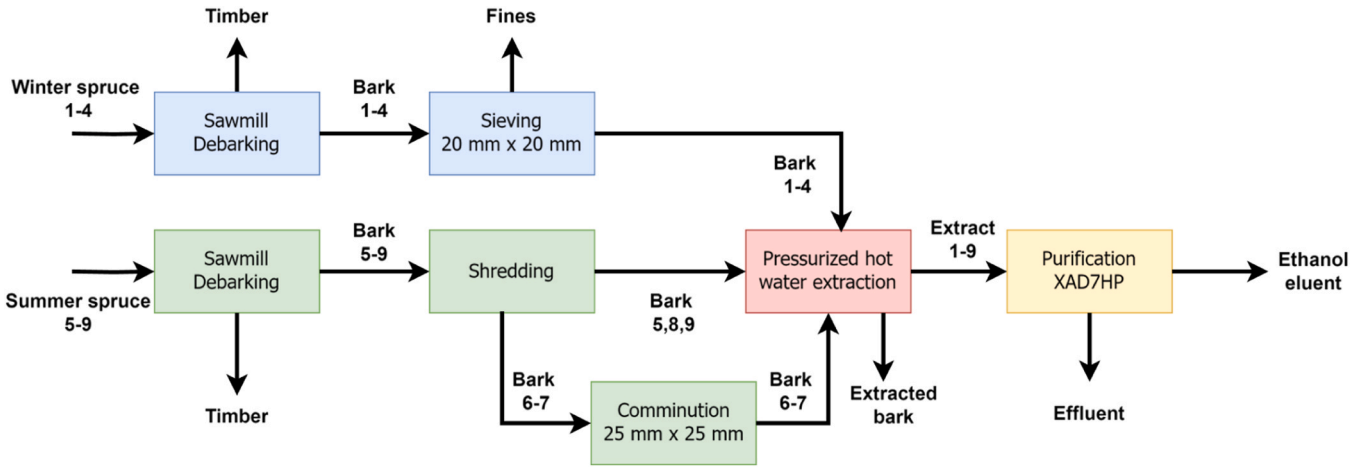

Fig. 1. Experimental design and processing routes for spruce bark fractions. Winter (samples 1–4) and summer (samples 5–9) spruce logs were debarked and the obtained bark fractions were subjected to size classification and size reduction (sieving, shredding, comminution). The resulting particle size fractions were processed by pressurized hot water extraction, followed by XAD7HP purification. The scheme highlights the different feedstock groups, pretreatment routes, and resulting extract fractions used for subsequent chemical analyses.

pieces were manually separated from samples dried at + 105 °C. After determining **the dry mass proportions** of wood and bark, the fractions were recombined for ash content analysis by particle size class. **Ash contents** were measured in duplicate for four composite bark–wood samples according to the standard SFS-EN ISO 18122:2015. The ash and moisture contents of the materials used in the extraction experiments is shown in Supplementary material (Table S1 and S2). For chemical analyses, three additional 1-L samples were collected from each batch. These samples were transported in insulated cool boxes and stored frozen prior to analysis.

For each pilot extraction batch, approximately 100 kg of bark was prepared. The samples for the first extraction series were collected from logs debarked on 15 February 2019. To prevent potential flow restrictions in the reactor, the amount of fine particles was controlled by screening the material with a 4 m^2 sieve with 20 mm × 20 mm mesh. The bark samples were delivered to the laboratory within four days, during which the ambient temperature ranged from + 5 °C to –7 °C. Prior to extraction, the material was stored under cold outdoor winter conditions. The samples for the second extraction series were composed of bark obtained from logs debarked on 4 September 2019. In this case, the bark was collected immediately after debarking and shredding using a wheel loader. To ensure sample homogeneity, each loader bucket was divided into five sampling piles of approximately 150 kg. Because flow restrictions were not observed during the winter extraction series, the summer bark was not screened. To examine the effect of particle comminution on extraction performance, the bark used in two extraction batches (no. 6–7) was further shredded using equipment fitted with a 25 mm × 25 mm screen (Eliet Super Prof 2000). Sample preparation for the summer bark was completed within four hours of the debarking process. During sampling, the ambient temperature was 12–15 °C, and the material was stored at + 5 °C prior to extraction.

2.2. Pilot-scale extraction

Based on the observations from the previously published laboratory-scale accelerated solvent extraction (ASE) optimization (Lintula, 2020), the parameters for pilot-scale extractions were selected. The optimum extraction temperature of the laboratory-scale study was found to be approximately 70 °C. Accordingly, 70 °C was included among the pilot-scale extraction temperatures. Higher temperatures of 90 and 120 °C were selected to examine whether the previously observed effect of increased extraction temperature also applies at a larger scale with a coarser, industrially sourced feedstock (Ding et al., 2017; Kemppainen et al., 2014). A 40 min extraction duration was used as the contact time for eight of the nine pilot-scale extractions, as it was considered sufficient to allow water penetration into the bark matrix and release of readily extractable tannins while maintaining practical process feasibility at pilot scale. In addition, one extraction was conducted for 80 min to determine whether prolonged contact time further increased tannin yield under otherwise comparable pilot-scale conditions (Table 1). Elevated pressure was used to conduct the extractions under conditions similar to the laboratory-scale ASE experiments.

Spruce bark samples were extracted with a 300-L hot water extraction system in batch mode (Kilpeläinen et al., 2014). The extraction system consisted of a reactor, a pump, heat exchangers, and a 150-kW heater. The system was controlled by a Siemens Simatic (S7–1200, v. 14) programmable logic controller. Extraction temperatures were measured inside the reactor using nine thermocouples, and the extraction temperature was calculated as the average of these measurements. The reactor was heated with hot water and, for extractions 5, 7, and 9 of the second series, steam was used to heat up the system and the samples before extraction. Make-up water that was pumped into the reactor was heated to a higher temperature than the actual extraction temperature to compensate for the heat needed to warm up the reactor. The reported extraction temperature refers to the time-averaged temperature over the entire extraction period.

The first extraction series consisted of four batches, which were conducted with 39 kg dry weight (DW) winter bark (102 kg fresh) in each batch (Table 1). The second extraction series included five batches with 34–39 kg DW summer bark (78–89 kg fresh) in each batch. Extracts were collected in containers, and samples were taken. These samples were frozen and stored at –20 °C in the dark. Extraction yields were calculated on a dry bark basis.

Bark residue was discharged from the extraction reactor to the bulk bag. The bulk bag containing the bark was weighed. After weighing the residual bark, it was thoroughly mixed, and three 1-L samples were taken for chemical analyses and immediately frozen.

2.3. Resin purification of extracts

Hot water extracts were purified using an XAD7HP adsorbent (7 kg, Sigma-Aldrich) packed into a 62 cm glass column that was pre-washed prior to use. The crude extract was loaded onto the column until the resin bed visibly turned reddish and the effluent became light brown, indicating saturation of the polyphenol-binding sites. Purification was performed as a two-step process for both winter and summer batches:

Table 1
Extraction parameters for the pilot-scale experiments. Bark was shredded at the mill prior to extraction. Temp = temperature.

Batch no.	Extraction timing	Extraction parameters						
		Time, min	Temp (planned), °C	Pre-steam, °C (min)	Temp (actual), °C	Pressure, bar	Weight dry, (fresh), kg	Solids, %
1	Winter (February)	40	70		58	20	39 (102)	12
2		80	70		69	20	39 (102)	13
3		40	70		70	20	39 (102)	12
4		40	90		87	20	39 (102)	13
5	Summer (September)	40	120	165 (**)	122	20	39 (89)	12
6		40	90		93	20	34 (78)	10
7		40	110	120 (10)	106	20	39 (89)	11
8		40	90		88	1.0 (*)	38 (89)	12
9		40	110	120 (10)	114	20	37 (89)	11

**Heated with steam until sample bed reached target temperature.
* Standard atmospheric pressure

the column was first rinsed with water to remove non-bound and weakly bound carbohydrates, after which tannins and other strongly adsorbing phenolics were eluted with 70% ethanol for the winter fractions and 94% ethanol for the summer fractions. The volume of rinse water differed between seasons. Winter extracts were rinsed with 5–10 L of water, while the more heterogeneous summer extracts required 20–30 L to achieve comparable removal of carbohydrate-rich solubles. This difference reflects the generally higher carbohydrate content and greater compositional variability observed in the summer bark extracts.

To assess the reproducibility and homogeneity of the purification process, two independent purification replicates were carried out for each summer bark batch, resulting in extract pairs (5–9.1 and 5–9.2). These replicates were also used to evaluate whether precipitate accumulation during extraction and storage influenced resin behavior or final extract composition. Following elution, all ethanol fractions were combined and concentrated by rotary evaporator.

2.4. Chemical analysis

Because the extraction trials were conducted in two stages with partially different objectives, the level of analytical detail varied across sample types. All sample streams, original bark, crude hot water extracts, XAD-purified extracts, and extraction residues were analyzed for condensed tannins and carbohydrates. More detailed analyses were focused on the summer bark fractions, which were produced under the final optimized pilot-scale conditions. Pyrolysis-GC/MS (for lignin-derived components) was carried out only for the XAD-purified fractions. Comprehensive extractives profiling was also performed exclusively for the XAD-purified summer bark extracts.

Condensed tannins (CT, proanthocyanidins) were determined by ultrahigh-performance liquid chromatography (UHPLC) after thiolytic degradation, as previously described (Korkalo et al., 2020). Briefly, 20–30 mg of each sample was weighed into 1.5 mL Eppendorf vials, after which 1 mL of depolymerization reagent (3 g cysteamine dissolved in 56 mL of methanol acidified with 4 mL of 13 M HCl) was added. The vials were incubated for 60 min at 65 °C, followed by rapid cooling in an ice bath. The chilled samples were then filtered into HPLC vials and analyzed using an Agilent 1290 Infinity UHPLC system equipped with diode array detection (DAD) and fluorescence detection (FLD). External standards, including catechin, epicatechin, gallocatechin, and epigallocatechin (Sigma Aldrich Oy, Espoo, Finland), as well as thiolyzed procyanidin B2 and procyanidin A2 (Extrasynthese, Lyon, France) were used to quantify free flavan−3-ols (terminal units) and their cysteaminyl derivatives (extension units).

The total polyphenol content was determined using a Shimadzu UV−2401PC spectrophotometer (Shimadzu, Kyoto, Japan) at 280 nm. Purified industrial profisetinidin-type tannin, similarly to Mimosa and quebracho tannins, (Tannino QL-SOL Silvateam, Italy), was used as an external standard and processed identically to the samples. Prior to analysis, samples were diluted in 0.1 M NaOH, and their UV absorbance at 280 nm was determined.

The amount and composition of sugars and hemicelluloses in the extracts were determined by acidic methanolysis (Raitanen et al., 2020; Sundberg et al., 1996). Acidic methanolysis depolymerises hemicelluloses and pectins and converts the released sugars into methyl glycosides. Solid samples were treated for 5 h and liquid extracts for 3 h. Resorcinol was used as an internal standard. After methanolysis, samples were silylated and analyzed with a Shimadzu GC−2010 gas chromatograph (Shimadzu, Kyoto, Japan) equipped with an HP−1 capillary column (25 m, 0.2 mm, and 0.11 m) and a flame ionization detector (FID). Hydrogen was used as the carrier gas.

Separation of extractives from the bark samples was performed using an Accelerated Solvent Extractor (ASE 100, Dionex, Sunnyvale, CA, USA) to sequentially remove lipophilic and hydrophilic compounds. Approximately 2 g of bark powder was loaded into a 34 mL stainless-steel extraction cell fitted with a cellulose filter. Samples were first extracted with n-hexane, followed by acetone–water (70/30, v/v). Extractions were carried out at 120 °C and 1500 psi with a static extraction time of 10 min, a 60% cell flush, and a nitrogen purge of 70 s. Each extraction cycle was performed once, and all procedures were conducted in duplicate. After extraction, the residues were lyophilized and stored for subsequent carbohydrate analysis.

Derivatization and Chromatographic Analysis of Extractives were performed as follows. Dried acetone–water extracts (3 mg) were combined with internal standards (100 μg each of heneicosanoic acid and betulinol in acetone). The mixture was dissolved in 500 μL of pyridine and 300 μL of silylation reagent (BSTFA/TMCS, 99/1, v/v; Sigma-Aldrich, Merck KGaA, Darmstadt, Germany) and incubated in an oven at 70 °C for 1 h to complete silylation. For quantitative analysis of the extractive groups, four internal standards were used: heneicosanoic acid, betulinol, cholesteryl margarate, and 1,3-dipalmitoyl−2-oleoylglycerol (100 μg each). When required, esterified lipophilic extractives were hydrolyzed by adding 1 mL of KOH in 90% ethanol and incubating at 70 °C for 3.5 h, followed by acidification to pH 3.5 and extraction with diethyl ether.

The derivatized samples were analyzed using gas chromatography with flame-ionization detection (GC-FID) for quantitative analysis and gas chromatography–mass spectrometry (GC-MS) for qualitative identification. GC-FID analyses were performed on an Agilent 6850 system equipped with either a short HP-1 simulated distillation column (7.5 m × 0.53 mm, 0.15 μm film) for extractive-group quantification or a long HP-5 column (30 m × 0.32 mm, 0.25 μm film) for individual compound analysis. GC-MS analyses were carried out using a Hewlett-Packard 5973 instrument fitted with the same long HP-5 column. Samples were injected at 290 °C and detected at 300 °C. The temperature program was as follows: 100 °C (1.5 min), ramp 6 °C min^{-1} to 180 °C, then 4 °C min^{-1} to 290 °C (hold 13 min), and finally 4 °C min^{-1} to 300 °C (hold 20 min). For short-column GC-FID, on-column injection was performed at 90 °C, with detection at 320 °C and a temperature ramp of 12 °C min^{-1} to 320 °C (hold 10 min).

HPLC analysis of phenolics was performed by dissolving predried extracts (4 mg for resin-treated samples or 1 mg/mL dilutions for bark hot water extracts) in MeOH/UHQ water (50/50, v/v) or UHQ water, followed by filtration through a 0.2 μm PTFE filter. Analyses were carried out using an Agilent 1290 LC system equipped with a ZORBAX StableBond column (80 Å C18, 2.1 mm × 100 mm, 1.8 μm, 1200 bar), a ZORBAX SB-C18 UHPLC guard column (2.1 mm, 1.8 μm), a 1290 Infinity II diode-array detector (DAD), and a 6460 triple-quadrupole mass spectrometer (LC/DAD/QQQ). The column temperature was maintained at 30 °C.

The mobile phase consisted of (A) 0.1% formic acid in UHQ water and (B) 0.1% formic acid in acetonitrile, with a flow rate of 0.3 mL min^{-1}. The gradient program was adapted from Gabaston et al., starting at 10% B and increasing stepwise to 100% B over 17 min, followed by re-equilibration to initial conditions (Gabaston et al., 2017). Mass spectrometry was performed in negative-ion mode (*m/z* 100–1200) using nitrogen as the drying gas (10 L min^{-1} at 365 °C), a nebulizer pressure of 40 psi, and a capillary voltage of 3100 V. Data were processed using Bruker Data Analysis 3.2 software. External standards were used for quantification: trans-piceid (0.05–0.8 mg/mL) for stilbenoids and polydatin for hydrophilic phenolic compounds.

For pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) analysis of purified, dried extracts, 100 μg of sample was deposited onto a platinum filament. Pyrolysis was performed using a Pyrola 2000 filament-pulse pyrolyzer equipped with an autosampler. The pyrolysis was conducted at 600 °C for 2 s under a helium atmosphere. The resulting pyrolysis products were analyzed using an Agilent 7890B gas chromatograph coupled to an Agilent 5977B single-quadrupole mass spectrometer. Chromatographic separation was achieved using an HP-5MS column (5% phenyl methyl siloxane; 30 m × 250 μm i.d., 0.25 μm film thickness). The GC oven program was as follows: initial temperature 50 °C (hold 0.5 min), ramp at 8 °C min^{-1} to 300 °C, and final hold at 300 °C for 6 min.

2.5. Antibacterial analysis

The antibacterial activity of XAD7HP-purified extracts was assessed using two recombinant bioluminescent indicator strains: *Escherichia coli* K12 + pcGLS11 (Gram-negative) and *Staphylococcus aureus* RN4220 + pAT19 (Gram-positive). These strains emit a continuous luminescent signal that decreases upon exposure to antibacterial compounds (Vesterlund et al., 2004), and the methodology has been described in detail previously (Tienaho et al., 2025). Briefly, strains were stored at −80 °C and revived by 16 h cultivation on lysogeny agar (tryptone 10 g L^{-1}, yeast extract 5 g L^{-1}, NaCl 10 g L^{-1}, agar 15 g L^{-1}) at 30 °C for *E. coli* or 37 °C for *S. aureus*. Plates were supplemented with 10% phosphate buffer (1 M, pH 7.0) and 100 μg mL^{-1} ampicillin for *E. coli*, and 5 μg mL^{-1} erythromycin for *S. aureus*. Stock cultures were prepared by inoculating a single colony into lysogeny broth containing the same supplements and incubating for 16 h at 300 rpm at the respective temperatures. Extracts were dissolved in dimethyl sulfoxide (DMSO) and diluted in double-distilled water to obtain final concentrations of 0.25, 0.5, and 1 mg/mL, with corresponding DMSO contents of 0.25, 0.5 and 1%, in opaque white microplate wells. Negative controls consisted of double-distilled water or DMSO at the same concentration present in the samples, whereas positive controls contained 8.75 or 17.5 vol% ethanol. Samples and controls were pipetted in triplicate and mixed with an equal volume of bacterial inoculum. Luminescence (wavelength range 360–670 nm) was recorded every 5 min for 60 min at room temperature using a Varioskan Flash reader (Thermo Fisher Scientific). Results are expressed as the percentage inhibition at 1 mg/mL after 50 min incubation, calculated as: inhibition% = $(1 - RLU_{sample}/RLU_{negative\ control}) \times 100$, where RLU denotes relative light units.

2.6. Statistical analysis

Correlations between antibacterial activity and of extract composition were evaluated using variables including condensed tannin content, prodelphinidin/procyanidin ratio, degree of polymerization, pyrolysis-derived variables, extraction temperature and extraction pressure. Statistical analyses were performed in RStudio 2025.05.1 Build 513 using R version 4.5.1. Correlations between variables and their statistical significance were calculated using the *rcorr* function from the Hmisc package.

Pyrolysis-GC-MS data were visualized using a heatmap. The ComplexHeatmap package in R (Gu, 2022; Gu et al., 2016) was used to classify samples based on their pyrolysis-GC-MS profiles. Peak areas were log-transformed to reduce the influence of a small number of dominant peaks. The 50 compounds with the highest variance were included in the heatmap. Compound values were scaled to a range from −2–2. Compounds were clustered using Euclidean distance and Ward's D2 method.

Differences in extractive composition among the XAD7HP-purified fractions 5.1–9.2 were also statistically evaluated. Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variance was evaluated using Levene's test. Based on these results, analysis of variance (ANOVA) was performed. Tukey's test was used for pairwise comparisons. Statistically significant values are indicated with different letters.

3. Results and discussion

The results are presented in accordance with the pilot-scale extraction workflow, beginning with characterization of the bark feedstock, which provides essential context for interpreting the extraction performance. Differences between winter- and summer-harvested bark, including particle size, bark–wood ratio, and ash content, are first examined to assess feedstock variability. This is followed by an evaluation of extraction yields, tannin enrichment during XAD-resin purification, and chemical composition of the resulting fractions. Finally, the bioactivity of the tannin-enriched extracts is assessed through antibacterial assays.

3.1. Bark feedstock

To support interpretation of extraction performance at pilot scale, the physical and compositional properties of the bark feedstock were characterized for both winter (batches 1–4) and summer (batches 5–9) material. The analyses focused on particle-size distribution, wood-to-bark ratio, as well as moisture and ash content, as these parameters influence heat transfer, diffusion, and the availability of tannin-rich tissues during hot water extraction.

Winter bark batches exhibited highly uniform particle-size distributions (Fig. 2). The dominant size class for all winter samples consisted of particles passing the 7 mm sieve but retained on the 3 mm sieve. Prior to extraction, winter bark was screened using a 20 × 20 mm sieve, effectively removing oversized chips and reducing the proportion of wood-containing fragments. In contrast, summer bark batches displayed much greater heterogeneity in particle size. Batches 6 and 7, which were subjected to an additional shredding step using a 25 × 25 mm screen, contained the smallest particles and only minor amounts of material retained on the 8 mm sieve. By comparison, batches 5, 8, and 9, which were only shredded once, contained substantial fractions (20–50%) of coarse particles, particularly in the 8 mm and 45 mm size classes. These differences indicate that bark moisture, debarking conditions, and shredding efficiency contributed to greater variability in the summer material.

Wood contamination varied between seasons and between size classes (Fig. 3). Winter bark contained consistently higher proportions of pure bark, reflecting effective initial debarking and the benefit of the

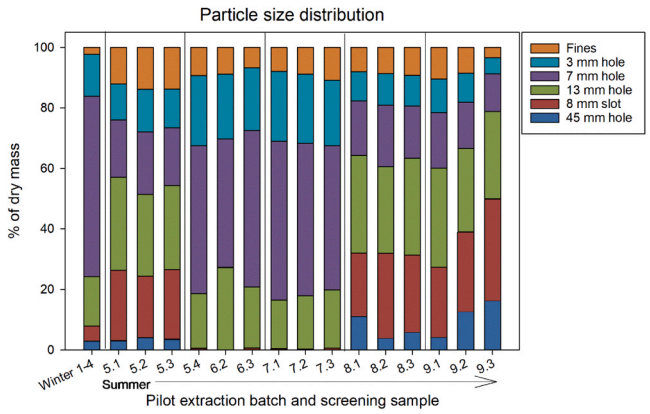

Fig. 2. Particle-size distributions of the Norway spruce bark winter fractions (1–4, combined distribution of five samples) and summer fractions (5–9, one randomly selected sample per batch; 5.1–9.3) used in the pilot-scale extraction experiments.

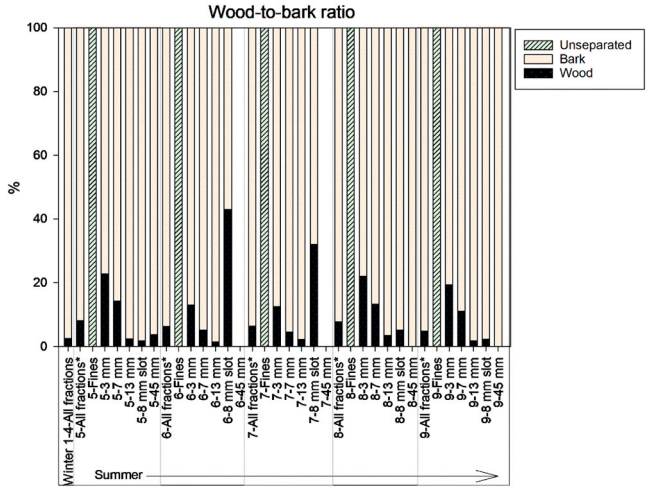

Fig. 3. Dry-mass-based wood-to-bark ratios of the bark samples used in the pilot extractions. Samples 1–4 are winter fractions (n = 20) and samples 5–9 summer fractions (one sample per extraction batch). Unseparated fine fractions are excluded from the total wood contents of batches 5–9.

screening step. In the summer bark, wood content was highest in the coarse fractions, particularly in the 8 mm slot class of batches 6 and 7, whereas the finer fractions (3 mm and 7 mm) showed only modest increases in wood content. Notably, in the largest particle class (45 mm), batches 8 and 9 consisted entirely of bark with no detectable wood, suggesting incomplete comminution of large outer bark fragments rather than bark–wood composites. These trends underscore the greater structural heterogeneity of the summer feedstock, with both larger chip sizes and more variable wood content than observed in the winter batches.

Ash contents of all bark and wood fractions are provided in Supplementary Table S1. The ash content of unsieved bark was relatively uniform across batches, ranging from 3.2 to 3.6 wt% (mean 3.34%, SD = 0.04 percentage points), whereas wood contained substantially lower ash levels (0.51%, SD = 0.04). In the summer bark (batches 5–9), the finest sieved fractions contained the highest ash contents, ranging from 3.9 to 5.9 wt%. This enrichment of ash in fine particles is consistent with earlier observations that mineral components are concentrated in the smallest bark particles (Miranda et al., 2012).

Moisture contents of the bark feedstocks are shown in Supplementary Table S2. Winter bark batches (1–4) exhibited slightly higher moisture contents (mean 63.0%) compared to the summer bark batches (55.6–58.4%). This difference likely reflects seasonal variation in bark hydration and storage conditions and may influence both bark brittleness during preprocessing and diffusion behavior during extraction. Wood contents determined for the feedstocks corroborated the particle-size and compositional trends described above: winter bark contained consistently lower wood proportions (2.7–3.3% of dry mass), whereas the summer bark batches showed higher and more variable wood contents (5.1–8.2%). These results further confirm that the summer bark feedstock was structurally and compositionally more heterogeneous than the winter bark, with potential implications for extraction efficiency and compositional uniformity in the resulting extracts.

Overall, winter bark showed greater uniformity in both particle-size distribution and wood-to-bark ratio, whereas the summer bark was more heterogeneous, particularly in the coarse fractions. Similar observations regarding seasonal differences and feedstock heterogeneity have been reported previously. Winter bark typically contains higher phenolic concentrations and exhibits greater structural uniformity than summer bark, whereas summer bark shows increased compositional degradation and variability (Halmemies et al., 2021; Jyske et al., 2024). Industrial debarking further contributes to heterogeneity in particle size and wood contamination (Jyske et al., 2024; Kemppainen et al., 2014). Moisture content, mineral content, and bark morphology are known to influence swelling behavior, water uptake, mass-transfer properties, and thermal penetration during extraction (Ilek et al., 2024; Kalagnanam et al., 2026; Le Normand et al., 2012). Also processing and the resulting micromorphology have a substantial impact on the material performance (Das et al., 2024; Gößwald et al., 2024). Previous work has generally relied on homogeneous and finely milled bark, often at laboratory scale (Barjoveanu et al., 2020; Le Normand et al., 2012), whereas emerging pilot-scale studies confirm that realistic industrial feedstock introduces significant structural and chemical variability (Tienaho et al., 2025). Our results align with these findings and extend them by demonstrating that such variability remains a critical determinant of extraction efficiency and purification behavior in pilot-scale spruce bark valorization.

3.2. Pilot-scale extractions

Pilot-scale extractions carried out in the 300 L reactor produced 232–248 kg of hot water extract per batch. Extraction conditions and corresponding extraction efficiencies, expressed as total dissolved solids (TDS) and yield, are summarized in Table 2. Extraction temperature was the main factor governing extraction performance. Increasing temperature enhanced both TDS and overall yield, most likely by improving water penetration into the bark matrix, accelerating diffusion and mass transfer, and increasing the solubilization of condensed tannins, carbohydrates, and other extractives. This effect was particularly evident in Sample 5, extracted at 122 °C, which produced the highest TDS (2.48 wt %) and yield (140.43 mg/g). The result indicates that sufficiently intense hot-water extraction conditions can partly overcome the mass-transfer limitations caused by coarse and heterogeneous industrial bark feedstock. However, the temperature effect should not be

Table 2
Extraction efficiency. TDS = total dissolved solids.

Sample		Extraction			TDS	Yield
		Time (min)	Temp (°C)	Pressure (bar)	wt%	mg/g
winter	1	40	58	20	1.47	89.33
	2	80	69	20	1.85	110.29
	3	40	70	20	1.65	104.92
	4	40	87	20	1.84	109.46
summer	5	40	122	20	2.48	140.43
	6	40	93	20	1.04	71.65
	7	40	106	20	1.32	85.10
	8	40	88	1.0	1.07	58.79
	9	40	114	20	1.51	96.20

interpreted solely as improved tannin recovery, because elevated temperatures may also increase the co-extraction of carbohydrates and other non-tannin components. Thus, extraction temperature represents a balance between maximizing overall solubilization and maintaining extract selectivity.

Carbohydrate contents of the original and extracted bark showed relatively small changes across most batches (Table 3), indicating that hemicelluloses and pectin were only partly solubilized under the extraction conditions used. The exception was Sample 5, where the higher extraction temperature resulted in a notable reduction in residual carbohydrates, suggesting enhanced removal of hemicelluloses and pectic substances. This observation aligns with previous reports indicating increased carbohydrate solubilization at elevated temperatures during pressurized hot water extraction.

Condensed tannin (CT) contents decreased in all extracted residues relative to the original bark, confirming that CTs were partially removed during hot water extraction. However, the persistent CT levels in most residues indicate incomplete recovery, especially of less mobile or more strongly matrix-bound tannins. The extraction of CTs is affected not only by their solubility but also by molecular size and diffusion within the bark matrix. Lower-molecular-weight CTs are expected to diffuse more readily through swollen bark tissues and dissolve into the aqueous phase, whereas higher-DP polymers may be retained because of slower diffusion, steric limitations, and stronger hydrogen-bonding or hydrophobic interactions with cell-wall carbohydrates, proteins, and lignin-derived structures. This size-dependent extraction behavior provides a mechanistic explanation for the DP patterns observed in the residues. The CT contents in residual bark also indicate that extraction parameters, particularly time, particle size, and temperature, could be further optimized to improve tannin recovery. In this context, Sample 5 again stands out: its residual CT content was drastically reduced, demonstrating that higher extraction temperature promotes more complete solubilization of CTs.

The degree of polymerization (DP) of CTs exhibited distinct seasonal patterns. Winter bark residues showed DP values similar to those of the original bark, suggesting that CTs of all molecular sizes were extracted relatively evenly. In contrast, the extracted summer bark samples displayed systematically higher DP values in the residues, indicating preferential solubilization of lower-molecular-weight CTs and retention of larger polymers in the biomass matrix. Sample 5 showed a markedly lower DP than the other summer extractions (6–9), supporting the interpretation that higher temperature facilitated the release of higher-molecular-weight CTs by enhancing solvent penetration, diffusion, and disruption of tannin-matrix interactions. The procyanidin-to-prodelphinidin (PC/PD) ratios remained consistent between original and extracted bark across all samples (Table 3), indicating that the extraction process did not selectively favor one tannin subclass over the other. This stability in tannin subunit composition supports the conclusion that the extraction conditions primarily influence the extent of CT solubilization rather than altering the underlying monomer distribution.

Similar temperature-dependent extraction behavior has been widely reported in earlier laboratory studies, where temperature is consistently the dominant parameter controlling both overall extraction yield and tannin solubilization from spruce bark (Kalagnanam et al., 2026; Kemppainen et al., 2014; Le Normand et al., 2012). Research has shown that industrial debarking introduces substantial variation in particle size, wood contamination, moisture, and chemical composition (Jyske et al., 2024; Kemppainen et al., 2014), yet high polyphenol yields can still be achieved at pilot scale (Tienaho et al., 2025). The strong extraction response observed in Sample 5 is consistent with these pilot-scale findings, indicating that even coarse, heterogeneous spruce bark fractions can be efficiently solubilized under sufficiently intense hot water extraction conditions.

3.3. Resin treatment purification using XAD7HP

Resin treatment was used to purify the tannin content in extracts. The XAD7HP purification step led to a consistent reduction in total carbohydrate content across all extracts (Table 4, Fig. 4). Carbohydrates in the crude bark extracts ranged from 34 to 45 mg/g in the winter extracts and 18–74 mg/g in the summer extracts but were reduced to 5–17 mg/g after purification. The most substantial reduction was observed in Sample 5, extracted at the highest temperature (122 °C), suggesting that the resin was particularly effective at removing hemicellulose-derived sugars when their initial concentrations were elevated.

Interestingly, all purified summer bark extracts contained similarly low carbohydrate levels (<10 mg/g) despite the fact that their crude extracts varied more than four-fold in initial carbohydrate content (18–74 mg/g). This convergence can be explained partly by the larger volume of rinse water applied to the summer batches, which enhanced the removal of freely soluble carbohydrates. However, the persistence of glucose across all purified samples (Fig. 4) suggests that some carbohydrates are not present as free sugars but are instead structurally linked to phenolic compounds.

Spruce bark is known to contain several classes of stilbene glucosides, including astringin, isorhapontin, and piceid, in which glucose is covalently attached to a phenolic aglycone. Multiple studies confirm the abundance of these glucose-linked phenolics in *Picea abies* bark. Previous work showed that industrial Norway spruce bark contains substantial levels of stilbene glucosides and that their concentrations vary strongly with season and industrial handling (Jyske et al., 2024). Additional studies on spruce roots and stumps similarly demonstrated that isorhapontin and astringin are the dominant glucosides, with piceid and catechin also present in measurable quantities (Latva-Mäenpää et al., 2013). High-resolution LC–MS analyses have likewise identified trans-astringin, piceasides, and other glycosylated phenolics in spruce bark, confirming that glucose-bearing phenolic structures are abundant components of the extractive fraction (Sut et al., 2021). Polymeric adsorbents such as XAD resins interact primarily with aromatic structures, meaning that glycosylated phenolics adsorb efficiently due to the affinity of their aromatic aglycones for the hydrophobic resin matrix. As a

Table 3
Bark total carbohydrates, condensed tannins, degree of polymerization (DP), procyanidin percentage (PC%), and prodelphinidin percentage (PD%) for the original bark (orig) and the extraction residual bark (res) expressed per dry original bark.

Sample bark		Total carbohydrates (mg/g)		Condensed tannins (mg/g)		DP		PC%		PD%	
		orig	res	orig	res	orig	res	orig	res	orig	res
winter	1	325.6	305.4	30.8	18.8	6.1	5.3	90.0	85.0	10.0	15.0
	2		305.6		22.0		5.7		87.0		13.0
	3	336.4	312.6	27.3	29.5	6.0	6.6	90.0	90.0	10.0	10.0
	4		313.3		28.6		6.6		91.0		9.0
summer	5	288.2	188.3	20.5	1.3	10.4	8.5	84.5	77.5	15.5	22.5
	6	267.9	243.6	19.9	13.5	9.9	15.3	80.5	81.5	19.5	18.5
	7	278.5	278.9	22.6	15.3	10.7	18.4	82.5	85.0	17.5	15.0
	8	279.7	276.3	20.4	20.7	9.9	13.7	82.5	87.0	17.5	13.0
	9	285.7	277.2	20.2	14.5	9.7	13.8	82.5	86.0	17.5	14.0

Table 4
Total carbohydrates, UV280 nm for total phenols, and condensed tannin content in mg/g of the extracts per dry extracted biomass for the original (ext) and XAD7HP-purified (XAD) extracts. # = sample number, DP = degree of polymerization, PC = procyanidin, and PD = prodelphinidin. Extracts 1–4 were obtained from winter bark and 5–9 from summer bark and results are expressed per dry original bark.

#		Total carbohydrates		UV280 nm for polyphenols		Condensed tannins		DP		PC		PD	
		ext, mg/g	XAD, mg/g	ext, mg/g	XAD, mg/g	ext, mg/g	XAD, mg/g	ext	XAD	ext, %	XAD, %	ext, %	XAD, %
1		33.6	11.7	29.2	23.5	2.6	35.2	6.6	4.6	94.3	98.6	5.7	1.4
2		40.7	16.5	41.1	33.7	3.8	46.6	6.6	4.9	95.2	98.3	4.9	1.7
3		37.6	11.7	38.5	25.6	3.6	37.7	7.2	5.0	94.5	99.0	5.6	1.0
4		44.8	15.6	42.6	38.4	4.4	59.2	6.3	5.2	94.0	98.5	6.0	1.5
5	.1	74.4	8.3	115.4	41.3	2.1	23.4	6.3	10.5	95.4	65.0	4.6	35.0
	.2		9.8		44.9		31.4		10.5		66.0		34.0
6	.1	22.0	5.2	47.6	24.9	3.7	39.5	6.0	7.3	95.2	75.0	4.8	25.0
	.2		6.9		40.2		48.4		6.9		79.0		21.0
7	.1	28.9	6.9	50.2	47.4	4.2	58.9	6.1	6.0	96.0	82.0	4.1	18.0
	.2		6.9		35.9		58.9		6.0		78.0		22.0
8	.1	17.9	5.5	33.0	26.7	2.5	35.4	5.9	6.3	93.6	86.0	6.4	14.0
	.2		5.2		37.2		32.9		5.6		86.0		14.0
9	.1	33.4	8.0	54.0	52.0	4.6	52.6	6.2	6.0	95.3	83.0	4.7	17.0
	.2		6.2		34.8		46.5		5.8		81.0		19.0

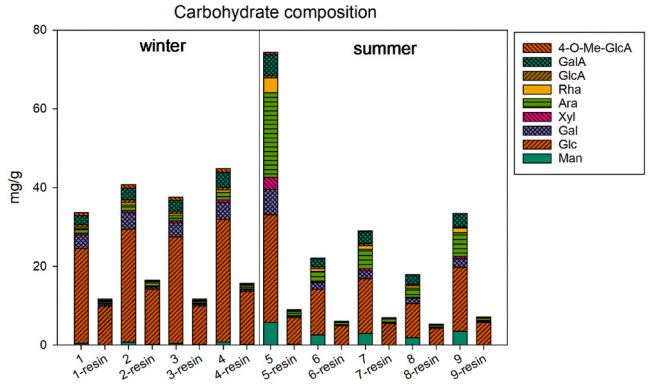

Fig. 4. Carbohydrate composition of the original and XAD7HP-purified extracts. Man = mannose, Glc = glucose, Gal = galactose, Xyl = xylose, Ara = arabinose, Rha = rhamnose, GlcA = glucuronic acid, GalA = galacturonic acid, 4-*O*-Me-GlcA = 4-*O*-methylglucuronic acid. Extracts 1–4 were obtained from winter bark and 5–9 from summer bark. Results are shown as means of two replicates per original dry bark.

result, enrichment steps remove most free carbohydrates but retain phenolics along with their bound glucose units.

UV-absorbing compounds measured at 280 nm (total phenolics) decreased during purification in both winter and summer extracts, with the strongest decline observed in the summer fractions (Table 4). This is consistent with the removal of non-tannin phenolics, small lignin fragments, and carbohydrate-linked UV-absorbing compounds that weakly bind to the resin. A larger reduction in UV280 absorbance was associated with greater feedstock heterogeneity and higher carbohydrate contamination prior to purification.

The XAD7HP step led to a clear enrichment in condensed tannins (CTs). All purified extracts showed approximately tenfold increases in CT concentrations relative to the crude extracts (Table 4). This indicates that the resin preferentially retains CT structures while allowing most sugars, organic acids, and small phenolics to be washed out during the water rinse and ethanol elution steps.

The average degree of polymerization (DP) of CTs was largely maintained between crude and purified extracts, suggesting that purification did not selectively retain specific CT size classes. However, notable seasonal differences emerged: the purified winter bark fractions showed consistently lower DP values than the corresponding summer bark fractions. This indicates that winter bark extracts are richer in lower-molecular-weight CTs, whereas summer bark extracts contain a higher proportion of large polymeric CT structures, consistent with the seasonal and particle-size differences observed in the feedstock.

The resin treatment also produced changes in tannin subunit composition. In the summer bark fractions, purification decreased the proportion of procyanidin (PC) units and increased the relative abundance of prodelphinidin (PD) units. This shift suggests differential adsorption of tannin structures on XAD7HP. As a non-ionic polymeric adsorbent, XAD7HP retains phenolic compounds mainly through hydrophobic interactions between the aromatic flavan−3-ol structures and the resin matrix, while hydrogen bonding and π–π interactions may further contribute to adsorption. PD-rich tannins contain more hydroxyl groups than PC-rich tannins, which may strengthen hydrogen bonding with the resin and surrounding phenolic structures, whereas their aromatic backbone still provides sufficient hydrophobic character for adsorption The observed enrichment of PD units after purification may therefore reflect combined effects of aromatic hydrophobic interactions, hydrogen bonding capacity, and tannin size or conformation. The PC/PD ratios of the winter extracts remained more stable, likely because their initial tannin profiles were more homogeneous and less affected by selective adsorption during resin treatment. However, because the winter and summer fractions were eluted with different ethanol concentrations, part of the observed compositional variation may reflect differences in elution strength rather than feedstock effects alone. Higher ethanol concentrations may improve desorption of more strongly retained phenolics from XAD7HP, whereas lower ethanol concentrations may favor recovery of more readily eluted compounds.

Overall, the XAD7HP purification step substantially improved extract quality by removing carbohydrates and low-molecular-weight UV-absorbing compounds while enriching condensed tannins. These results are consistent with earlier uses of non-ionic polymeric resins for plant material purification, which similarly report strong phenolic retention and efficient carbohydrate removal (Seif Zadeh and Zeppa, 2022). The global tannin market is growing rapidly, with an estimated compound annual growth rate of 5.8% from 2016 to 2025 (Singh and Kumar, 2020) and 6.6% from 2023 to 2030, reaching around USD 4.12 billion by 2030 (Ozogul et al., 2025). Our results indicate that resin treatment is a viable possibility for obtaining high-purity tannin fractions from spruce bark. The strong purification performance of Sample 5 highlights an important process insight: higher extraction temperatures (≥120 °C) produce more carbohydrate-rich extracts, but these carbohydrates are efficiently removed during resin treatment, up to a limiting level determined by sugar–phenolic interactions. The seasonal differences observed in CT DP and PC/PD ratio suggest that resin purification may interact with feedstock-dependent CT structures, which is a relevant consideration for industrial fraction standardization. During the purification series, no clear decline in separation quality was observed, as carbohydrate removal and CT enrichment remained consistent across

the purified fractions. The resin was regenerated between runs by ethanol elution followed by water rinsing. However, long-term resin stability, fouling, and regeneration efficiency were not systematically evaluated and should be addressed in future continuous or repeated-cycle studies. Additionally, a full techno-economic assessment was beyond the scope of the present study. The techno-economic feasibility and industrial constraints of spruce bark extraction and downstream processing have been evaluated in previous studies (Ruuttunen et al., 2024; Tienaho et al., 2025). In this context, the present results provide complementary pilot-scale evidence on process performance, extract purification, and product quality.

3.4. Detailed chemical composition of the purified extracts

3.4.1. Pyrolysis-GC/MS

Pyrolysis–GC/MS was conducted on the XAD7HP-purified extracts to characterize the aromatic structures and lignin-derived components present in the final tannin-rich fractions (Fig. 5). In addition, model compounds were analyzed to aid interpretation: catechin as a representative condensed tannin monomer, picein as a stilbene glucoside, and PHWE-lignin as a softwood lignin reference. Catechin pyrolysis produced aromatic fragments not associated with typical lignin units (H, G, or S), whereas picein yielded a mixture of aromatic and carbohydrate-derived compounds. PHWE-lignin produced a profile dominated by guaiacyl (G) units, with minor amounts of syringyl (S) units and non-lignin aromatics, consistent with the known guaiacyl-rich composition of spruce lignin.

The purified winter and summer bark extracts all contained high amounts of carbohydrate-derived fragments and non-lignin aromatic units, reflecting their polyphenol-rich composition after resin purification. However, clear seasonal differences were observed. Winter extracts contained small but detectable amounts of S-units, whereas S-units were absent from all summer extracts. Because syringyl-type lignin is not characteristic of spruce (Neiva et al., 2020), these minor S-unit signals likely arise from trace contributions of methoxy-substituted phenolics, non-lignin aromatic structures, or small amounts of hardwood contamination. Their presence does not appear to substantially influence the overall aromatic profile.

Summer extracts generally exhibited higher proportions of other aromatic fragments and terpene-originated units than the winter extracts, likely reflecting the greater structural heterogeneity of the summer feedstock. Multivariate analysis of the 50 most discriminating pyrolysis compounds (Supplementary Fig. S1) confirmed a clear separation between winter and summer extracts. This indicates that chemical heterogeneity originating from feedstock and extraction conditions is retained even after resin purification.

Overall, the pyrolysis–GC/MS data demonstrate that the purified extracts are composed primarily of condensed-tannin-related and non-lignin aromatic structures, with some contributions from lignin-derived components. This is also supported by the earlier literature on softwood bark pyrolysis products (Case et al., 2014; Pinto et al., 2018). The observed seasonal differences in aromatic composition are consistent with the feedstock variability described earlier and highlight the influence of raw material heterogeneity on the chemical characteristics of the final purified extracts.

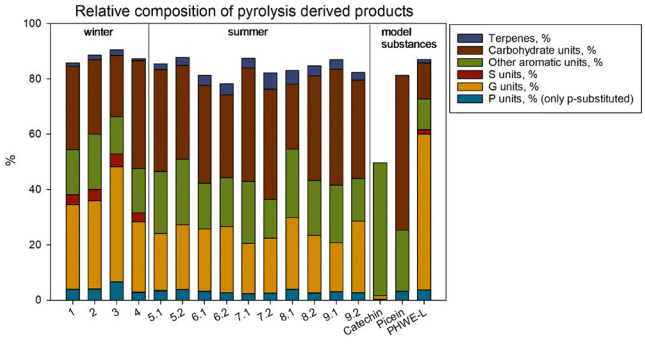

Fig. 5. The relative composition (%) of pyrolysis-derived products for winter and summer bark extract XAD7HP-purified fractions and model substances. Results are shown in P-hydroxyphenyl (P), guaiacyl (G), and syringyl (S) units, while also showing other aromatic units, carbohydrates and terpenes. PHWE-L = pressurized hot water extracted lignin.

3.4.2. Extractives

Comprehensive extractives profiling was conducted only for the purified summer bark extracts, as these batches displayed the greatest variability in tannin content and represented the higher, optimized extraction temperatures. The quantified extractive groups are shown in Fig. 6 and more detailed analysis of the statistical differences can be found in Supplementary Fig. S2. Total extractives ranged from 6.7 to 16.9 mg/g dry bark, indicating moderate variability among the purified summer fractions. Notably, substantial differences were observed between the replicate extracts (e.g., 6.1 vs. 6.2 and 9.1 vs. 9.2), reflecting the structural heterogeneity of the summer feedstock (particle size, wood content) and the compositional variation already evident in the crude and purified extracts. As expected, extractive contents were low compared with the condensed tannin yields (Table 4), confirming that small phenolic and non-phenolic extractives represent only a minor portion of the purified fractions. Samples 7.1, 8.1, and 9.1 exhibited the highest total extractives among the summer batches.

The extract profiles obtained after XAD7HP purification were consistently phenolic-rich and stilbene-dominated, with stilbenoids emerging as the most abundant class across samples. This compositional pattern aligns with extensive evidence that Norway spruce bark inherently accumulates high levels of stilbene glucosides and that pilot-scale handling and fractionation largely preserve a stilbene-rich signature despite quantitative fluctuations across seasons and supply chains (Dou et al., 2022; Jyske et al., 2024). Alongside stilbenes, alcohols, organic acids, and carbohydrate-associated signals remained relatively abundant, supporting the interpretation that a fraction of carbohydrates is conjugated to aromatics and thus co-elutes through the non-ionic resin step. In spruce bark, the predominant stilbenes occur as glucosides, and enzymatic deglycosylation is required to fully liberate aglycones, direct evidence that sugar–phenolic conjugation is intrinsic to the matrix and can carry carbohydrate equivalents through purification.

The detection of flavonoids and lignans at sub-stilbene levels ($\leq$1.8 and $\leq$1.6 mg/g dry bark, respectively) is consistent with literature showing that spruce bark hosts a broader phenolic repertoire beyond stilbenoids and that extraction and fractionation conditions modulate their relative abundances (Hammerbacher et al., 2020; Metsämuuronen and Sirén, 2019). Fractions produced under higher extraction intensity and greater purification efficiency exhibited elevated stilbene contents and an increased share of aromatic extractives relative to lipid-derived

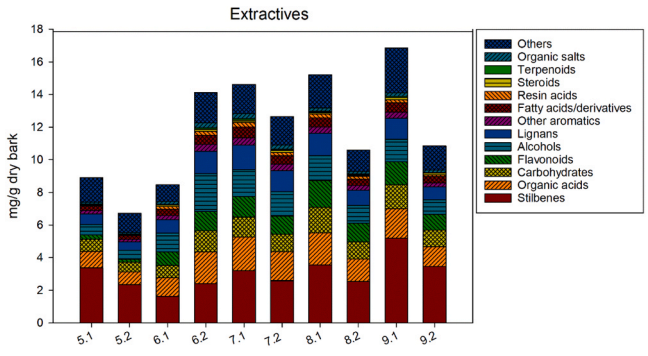

Fig. 6. Chemical composition of XAD7HP-purified summer bark extracts. Results are presented as mg/g of original dry bark.

components, an outcome in line with pilot-scale observations that process severity and solvent access parameters favor phenolic enrichment and can suppress the proportional contribution of lipophilic resin and fatty acids (Suprun et al., 2021).

Despite quantitative variability, the qualitative composition of the extractives was broadly similar among the summer fractions. This pattern indicates that, under the pilot-scale conditions applied, the chemical profile of extractives is robust to feedstock variability, even though the relative abundances of individual groups respond to extraction intensity and upstream bark heterogeneity. The prevalence of stilbenoids, together with condensed tannins, supports the phenolic-rich character of the purified extracts. The presence of resin acids and fatty acids at modest levels suggests that further tuning of purification, e.g., rinse volume, bed volumes, or eluent strength, could reduce residual lipophilic components while maintaining high recovery of phenolics.

3.5. Antibacterial activity

Antibacterial testing was limited to the XAD-purified extracts, as these represent the concentrated polyphenol fractions intended for industrial use. Activity was evaluated against two strains: *Escherichia coli* K12 + pcGLS11 (Gram-negative) and *Staphylococcus aureus* RN4220 + pAT19 (Gram-positive) (Fig. 7). DMSO at the concentrations present in the extract dilutions showed low inhibition and therefore did not explain the observed antibacterial effects. Ethanol was included as a positive control because it produces a rapid reduction in bacterial luminescence through membrane disruption and metabolic stress. Several purified bark fractions approached or exceeded the inhibition levels observed for the lower ethanol control, particularly against *S. aureus*, indicating that the observed activity was biologically relevant However, conventional antibiotic standards were not included in the assay, and the results should be interpreted as comparative antibacterial potential within this extract set rather than as potency relative to clinically used antimicrobials.

Seasonal differences were evident for *S. aureus*: summer bark fractions (5–9) generally displayed higher inhibition than winter fractions (1–4) (Fig. 7**B**). This is consistent with seasonal variation in the concentration of phytochemicals with antibacterial activity (Hammerbacher et al., 2020; Jyske et al., 2015). The main antimicrobial compounds in plants have previously been identified as phenolic compounds, terpenoids, alkaloids, carotenoids, and sulfur-containing phytochemicals (Barbieri et al., 2017; Muilu-Mäkelä et al., 2022; Tienaho et al., 2026). In contrast, no consistent seasonal trend was observed for *E. coli* (Fig. 7**A**). Instead, for *E. coli* the lowest activities were observed with extracts 4, 5 and 9 with 0.5 and 1 mg/mL concentration and 2, 4, 5 and 9 in the case of 0.25 mg/mL. These are predominantly the extracts with highest temperatures and/or longest times for the extraction procedure and also contained the lowest amount of flavonoids and other aromatic compounds.

To relate composition to activity, pairwise correlations were calculated between bacterial inhibition and extract composition for the XAD-purified fractions (Supplementary Table S3). *E. coli* inhibition showed the strongest positive correlations with resin acids ($r \approx 0.92$–0.95 at 0.25–1 mg/mL), lignans and other aromatic compounds (both $r \approx 0.90$ at 0.25 mg/mL), fatty acids/derivatives (up to $r \approx 0.86$), steroids ($r \approx 0.85$–0.88), and terpenoids ($r \approx 0.79$–0.86). Among phenolics, flavonoids, lignans, and other aromatic compounds showed significant positive correlations (Table 5). In contrast, *E. coli* inhibition was strongly negatively correlated with stilbenes ($r \approx -0.83$ to -0.94), as were the unidentified "other" compounds ($r \approx -0.91$ at 0.5–1 mg/mL) and several variables including degree of polymerization (DP) of tannins (–0.90), p-units (–0.95 to –0.90), and the sum of extractives ($r \approx -0.78$ to -0.82). These patterns suggest that Gram-negative susceptibility was primarily associated with lipophilic and amphiphilic extractives, such as resin acids, fatty acids, terpenoids, and certain sterols, which may interfere with outer membrane organization. Conversely, enrichment in small polar aromatics such as stilbenes, or loss of membrane active lipophiles, corresponded to weaker activity. This is consistent with the observation that batches produced under harsher conditions exhibited depletion of labile membrane active components and concurrently reduced *E. coli* inhibition.

For *S. aureus*, the pattern differed markedly. Positive correlations were strongest for prodelphinidin percentage (PD%) ($r \approx 0.87$–0.91), degree of polymerization (DP) (up to $r \approx 0.75$ at 1 mg/mL), and the "Other" fraction ($r \approx 0.90$ at 1 mg/mL). Extraction conditions also played a notable role, as temperature ($r \approx 0.92$) and pressure ($r \approx 0.91$) correlated positively with *S. aureus* inhibition. Significant positive correlations were additionally observed with stilbenes ($r \approx 0.32$–0.67) and UV280 (up to $r \approx 0.52$). Conversely, PC (procyanidin) (≈ -0.90) and sugars (≈ -0.86 to -0.90) correlated negatively with *S. aureus* inhibition. Overall, this implies that Gram-positive activity aligns with phenolic intensity and phenolic structure rather than lipophilic extractives. Higher PD% and higher DP support stronger protein-binding and metal-chelating interactions typical of trihydroxylated PD-type condensed tannins, mechanisms compatible with the protein-rich peptidoglycan/teichoic-acid matrix of *S. aureus*. Positive associations with stilbenes and

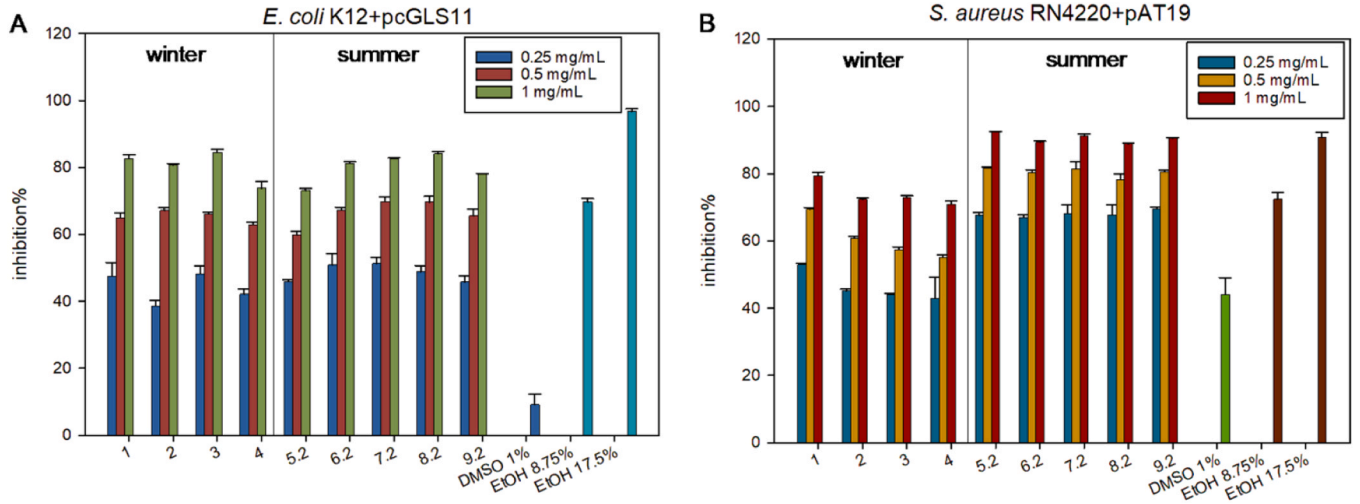

Fig. 7. Inhibition percentages of three concentrations (0.25, 0.5, and 1 mg/mL) of the XAD7HP-purified bark extracts against *Escherichia coli* (A) and *Staphylococcus aureus* (B). For the summer fractions, the tested fractions were 5.2–9.2. Results are presented in means of three technical replicates ± standard deviation. DMSO 1% was included in the 1 mg/mL extracts and shown as negative controls along with two ethanol concentrations.

Table 5
Statistically significant ($p < 0.05$) correlations between bacterial inhibitions and extractive results. DP = degree of polymerization, PC = procyanidin, PD = prodelphinidin, p-units = p-hydroxyphenyl-derived pyrolysis products.

	E. coli 0.25 mg/mL	*E. coli* 0.5 mg/mL	*E. coli* 1 mg/mL	*S. aureus* 0.25 mg/mL	*S. aureus* 0.5 mg/mL	*S. aureus* 1 mg/mL
Stilbenes	−0.94					
Flavonoids		0.89				
Lignans	0.90					
Other aromatic compounds	0.90					
Resin acids	0.92	0.93	0.95			
Organic salts	0.94					
Other		−0.91	−0.91			0.90
DP		−0.90				
PC						−0.90
PD						0.90
Temperature						0.92
Pressure					0.91	
p-units		−0.95	−0.90			

UV-absorbing phenolics further corroborate a phenolic-dominated driver of Gram-positive activity, whereas residual sugars appear antagonistic or dilutive.

The different response patterns of *E. coli* and *S. aureus* are consistent with their distinct cell envelope structures. The outer membrane of Gram-negative *E. coli* acts as an additional permeability barrier that can restrict the access of large polar polyphenols, including polymeric condensed tannins, to the cytoplasmic membrane and intracellular targets. Therefore, activity against *E. coli* is more likely to depend on smaller or more lipophilic constituents capable of disturbing the outer membrane, such as resin acids, fatty acids, terpenoids, sterols, and selected flavonoids. In contrast, Gram-positive *S. aureus* lacks an outer membrane and has a thick, protein- and teichoic-acid-rich peptidoglycan layer that is more directly exposed to phenolic compounds. This may explain why *S. aureus* inhibition correlated more strongly with phenolic variables, including PD content, DP, stilbenes, and UV280-absorbing compounds.

The structure–activity relationships observed here suggest that antibacterial activity was not governed solely by total condensed tannin concentration. Instead, tannin structure appeared important. Higher prodelphinidin content may enhance antibacterial activity because PD-type units contain additional hydroxyl groups, increasing their capacity for hydrogen bonding, protein complexation, and metal chelation. These interactions can interfere with bacterial surface proteins, enzymes, nutrient acquisition, and membrane-associated processes. The positive association between *S. aureus* inhibition and DP further suggests that larger tannin oligomers or polymers may be more effective in forming multivalent interactions with cell wall components and extracellular proteins. However, very high molecular size may also limit diffusion, particularly through the Gram-negative outer membrane, which may partly explain the weaker and less consistent relationship between tannin variables and *E. coli* inhibition.

These findings are consistent with previous reports showing that plant polyphenols can inhibit bacteria through multiple mechanisms, including membrane perturbation, protein binding, enzyme inhibition, metal chelation, oxidative stress modulation, and interference with energy metabolism (Chen et al., 2024). Condensed tannins show structure-dependent antibacterial activity, with polymer size and hydroxylation pattern influencing their interactions with bacterial membranes, cell wall components, and proteins (Qiao et al., 2026). In the present study, the association of PD-rich, higher-DP condensed tannins and stilbenoids with stronger antibacterial activity against Gram-positive bacteria is indeed compatible with prior structure–activity studies, which show that medium-to-high polymerization degree CTs exhibit higher bioactivity than low-DP forms, partly through membrane-disruptive or protein complexing mechanisms (Andersen-Civil et al., 2021; Liang et al., 2024; Wang et al., 2021). Similarly, stilbenoids from spruce bark and other plant sources have also been associated with antimicrobial effects, although their activity depends strongly on concentration, glycosylation, and the target organism (Li et al., 2024). In contrast, the positive correlations between *E. coli* inhibition and lipophilic extractives in the present study are in line with reports that terpenoids, resin acids, and fatty-acid derivatives may increase membrane permeability or disturb membrane integrity, effects that are particularly relevant for Gram-negative bacteria (Ergüden, 2021; Muilu-Mäkelä et al., 2022).

Thus, retaining a controlled fraction of the lipophilic constituents, rather than removing them in harsh purification, may enhance overall antibacterial potency, consistent with documented membrane-damaging behavior of plant polyphenol and lipid-soluble fractions (Wang et al., 2021). However, interpretation is complicated by the co-variation of several structural variables, including DP, monomer composition, PD/PC ratio, and lipophilic content. In addition, analytical fractionation inherently enriches certain molecular-size classes, and the limited number of observations restricts detailed statistical analysis. Therefore, the correlations observed here should be interpreted as associative rather than causal, in line with recent reviews emphasizing the methodological and biosynthetic complexity of CT systems (Lu,

2024; Luca et al., 2020).

4. Conclusions

This study demonstrates the technical feasibility of producing polyphenol-rich fractions from industrial Norway spruce bark using pilot-scale pressurized hot water extraction followed by XAD7HP resin purification. Across nine winter- and summer-harvested batches, extraction yields were strongly governed by extraction temperature and feedstock variability, while resin treatment consistently enriched condensed tannins and reduced carbohydrate contamination to low levels. Feedstock characteristics had a clear influence on extraction outcomes. Winter bark showed more uniform particle-size distribution, lower wood contamination, and higher compositional consistency, whereas summer bark exhibited greater heterogeneity. This variability was reflected in extraction performance, particularly at higher temperatures, and in the composition of the purified extracts. Purification with XAD7HP effectively concentrated tannins and reduced non-tannin components, producing fractions dominated by condensed tannins, stilbenoids, and other phenolic compounds. Resin treatment also normalized carbohydrate levels across summer fractions and generated reproducible chemical profiles for most batches. Seasonal differences were retained in the fine-scale composition: winter extracts primarily contained lower-DP tannins, whereas summer extracts showed higher DP and higher proportions of prodelphinidin units.

Advanced chemical analyses confirmed the structural complexity and phenolic richness of the purified extracts. Pyrolysis–GC/MS revealed mixtures dominated by tannin-derived and non-lignin aromatic units, with modest lignin contributions and clear seasonal signatures. Detailed extractives profiling demonstrated stilbenes as the major non-tannin phenolics. The purified fractions exhibited strong antibacterial activity, with distinct structure–activity relationships for Gram-negative and Gram-positive bacteria. *E. coli* inhibition correlated with lipophilic and membrane-active constituents such as resin acids, terpenoids, steroids, fatty acids, and flavonoids. In contrast, *S. aureus* inhibition correlated positively with phenolic intensity and structure, including prodelphinidin content, tannin degree of polymerization, stilbene concentration, and UV-absorbing phenolics. These patterns align with the differing cell-envelope architectures of Gram-negative and Gram-positive bacteria.

Overall, the results show that pilot-scale extraction and purification of spruce bark can produce chemically consistent, bioactive polyphenol fractions even from heterogeneous industrial feedstocks. The tannin- and stilbene-rich extracts exhibit promising antibacterial properties, supporting their potential applicability in biobased industrial products, including cosmetics, feed additives, and water-treatment formulations. Future work should focus on techno-economical assessment, process optimization considering extraction severity, resin loading, and elution strategies as well as long-term reproducibility, and scaling toward continuous or semi-continuous operation to further advance the industrial readiness of spruce bark valorization.

CRediT authorship contribution statement

Petri Kilpeläinen: Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Investigation, Funding acquisition, Conceptualization. **Jenni Tienaho:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Eelis Halmemies:** Validation, Methodology, Investigation, Formal analysis. **Hanna Brännström:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Data curation, Conceptualization. **Risto Korpinen:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition. **Jarkko Hellström:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis. **Paula Jylhä:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Data curation.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2026.123988.

Data availability

Data will be made available on request.

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