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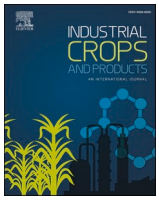

Chemical and bioactivity profiles of needle-rich Norway spruce logging residue fractions

Jenni Tienaho [a,*], Marina Fidelis [a], Anni Perämäki [b], Leif Hed [c], Hanna Brännström [d], Magnus Rudolfsson [e], Jaana Liimatainen [a], Marjo Haapakoski [b], Varpu Marjomäki [b], Risto Korpinen [a], Tuula Jyske [a,1], Magnus Matisons [f], Petri Kilpeläinen [a]

[a] Production Systems, Natural Resources Institute Finland (Luke), Latokartanonkaari 9, Helsinki FI-00790, Finland
[b] Department of Biological and Environmental Sciences and Nanoscience Center, University of Jyväskylä, Jyväskylä FI-40014, Finland
[c] Centria University of Applied Sciences, Talonpojankatu 2, Kokkola FI-67100, Finland
[d] Production Systems, Natural Resources Institute Finland (Luke), Teknologiakatu 7, Kokkola FI-67100, Finland
[e] Swedish University of Agricultural Sciences, Unit of Biomass Technology and Chemistry, Umeå SE-90183, Sweden
[f] BioFuel Region AB, Storgatan 35, Umeå SE-90325, Sweden

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ABSTRACT

Norway spruce logging residues, an abundant byproduct of timber harvesting for wood products, pulp, and paper industries, are primarily composed of wood, needles, and bark. The latter two are rich in phenolic compounds, known for their antioxidant and antimicrobial properties. However, variations in compound profiles and bioactivities can arise due to differences in plant organ composition, seasonal changes, and processing conditions. In this study, the aim was to evaluate the differences in fractions obtained across different seasons and through three processing/assortment methods. The effectiveness of two industrial assortment procedures was compared with that of hand-picked pure fractions of needles, branch bark, and branch wood, and the volatile organic compound (VOC) profile of pure fractions was analyzed. As expected, the hand-picked needle fraction exhibited high antioxidant and antibacterial properties, and these activities correlated positively with the amount of stilbenes and flavonoids in the extracts. Additionally, an assortment processed without a drying cyclone preserved the freshness of the fractions and produced needle-rich fractions with a bioactivity profile comparable to hand-picked needles. Due to seasonal differences and assortment efficiency, antiviral efficacy against enterovirus (coxsackievirus A9) was lower in both the first assortment and hand-picked fractions, both obtained in spring, while even low concentrations of fall assortment fractions showed high efficacy. VOC profiles of the hand-picked fractions revealed that needles contained the highest number and amount of compounds. The results highlight that mechanical separation enables efficient recovery of valuable needle fractions, offering valuable insights for the future utilization of spruce logging residues in biorefineries.

1. Introduction

Norway spruce (*Picea abies* [L.] Karst.) is an evergreen coniferous tree species widely distributed in Northern and Central Europe and well adapted to cold and temperate climates. Being one of the most important commercial timber species for wood products and pulp and paper, Norway spruce harvesting results in high volumes of felling side-streams also known as logging residues. The most productive forest sites for logging residue procurement in Finland and Sweden are Norway spruce-dominated, and logging residues are mainly recovered from the final felling of the sites (Routa et al., 2013). Logging residues include treetops, branches, needles/leaves, and unmerchantable stemwood, with a typical removal rate of 35–50 m^3 per hectare (Routa et al., 2013). According to web-based GIS service Biomass Atlas (Lehtonen et al., 2024), a maximum yearly amount of 48.5 × 10^5 m^3 of spruce logging residues can be sustainably gathered from all around Finland. There are however regional differences in the availability, and also the resulting transport costs need to be acknowledged to achieve economically feasible

* Corresponding author.
E-mail address: jenni.tienaho@luke.fi (J. Tienaho).
[1] Current address: Department of Forest Sciences, University of Helsinki, PO Box 27 (Latokartanonkaari 7), FI-00014, Helsinki, Finland

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procurement (Laitila et al., 2025). However, it is safe to say that logging residues represent an abundant biomass resource with potential for high-value applications in biorefineries.

When considering industrial feasibility and sustainability of procurement, it needs to be acknowledged that while the removal of stemwood does not pose a threat to the long-term productivity of forests, the removal of logging residues can be problematic on poor soils because most of the nitrogen is stored in the needles. Accordingly, Best Practices for Sustainable Forest Management in Finland instruct that at least 30 % of the logging residues should be left on-site to help preserve the nutrient balance (Best Practices for Sustainable Forest Management, 2023). Logging residues should also only be removed from relatively fertile spruce-dominated forests, while the removal is not recommended at all in pine-dominated forests on nutrient-poor forest land. Additionally, to prevent a negative impact on soil fertility in nutrient-poor stands, it is recommended to leave logging residues in the forest during one season to dry and to drop as much as possible of the nutrient-rich needles.

When logging residues are left to dry in small piles on the logging site, substantial portions of needles, along with some bark and thin branches, are indeed lost. Simultaneously, proportional wood content increases, moisture content decreases and the amount of recoverable material can decrease as much as 20–30 %, mainly due to loss of needles. The supply chain in Finland and Sweden is most commonly based on a roadside comminution of logging residues (Routa et al., 2013), which is also the most common practice all over Scandinavia and Central Europe (Moskalik and Gendek, 2019). When planning supply chains for biorefineries using needles as feedstock, the significant loss of needle biomass during the drying of logging residues and the potential reduction in extraction yield and microbial quality due to storage should be considered. In addition, feasible industrial-scale processing methods are yet scarce, further limiting the use of fresh needle biomass in biorefineries.

Spruce needles and bark are known to be rich in phenolic compounds such as stilbenes, flavonoids, simple phenolic compounds, and tannins (Gabaston et al., 2017; Jyske et al., 2023; Krogell et al., 2012; Nybakken et al., 2018; Slimestad and Hostettmann, 1996). There is a complex interplay of age, species, and environmental factors in shaping the chemical composition and qualitative characteristics in different organs of conifers, namely foliage, branch wood, and bark, due to their adaptive strategies and ecophysiological roles in living tree stems (Hammerbacher et al., 2020; Jaakola and Hohtola, 2010; Taiz and Zeiger, 2010). The biosynthesis of many secondary metabolites is influenced by gene-environment interactions that include the impacts of seed origin, growing site, light, temperature, and photoperiod (Jaakola and Hohtola, 2010; Virjamo and Julkunen-Tiitto, 2016; Yan et al., 2020). Furthermore, the age of the tissue, the location within the tree stem, and potential defense reactions caused by abiotic/biotic factors also affect the primary and secondary metabolite content and composition (Ahlawat et al., 2023; Netherer et al., 2024), as well as the freshness, handling, and storage within the supply chain of raw materials (Jyske et al., 2024, 2020c, 2020b, 2020a; Zommere et al., 2024).

Foliage typically contains high levels of nitrogen and phenolic compounds, which are essential for photosynthesis and defense against pests (Barton et al., 2019). Foliage generally has higher water content compared to wood and bark, which are more fibrous and drier, allowing foliage to maintain photosynthetic efficiency while also impacting the overall water balance in the tree. Wood is rich in carbohydrates and lignin, providing structural support, while bark has a higher concentration of secondary metabolites (Wenig et al., 2021). Additionally, the bark of Norway spruce has a complex structure with a significant amount of phenolics, such as tannins and stilbene glucosides, offering protection against environmental stressors (Axelsson et al., 2020; Kemppainen, 2015).

The organ physiological age also plays an important role, i.e., the characteristics of mature and immature needles, wood, and bark differ in chemical composition and physical properties (Jyske et al., 2015). For example, immature needles have been demonstrated to show distinct biochemical profiles in comparison to mature needles (Virjamo and Julkunen-Tiitto, 2014). Notably, the immature needles of some species were shown to have a high concentration of secondary metabolite compounds, which decreased as the needles matured, while other compounds showed the opposite trend (Slimestad and Hostettmann, 1996; Virjamo and Julkunen-Tiitto, 2014). These seasonal trends highlight that environmental factors and physiological processes may influence the chemical composition of stem parts (Bag et al., 2021), warranting further investigation into property variability to optimally utilize them as harvesting residues in biorefinery concepts.

Plant-based phenolic compounds are known to exhibit antioxidant properties and potential health benefits. In a study by Kähkönen et al., a total of 92 extracts of Finnish plant materials were examined for their total phenolic content and antioxidant activity through autoxidation of methyl linoleate (Kähkönen et al., 1999). The results showed that spruce needle extract stood out with the highest phenolic content, and it had one of the highest antioxidant activities among the extracts. The antioxidant activity of needle extract was also high in ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) tests and was higher than that of corresponding spruce sprouts extracts (water and aqueous ethanol extracts) (Jyske et al., 2020b). Nisca et al. investigated the antioxidant activity of ultrasound-assisted and microwave-assisted extracts of spruce bark (Nisca et al., 2021). The extracts indicated relatively high antioxidant activities in free radical scavenging ability (2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) tests) and low minimum inhibitory concentrations (MIC) against gram-positive *S. aureus* bacteria, including methicillin-sensitive and resistant strains, highlighting their potential as natural antibacterial agents. In previous studies, pressurized hot water extracts from Norway spruce bark contained high phenolic content and showed excellent antiviral activity against stable non-enveloped enteroviruses, whereas hemicellulose-rich fractions were poor in antiviral efficacy (Granato et al., 2022). In addition, the results with berries rich in total phenolics as well as purified polyphenols showed direct and efficient inactivation of tested enteroviruses, further demonstrating that phenolic compounds are promising candidates for antiviral applications (Pap et al., 2021; Reshamwala et al., 2021).

In our previous study (Tienaho et al., 2024), we used response surface methodology to optimize fresh industrially assorted needle-rich fraction solvent extraction with water, aqueous ethanol, water with Na_2CO_3 and $NaHSO_3$ addition, and limonene. Multiple responses, such as phenolic and condensed tannin content as well as antibacterial and antioxidant activities, were used simultaneously, and the optimum extraction temperature and time were determined for each of the extraction solvents. Because it was shown that aqueous ethanol yielded the highest antioxidant and antibacterial phenol-rich products, it was chosen as the extraction solvent for the present study.

In this study, our specific aims were to investigate: 1) the differences between fresh fractions obtained during different seasons and with different processing/assortment methods; 2) the differences between industrially assorted and pure hand-picked fractions; and 3) the main differences in the volatile organic compounds (VOCs) and phenolic compounds of the pure fractions. Analysis was performed by screening the different fractions obtained by two industrial-scale assortment procedures (Spring 1–4 and Fall 1–4) for their antibacterial (gram-negative *Escherichia coli* and gram-positive *Staphylococcus aureus*), antiviral (non-enveloped CVA9 and lipid-enveloped OC43), and antioxidant (FRAP and ORAC) activities, as well as for their total phenolic content (Prussian blue method) and compound profiles by gas chromatography mass spectrometry (GC-MS). Furthermore, these were compared to the hand-picked fractions of needles, branch wood, and branch bark, all obtained during spring, for which we also present more comprehensive metabolite profiles using Headspace-GC-MS for the volatile compounds. The extracts of the pure fractions were also further analyzed using ultra-high

performance liquid chromatography mass spectrometry (UHPLC-MS) for phenolic compounds.

2. Materials and methods

2.1. Collection and assortment of raw materials

In both the spring and fall material batches, branches from felling residues taken from a 70-year-old stand in Håknäs, Sweden (63°54'0"N, 19°74'1"E) were used. The branches used in hand-sorting were taken at three different heights and in four directions to represent the entire tree as well as possible. The separation of the needles from the harvested branches was done immediately after the felling. Felling and sorting were carried out on two occasions, spring (May 2021) and fall (September 2021). The fractions were frozen immediately after separation, and the separation work was carried out at the Biomass Technology Center (BTC), Swedish University of Agricultural Sciences (SLU), Umeå, Sweden. Because the industrially assorted fractions likely contained varying amounts of branch organ tissues, reference fractions consisting of needles, branch bark, and branch wood were manually sorted from the spring-harvested material (Fig. 1).

Spring procedure: To enable separation of the green needles from the rest of the branch material, the branches were chipped using a chipper (Edsbyhuggen, Woxnadalens Energi AB, Sweden). This material was then be fed into a cyclone (Mäkelä et al., 2016), where the needles were further separated from the small branch parts. This allowed for mechanically screening (Fredrik Mogensen AB, Mogensen Sizer E0554) of the material.

Fall procedure: For the separation of the autumn samples, a new two-stage method was used, consisting of a grinding stage (Das et al., 2021) and subsequent mechanical sieving. The mill comprised shafts with saw blades packed against each other without gaps. The branch material was fed perpendicular to the shaft and sawn into a powder. The mill lacks a sieve, which enables grinding of fresh materials with a high moisture content. Conventional mills (e.g., hammer mills) with built-in sieves that regulate the particle size of the ground material are typically not able to grind fresh, green materials. The degree of grinding was regulated in this mill by changing the blade speed or feed speed. This means that with the correct setting, the woody part of the branch was ground while the needles passed through the mill without being damaged and were separated from the other branch parts. The mechanical sieving was carried out in the same way as before, but with sieve sizes adapted to the ground input material.

2.2. Extraction and total dissolved solids (TDS)

The sorted logging residues and hand-picked samples were extracted without comminution using an accelerated solvent extractor (ASE-350, Dionex, USA). Batch extractions were carried out at 110 °C for 60 min using ethanol/water (90/10, v/v) as the extraction medium. Ethanol was obtained from Altia, Finland. The system operating pressure was 103.4 bar (1500 psi). The moisture content of the samples was determined by oven drying (105 °C until constant weight), and the sample amount was adjusted so that each extraction had 10 g of dry material in 100 mL extraction vessel. After extraction, samples were collected and stored in a freezer (-20 °C) until further analysis. Total dissolved solids (TDS) of the extract were determined by drying the extract in an oven at 105 °C, and after drying, samples were placed into a desiccator to cool down. After cooling down samples were weighed, and TDS was calculated.

2.3. Antioxidant analyses

In the **ferric reducing antioxidant power (FRAP)** methodology, the single-electron transfer (SET) mechanism is used to measure the antioxidant's ability to reduce the iron complex $[Fe(III)(TPTZ)_2]^{3+}$. The methodology is based on the capacity of antioxidants to reduce chelated metal ions (Fe^{3+}) and is slightly modified from the originally reported method (Benzie and Strain, 1996). If not otherwise mentioned, all chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich Chemie GmbH, Steinheim, Germany). As previously published, three technical replicates of 25 μL were added to a reaction mixture containing 20 mM $FeCl_3 \bullet 6\ H_2O$ and 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) in 300 mM acetate buffer (pH 3.6) in transparent, flat-bottom 96-well plates (Tienaho et al., 2021). The absorbance was measured at 593 nm with a microplate reader (Varioskan Flash, Thermo Scientific) to monitor the formation of the blue ferrous-tripyridyltriazine complex in the dark. A series of $FeSO_4 \bullet 7\ H_2O$ dilutions were used to draw a standard curve, against which all the results were compared, and the sample color effect was minimized by blank subtraction. The positive control, L (+)-ascorbic acid (150 μM and 800 μM) (VWR Chemicals), was also measured, and the results are expressed as μM Fe(II) equivalents per gram of extracted biomass dry weight (DW).

Oxygen radical absorbance capacity ($ORAC_{FL}$) methodology follows the hydrogen atom transfer (HAT) mechanism and is originally described by Huang, Prior, and co-authors (Huang et al., 2002; Prior et al., 2003). In this method, antioxidants prevent peroxyl radicals from damaging the fluorescent molecule fluorescein. Samples were measured in five dilutions and two technical replicates as previously reported (Tienaho et al., 2020). Additional dilutions were prepared when needed to fit the samples to the standard curve. The sample, in 75 mM phosphate buffer (PB), pH 7.5 (Merck), 0.0816 μM fluorescein, and the peroxyl radical generator 2,2'-azobis(2-methylpropionamidine) dihydrochloride were added to a black, opaque, flat-bottom 96-well plate. The fluorescence (emission 538 nm; excitation 485 nm, bandwidth 12 nm) was monitored with a microplate reader (Varioskan Flash, Thermo Scientific) every 2 min for 21 measurements, while maintaining the temperature at 37 °C. A brief shake was given to the plate prior to every measurement. Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), a vitamin E analogue, was used at various concentrations to draw the standard curve, against which all results were compared. Results are expressed as Trolox equivalents (TE) per

Fig. 1. Pure hand-picked reference fractions from the spring batch.

gram of extracted biomass (μM TE / g DW).

2.4. Total phenolic content (TPC)

The total phenolic content (TPC) of the samples was measured using the **Prussian blue assay** (Margraf et al., 2015). Similar to the FRAP method described above, this method is based on the ability of phenolic compounds to reduce chelated hexacyanoferrate(III) ions, forming a Prussian blue color. As previously described, the samples were pipetted in technical triplicates and at least three dilutions into a microplate containing 0.50 mM $FeCl_3$•6 H_2O diluted in 0.01 M HCl (Tienaho et al., 2024). An aliquot of 0.50 mM $K_3[Fe(CN)_6]$ solution was injected into the microplate to initiate the reaction, and the plate was briefly shaken before incubating in the dark. Absorbance was measured at 725 nm after 15 min using a microplate reader (Varioskan Flash, Thermo Scientific). The results are expressed as mg gallic acid equivalents (GAE) per gram of extracted biomass (mg GAE / g DW).

2.5. Antibacterial analyses

Two recombinant, bioluminescent indicator strains were used to determine the antibacterial properties of the extracts: *Escherichia coli* K12 +pcGLS11 (gram-negative) and *Staphylococcus aureus* RN4220 +pAT19 (gram-positive). These strains produce a continuous luminescent light signal as part of their normal metabolism, and a measurable decrease in the signal occurs when the strains are in contact with antibacterial substances (Vesterlund et al., 2004). As previously published, the strains were stored at –80 °C and revived by approximately 16 h of cultivation at 30 °C (*E. coli*) and 37 °C (*S. aureus*) on lysogeny agar plates (tryptone 10 g/L; yeast extract 5 g/L; NaCl 10 g/L; and agar 15 g/L) (Tienaho et al., 2021). Because of the pH sensitivity of the strain, the *E. coli* plates were supplemented with 10 % (v/v) sterilized phosphate buffer (PB) (1 M, pH 7.0) and 100 μg/mL of ampicillin to maintain selection pressure. Similarly, the *S. aureus* plates were supplemented with 5 μg/mL erythromycin. Stock solutions were prepared by inoculating a single colony of bacteria into lysogeny broth with the identical supplements as for the plates and cultivating for approximately 16 h at 300 rpm shaking at 30 °C (*E. coli*) and 37 °C (*S. aureus*). All samples were diluted in water to obtain four concentrations between 0.9 and 2.8 mg/mL per opaque, white polystyrene microplate well. Double-distilled water was used as the negative control, and the same concentration of ethanol as in the samples was also measured to witness any solvent effect. The samples and positive controls (8.75 and 17.5 vol % ethanol) and negative controls were pipetted in triplicates into the same plate, and measurement was initiated by adding an equal volume of the bacterial inoculum. The luminescent light signal (wavelength range 360–670 nm) was monitored using a microplate reader (Varioskan Flash, Thermo Scientific) once every 5 min for 60 min at room temperature, with brief shakes before every measurement. Results are expressed as inhibition percentages (inhibition%) at 1 mg/mL (DW of extracted biomass) after 50 min of incubation to ensure luminescent signal stabilization (Tienaho et al., 2015). Inhibition percentages were calculated as follows,

$$\text{inhibition\%} = (1\text{-}RLU_{sample}/RLU_{neg\_control}) \times 100\%,$$

where RLU refers to relative light units obtained from the microplate reader.

2.6. Antiviral analyses

The antiviral properties of the samples were tested against two viruses: human coronavirus HCoV-OC43 (ATCC®VR-1558™, 7.43 $\times 10^7$ plaque forming unit (PFU)/mL) and human enterovirus coxsackievirus A9 (CVA9, ATCC®VR-186™, 1.94 $\times 10^7$ PFU/mL) as previously described (Haapakoski et al., 2023; Reshamwala et al., 2025). After incubating HCoV-OC43 or CVA9 with the test samples for 1 h at 37 °C (enteroviruses) or at 34 °C (coronaviruses), the remaining viral infectivity was assessed on MRC-5 cells (ATCC®CCL-171™) or A549 cells (ATCC®CCL-185™), respectively. MRC-5 cells were cultured in Minimum Essential Medium (MEM) and A549 cells in Dulbecco's Modified Eagle Medium (DMEM), supplemented with 10 % fetal bovine serum (FBS), 1 % penicillin-streptomycin, and 1 % GlutaMAX, under 5 % CO_2. For the experiments, cells were plated in 96-well flat-bottom microtiter plates at a density of 15,000 (MRC-5) or 12,000 (A549) cells per well on the previous day. The 1 % and 10 % (v/v) extract concentrations were diluted in phosphate-buffered saline with magnesium chloride (PBS-$MgCl_2$ buffer). After incubating viruses with test solutions, the samples were diluted with growth medium to prevent cytotoxic effects on the cells. Diluted test samples were then added to the cells for 2 days (enteroviruses) or 5 days (coronaviruses). Cells without viruses or samples served as cell controls, and viruses incubated only with PBS-$MgCl_2$ served as virus controls. Once the cytopathic effect (CPE) was observed, the staining protocol was carried out. Cells were washed twice with PBS, then fixed and stained with crystal violet as described (Haapakoski et al., 2023). The absorbance of the samples at 570 nm was measured spectrophotometrically with VICTOR™ X4 multilabel reader (Perkin Elmer). Absorbance data from the CPE experiments were analyzed using GraphPad Prism 6 program. The results of three replicates were plotted as an average bar chart with standard error of the mean.

2.7. Characterization with gas chromatography

Compounds in the extracts were determined with gas chromatography–mass spectrometry (GC–MS). Before GC analysis, aliquots of the extracts were dried by heating under a nitrogen flow and further dried in a vacuum oven for 15 min. Samples were silylated by adding 150 μl of pyridine, 100 μl of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA), and 50 μl of chlorotrimethylsilane (TMCS). Heneicosanoic acid (C21:0) and betulinol were used as internal standards. The silylated samples were analyzed using a GC-MS (HP6890–5973, Hewlett Packard, Palo Alto, CA, USA) equipped with a Zebron, ZB-semivolatiles column (30 m × 0.25 mm i.d., film thickness 0.25 μm) (Fidelis et al., 2023). Helium was used as the carried gas, and injector and MS interface temperatures were 280 °C and 300 °C, respectively, during the analysis. A split injection ratio of 1:15 was used. Mass spectra were obtained in electron ionization (70 eV) mode. Compounds were identified by comparing fragmentation patterns with commercial NIST14 and Wiley11 libraries, as well as the laboratory's own MS libraries.

2.8. UHPLC-Orbitrap-MS analysis for the extracts of the hand-picked fractions

The chemical composition of pure, hand-picked fractions (spring 5–7) was screened using ultra-high-performance liquid chromatography–mass spectrometry (UHPLC–MS). Extracts were diluted and filtered (0.2 μm) prior to analysis. The UHPLC system consisted of a Thermo Fisher Vanquish UHPLC device equipped with a diode array detector (model VF-D11-A) and a Hypersil Gold Vanquish C18 column (Thermo Fisher Scientific Inc., Waltham, MA, USA; 150 × 2.1 mm i.d., 1.9 μm). The autosampler temperature was set at 15 °C and the column temperature at 40 °C. The binary mobile phase consisted of 0.1 % formic acid in water (A) and 0.1 % formic acid in acetonitrile (B). Elution started at 2 % B, followed by a linear gradient to 95 % B in 15 min, held isocratically at 95 % B for 7 min, then a linear gradient back to 2 % B in 0.5 min, and held isocratically at 2 % B for 5.5 min. The flow rate was 0.35 mL/min and the injection volume was 1 μL. The wavelength range was 190–800 nm and a bandwidth of 4 nm. Full-scan mass spectra were recorded using electrospray ionization in both positive and negative polarity, with spray voltages of +3.5 kV and −3.0 kV, at a scan range of m/z 80–1000, on an Orbitrap Exploris 240 mass spectrometer (Thermo

Fisher Scientific Inc., Waltham, MA, USA). Sheath, aux, and sweep gas (N_2) flow rates were set at 50 L/min, 10 L/min, and 1 L/min, respectively. The vaporizer temperature was 250 °C and the ion transfer tube temperature was 280 °C. The resolution was set at 120,000. For data dependent MS^2 spectra, the five most intense signals at each time point were selected using dynamic exclusion; that is, parent ions (MS) with the same *m/z* values (within 2.5 ppm mass tolerance) were fragmented only once (MS^2) during a 3 s scanning window. Stepped HCD collision energies of 20, 40, and 80 % were applied, and the resolution was set at 60,000 for MS^2. The data were processed with Thermo Scientific FreeStyle 1.8 SP2 QF1 (version 1.8.65.0) software.

2.9. Statistical analysis

The experimental data were expressed as means ± standard deviation. The Shapiro–Wilk test was used to assess the data normality and the Brown-Forsythe test to assess the homogeneity of variance. One-way analysis of variance (ANOVA) and the Tukey's post hoc test were used to compare mean values. Differences reaching a confidence level of 95 % ($p < 0.05$) were considered statistically significant. To establish correlations between chemical composition and bioactivities, bivariate Pearson correlation analysis was performed using a two-tailed test. Statistical analyses were conducted using TIBCO Statistica v.13.3 and Sigmaplot v.14.0.

2.10. HS-GC–MS analysis of hand-picked biomass fractions

The original samples were placed in headspace vials (Agilent 7697 A headspace sampler), and released volatiles were analyzed by GC–MS (Agilent 8890 GC with Agilent 5977B GC/MSD). Compounds were separated on a capillary column (Agilent Technologies HP-5MS UI; 30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium at 1 mL/min was used as the carrier gas. The injector temperature was 270 °C, with a split injection ratio of 20:1, and the transfer line temperature to the MS was 250 °C. Initial oven temperature was 40 °C for 4 min, then increased at 15 °C/min to 280 °C and held for 1 min. Compounds were identified using Agilent Masshunter Unknown Analysis software with the NIST17 mass spectral library and by comparison with literature data.

3. Results and discussion

3.1. Industrial assortment

The resulting assorted logging residue fractions are presented in Fig. 2. Both spring and fall procedures resulted in four fractions with differing particle sizes and proportions of green needles. Visually fall fractions seem to contain larger proportion of green needles than spring fractions. The particle size distribution for the spring and fall procedures can be found in the Supplements (Supplementary Table S1 and Supplementary Table S2, respectively). Efficient industrial-scale screening and handling of the logging residue biomass is important because of the differences between the plant tissues. Bark and especially needles can be problematic during combustion or for many biorefining processes, and separation of the needles and/or bark for the extraction of high value chemicals can improve the quality of the remaining fraction that can be used by other biorefining processes (bio coal, biofuels, etc.). Lestander et al. proposed that assortment can improve fuel production because pure fractions with different ash content and chemical composition are more resource-efficient to gasify separately (Lestander et al., 2022). This proves that pure components are better for extraction and handling as well as for energy use and highlights that industrial assortment is important for achieving the economic viability and resource efficiency for the future biorefineries. Currently, logging residues are also used to replace fossil-fuels and provide renewable energy (Anttila et al., 2018). This logging residue-based energy could also be utilized to heat up the extraction equipment after removing the valuable extractables.

3.2. Extraction yield and TDS

The lowest extraction yield (24.36 mg/g) was obtained from a hand-picked wood sample. The yield (171.15 mg/g) of the hand-picked branch bark sample was higher than that of the wood sample but

Fig. 2. Assorted fresh logging residue fractions: Spring 1–4 and Fall 1–4.

lower than all other assorted samples and the pure needle fraction (Table 1). The yields of the needle-rich fraction were generally higher than those of the other fractions. Spring sample yields ranged from 184 to 248 mg/g and fall sample yields from 211 to 253 mg/g. Slight differences in yields could be due to different pretreatment methods with varying separation efficiencies. Spring samples were passed through a cyclone and were drier than the fall samples, which were merely milled and assorted (Supplementary tables S1 and S2). Visually, spring fractions 1 and 4 contained wood and bark contamination (Fig. 2), which also affected extraction yield (Table 1). In the case of all fall fractions and spring fractions 2 and 3, the yields were comparable to those of hand-sorted needles.

3.3. Total phenols and antioxidant activity

The total phenolic content (TPC) of the extracts was measured using the Prussian blue assay. The assay is rapid and simple and has been found to correlate closely with the widely used Folin-Ciocalteu method. However, the Prussian blue method typically yields slightly lower activity values, likely due to its higher selectivity (Margraf et al., 2015; Pap et al., 2021). Results are shown in Fig. 3.

The highest total phenolic content was observed in Fall fraction 1, which had the largest particle size and lowest moisture content of the fall fractions (Supplementary Table S2). Among the hand-picked fractions, needles exhibited the highest activity. For the assorted spring fractions, TPC ranged from 10.0 to 16.2 mg GAE/g, whereas for the fall fractions, the range was 12.2–19.9 mg GAE/g. The lower TPC range in the spring fractions may be attributed to the use of an additional drying cyclone during processing. The result obtained for the pure needle fraction aligns well with the optimized theoretical expectations previously reported (Tienaho et al., 2024). The Prussian blue method has also been applied to pressurized hot-water extracts of *Picea abies* stem bark (Pap et al., 2021) and the branch bark activity observed in this study was slightly lower. Given the use of aqueous ethanol, the activity would have been expected to be higher than in the previous study, suggesting that using branch bark instead of stem bark influences the activity. However, in biological matrices, numerous factors can affect the chemical composition and biological activity. Therefore, making highly detailed comparisons with other literature sources often speculative.

In addition to total phenols, antioxidant activity was also measured for the extracts, and the results are presented in Fig. 4. Two methodologies with differing mechanisms were used: FRAP and ORAC. Using the FRAP method, the response of the hand-picked needles was superior to the others, with a signal of 599 μM Fe(II) eq./g (Fig. 4**A**). The assorted spring fractions yielded FRAP values in the range of 338–527 μM Fe(II) eq./g, and fall fractions 372–499 μM Fe(II) eq./g. Except for the wood fraction, all fractions gave values within a similar range. The FRAP value reported in the literature under the same extraction conditions was 412 μM Fe(II)/g (Tienaho et al., 2024), indicating that the values obtained in this study are within the same range and even higher than previously reported. FRAP results were found to be sensitive to changes in sample heterogeneity and assortment in our previous study (Tienaho et al., 2024), which can explain the differences. Previously, values up to 800 μM Fe(II) eq. have been reported from freshly frozen, pure needle biomass extracted with water and aqueous ethanol (70:30, vol%) from young trees (Jyske et al., 2020b). For the ORAC method (Fig. 4**B**), the two highest results were obtained with hand-picked needles and the Fall 1 fraction: 3141 and 3204 μM TE/g, respectively. Overall, the assorted spring fractions yielded values in the range of 2006–2852 μM TE/g and fall fractions 2472–3204 μM TE/g. The previously published expected ORAC value of 2664 μM TE/g falls within the range of both spring and fall fractions (Tienaho et al., 2024) and is supported by earlier results (Jyske et al., 2020b). However, as with total phenolic content, it should be acknowledged that several factors can affect the observed bioactivities, and overly detailed comparisons to earlier literature may be misleading.

3.4. Antibacterial and antiviral analyses

The antibacterial activity of the fractions was evaluated against gram-negative and gram-positive bacterial strains, *E. coli* and *S. aureus*. Both strains pose clinical and epidemiological challenges as pathogens and are therefore excellent model organisms for assessing the antibacterial efficacy (Abban et al., 2023). The results are presented in Fig. 5. In general, higher efficacy was observed against the gram-positive *S. aureus*. This is not unexcepted and is likely due to the absence of

Table 1
Total dissolved solids (TDS) and extraction yield of the original dry sample. Pure, hand-picked spring fractions (needles, branch wood, and branch bark) were used as controls and are highlighted with green.

Sample	TDS (wt%)	Yield (mg/g)
Spring 1	2.75	199.32
Spring 2	3.42	244.46
Spring 3	3.55	247.74
Spring 4	2.54	183.93
Needles	3.25	245.10
Branch wood	0.32	24.36
Branch bark	2.36	171.15
Fall 1	3.66	248.71
Fall 2	3.07	211.38
Fall 3	3.58	252.80
Fall 4	3.38	238.40

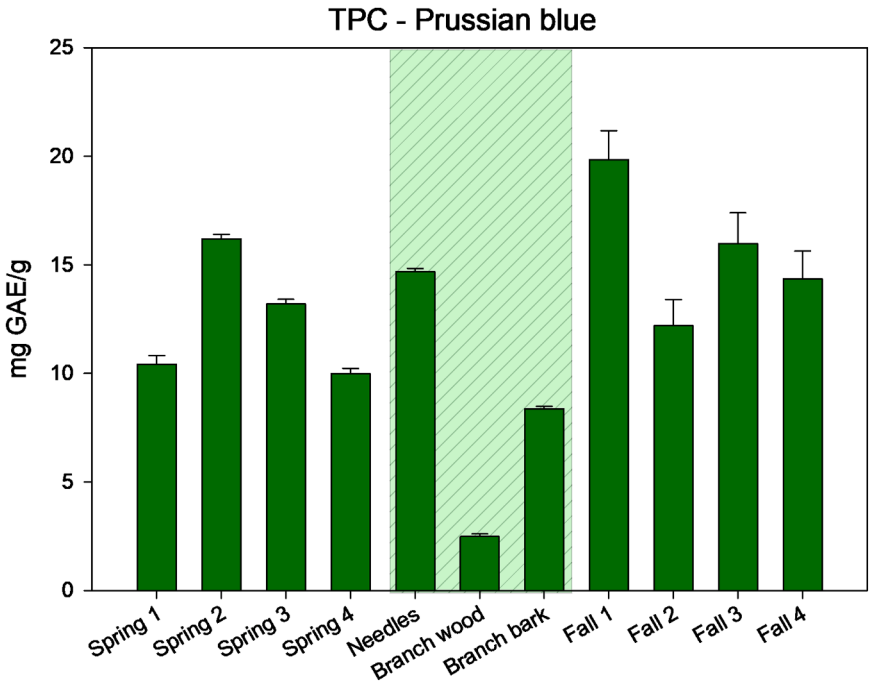

Fig. 3. The total phenolic content (TPC) determined using the Prussian blue method in aqueous ethanol extracts of logging residues fractions. Results are shown as means of sample replicates ± standard deviations (n = 3). Pure, hand-picked spring fractions are indicated by a line pattern in the background.

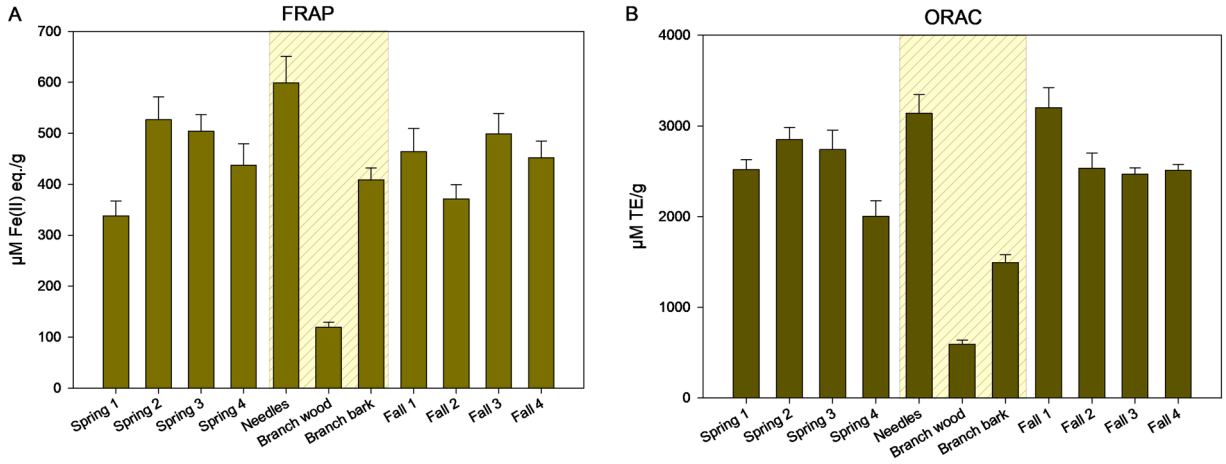

Fig. 4. The antioxidant content in aqueous ethanol extracts of logging residues fractions, determined by ferric reducing antioxidant power (FRAP) methodology (A) and oxygen radical absorbance capacity (ORAC) assay (B). Results are shown as means of sample replicates ± standard deviations (FRAP: n = 5; ORAC: n = 4). Pure, hand-picked spring fractions are indicated by a line pattern in the background.

the protective lipopolysaccharide layer in the cell wall of the gram-positive bacteria (Kunnas et al., 2024). For both strains, the pure hand-picked needle fraction displayed high efficacy, 33 % and 60 % inhibition against *E. coli* and *S. aureus*, respectively. For the assorted spring fractions, the inhibition ranged from 3.8 to 19.8 % against *E. coli* and 0–51.3 % against *S. aureus*, whereas for the fall fractions the ranges were 13.6–25.0 % and 11.2–58.7 %, respectively. This indicates that the fresher fall fractions obtained without the drying cyclone exhibited elevated activity against both strains. Interestingly, the pure hand-picked branch bark fraction showed no activity against *S. aureus*, which, in light of previous results for spruce bark, is surprising. This is likely due to the use of branch bark instead of stem bark in the present study. Indeed, studies have indicated that the yield and composition of softwood bark extractives vary longitudinally within the stem. Silver fir stem bark contained higher total phenolic content than branch bark, and the base of the branch appeared richer in extractives than samples collected farther from the stem (Vek et al., 2022). Brennan and co-authors investigated bark phenolic extractives from three softwood species, including *Picea abies*, and found that while flavonoid content tends to increase with height, compounds such as epi-gallocatechin gallate were more abundant in bark at the base of the stem (Brennan et al., 2020). Thus, as the compound profile in branch bark differs from that of stem bark, it is unsurprisising that biological activities also differ. Fall fraction 4 was the assorted fraction that yielded a result very similar to that of pure needles. The particle size of fall fraction 4 was the smallest among the fall fractions, increasing the contact surface area of the biomass, which may have influenced extraction efficiency.

The cytotoxicity of the spring and fall samples was evaluated to confirm that concentrations used did not mask the antiviral efficacy tested in human cell lines. The concentrations were not toxic to cells after incubation for 2 days (A549 cells) or 5 days (MRC-5 cells) in cell culture (Fig. 6).

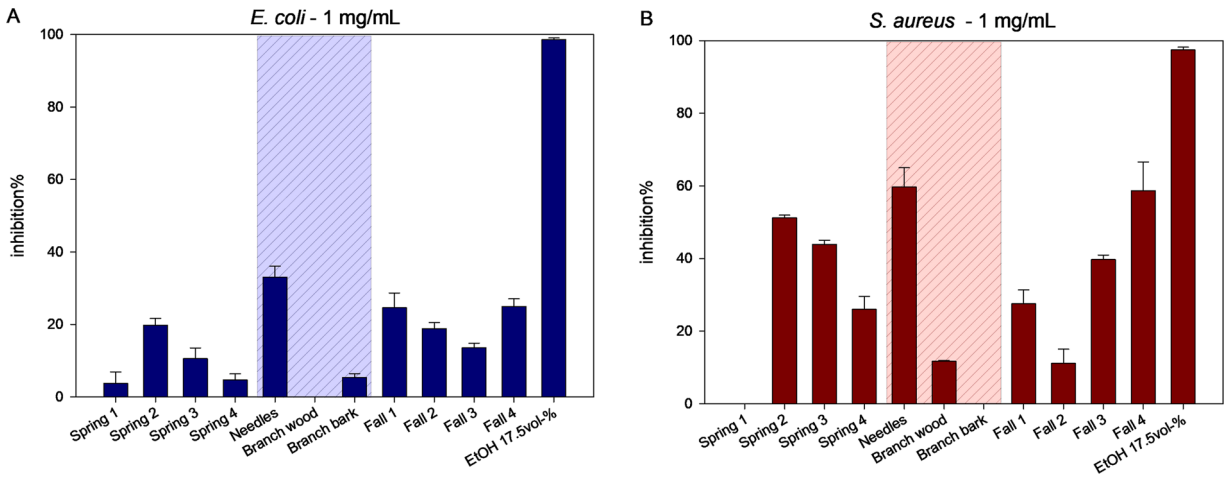

Fig. 5. The antibacterial activity of aqueous ethanol extracts from logging residues fractions and a 17.5 vol% ethanol control, tested against gram-negative *E. coli* (A) and gram-positive *S. aureus* (B) indicator strains. Results are shown as means of sample replicates ± standard deviations (n = 3). Pure hand-picked fractions are indicated by a line pattern in the background.

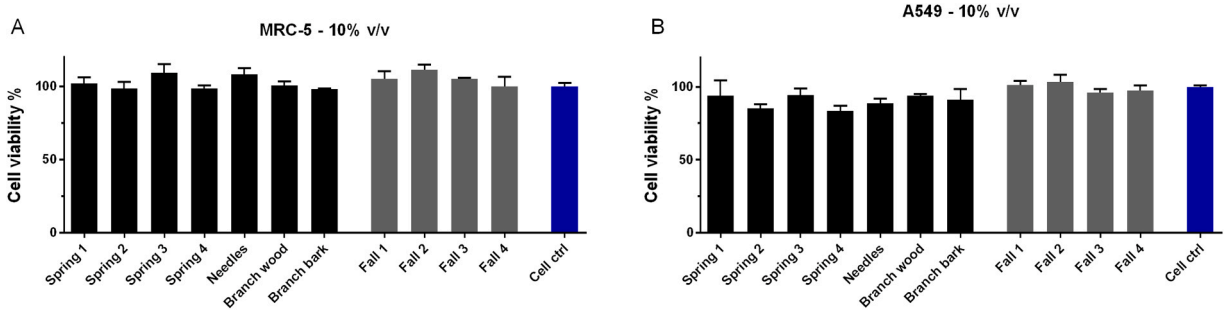

Fig. 6. No cytotoxicity was detected at a 10 % v/v concentration of extracts in tests with MRC-5 cells (A) or A549 cells (B). Results are shown as means of sample replicates ± standard error of the mean (n = 3).

The samples were tested at two concentrations, 10 % and 1 % (v/v), in direct contact with non-enveloped enteroviruses, Coxsackievirus A9 (CVA9), and a member of the beta-coronaviruses, the seasonal coronavirus HCoV-OC43. The samples were incubated with the viruses for 1 h

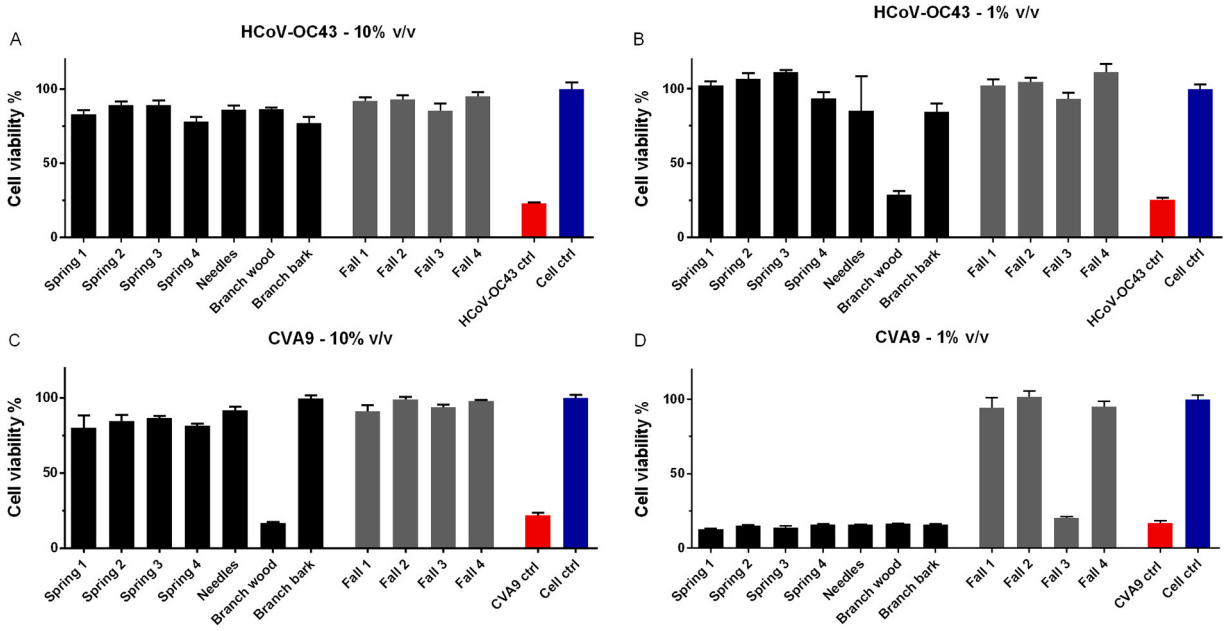

Fig. 7. Antiviral activity of 10 % (v/v) and 1 % (v/v) solutions tested against human coronavirus HCoV-OC43 (A, B) and human enterovirus coxsackievirus A9 (C, D). Results are shown as means of sample replicates ± standard error of mean (n = 3).

and then diluted in cell culture to assess direct effects on the virions. The results with the 10 % solution clearly show that all samples eliminated coronavirus infectivity (Fig. 7**A**). When testing the more diluted 1 % solution (Fig. 7B), the hand-picked branch wood showed lower efficacy. The same branch wood sample showed no efficacy against enteroviruses even at 10 %, further confirming that it is low in antivirally active components (Fig. 7C). The 1 % solution revealed that all spring samples had lower antiviral efficacy compared to fall samples (Fig. 7D), likely due to the use of a drying cyclone in the spring fraction assortment. In addition, among fall samples, sample 3 also showed no efficacy at 1 %. Interestingly, the low efficacy also applied to the hand-picked fractions. Seasonal differences may explain the variation between spring and fall fractions. For example, extractable terpene content has been found to accumulate from summer to fall (Večeřová et al., 2021) and monoterpenes and sesquiterpenes have been reported as active against multiple viruses (Cox-Georgian et al., 2019). This assumption could have been verified by including hand-picked samples from the fall fraction as a reference. However, the industrially assorted fall fractions demonstrated high antiviral efficacy. Therefore, it can be concluded that realistic industrial practices can be employed to obtain broadly acting antimicrobial products, which can be recovered regardless of seasonal changes or processing steps.

3.5. *Chemical analysis of extracts and correlation with bioactivity*

Correlations between the individual tests can be found in the Supplementary file (Supplementary Table S3) and the total phenolic content correlated well with all other activities, indicating that phenolic compounds are likely responsible for these activities. To further support this, additional chemical analysis was performed. Classes of compounds identified from the extracts by GC-MS in this study, fatty acids, diterpenoids/resin acids, lignans, stilbenoids, flavonoids, and sterols, are shown in Fig. 8. More detailed chemical characterization information can be found in Supplementary Table S4 and Supplementary Fig. S1. The identified compounds were typical metabolites of Norway spruce (Hammerbacher et al., 2020). Among the compounds with the highest total contents were dehydroabietic acid, catechin, isorhapontin, isopimaric acid, and abietic acid. The highest total content was found in fall fraction 1 (11.6 mg/g, average of two runs), which had the largest particle size and lowest moisture content among the fall fractions. Unlike the other fall factions, this fraction contained mostly branches and branch wood and consequently more lignans. For pure fractions, the hand-picked needles yielded a similar content compared to the hand-picked branch bark. The total content of the identified compounds in the spring fractions ranged from 6.6 to 9.6 mg/g, while in the fall fractions, it ranged from 6.8 to 11.6 mg/g. Differences in compound content and composition could be attributed to the nature of each fraction and the different pretreatment methods (i.e., assortment, drying with a cyclone, milling).

Pearson's correlations between compounds and TDS, phenolic content (TPC), antioxidant, and antibacterial activities are presented in Table 2. Since all extracts showed strong antiviral activity at certain concentrations, and the results were either fully active or completely inactive, the antiviral data were excluded from statistical analysis, which requires a normally distributed dataset. The compounds contributing most strongly to the observed positive correlations included isorhapontin, catechin, β-sitosterol, piceatannol, isorhapontigenin, trans-piceid, and (icosanoic) acid 20:0. Interestingly, the lignans hydroxymatairesinol isomer 1, hydroxymatairesinol isomer 2, and matairesinol negatively correlated with TDS, FRAP, ORAC, and *E. coli*. The consistently high correlation coefficients suggest that isorhapontin exhibits strong antioxidant activity and inhibition against *E. coli* and *S. aureus*. Stilbenes, such as isorhapontin, piceid, piceatannol, and isorhapontigenin, are known to act as phytoalexins, meaning they are biosynthesized in plants in response to biotic and abiotic stress (Jeandet et al., 2010). Therefore, it is unsurprising that these compounds have also been found to be highly antioxidant and antimicrobial in numerous previous studies (Albert et al., 2011; Biais et al., 2017; Chalal et al., 2014; Jeandet et al., 2010; Plumed-Ferrer et al., 2013; Rodríguez-Bonilla et al., 2017; Stojanović and Brede, 2002; Tran et al., 2023; Valletta et al., 2021). Flavonoid biosynthesis is enhanced in plants under oxidative stress, and flavonoids such as catechin represent another compound class with well-known bioactivities (Kumar and Pandey, 2013). The negative correlation between lignans and

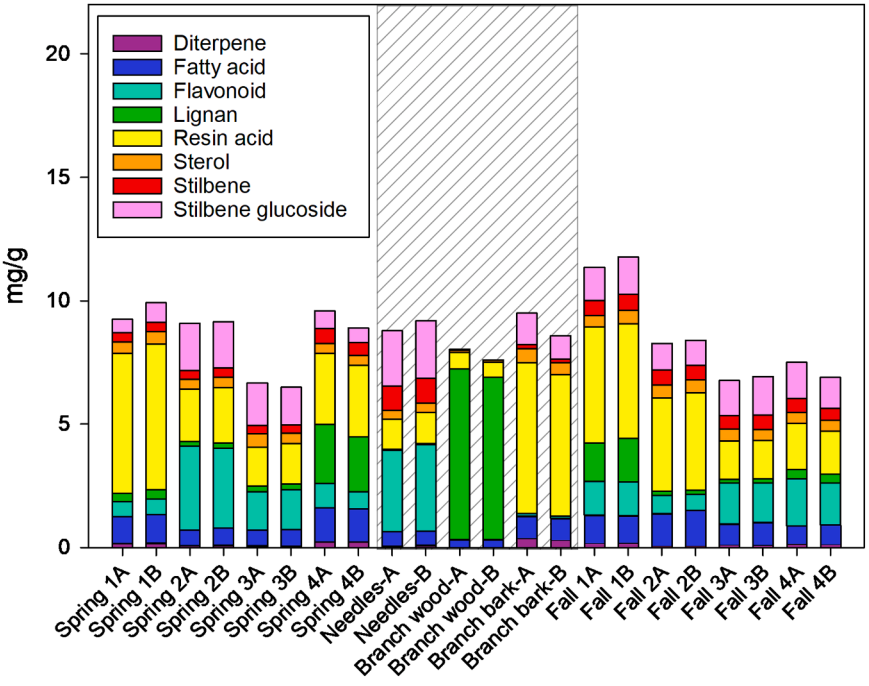

Fig. 8. Extractable compound classes identified by GC-MS, expressed as mg/g per dry original biomass for each fraction. All fractions were analyzed in duplicate (A and B). Pure, hand-picked fractions are indicated by a line pattern in the background.

Table 2
Pearson correlation coefficients between compounds and bioactivity assays in logging residue extracts. TDS= total dissolved solids; TPC = total phenolic content (Prussian blue method); FRAP= ferric reducing antioxidant power; ORAC=oxygen radical absorbance capacity.

Compound	TDS	TPC	FRAP	ORAC	*E. coli*	*S. aureus*
Cembrene	0.141	0.043	0.145	-0.050	0.025	-0.525
Acid 16:0	0.446	0.406	0.241	0.465	0.284	-0.043
Acid 18:2	0.224	0.254	-0.019	0.286	0.209	-0.175
Acid 18:1	0.234	0.164	0.028	0.238	0.133	-0.246
Stearic acid	0.040	-0.094	-0.018	-0.049	-0.202	**-0.629***
Pimaric acid	0.101	0.036	-0.149	0.154	-0.077	**-0.650***
Isopimaric acid	-0.020	-0.119	-0.148	-0.073	-0.122	**-0.836****
Dehydroabietic acid	0.303	0.317	0.050	0.381	0.182	-0.473
Abietic acid	-0.034	-0.132	-0.121	-0.148	-0.128	**-0.817****
Acid 20:0	0.434	0.342	0.173	0.394	0.287	-0.370
Acid 22:0	0.237	0.104	0.127	0.103	0.062	-0.527
Isorhapontigenin	**0.676***	**0.700***	**0.676***	**0.729***	**0.810****	0.570
Piceatannol	**0.630***	0.580	**0.702***	**0.753****	**0.787****	0.491
Catechin	**0.651***	**0.671***	**0.789****	**0.713***	**0.748****	**0.821****
Hydroxymatairesinol isomer 1	**-0.845****	-0.581	**-0.787****	**-0.679***	**-0.645***	-0.171
Hydroxymatairesinol isomer 2	**-0.867****	**-0.650***	**-0.775****	**-0.727***	**-0.720***	-0.159
Matairesinol	**-0.788****	-0.564	**-0.704***	**-0.642***	**-0.659***	-0.078
Campesterol	-0.062	-0.110	-0.151	-0.086	-0.091	**-0.740****
Beta-sitosterol	**0.847****	**0.678***	**0.651***	**0.695***	**0.608***	0.012
Trans-piceid	**0.670***	0.477	**0.776****	0.562	**0.693***	0.219
Isorhapontin	**0.813****	**0.804****	**0.909****	**0.808****	**0.857****	**0.704***
Total compounds[a]	0.057	0.252	0.043	0.253	0.188	-0.299

[a] Sum of concentrations of compounds analyzed by GC-MS.
* Correlation is significant at the 0.05 level (two-tailed).
** Correlation is significant at the 0.01 level (two-tailed).

bioactivities is likely due to most of the lignan content being found in the hand-picked branch wood sample, which exhibited the lowest bioactivities in most of the tests.

3.6. Phenolic compounds in the hand-picked fraction extracts

In this study, chemical characterization and quantification of compounds was performed using GC-MS. Since GC analysis is limited to small and volatile molecules, the extracts of hand-picked fractions were

Table 3
VOC profiles of the pure hand-picked fractions. Compounds representing more than 1 % of the total peak area are shown. The full VOC profile is available in the Supplementary file (Supplementary Table S6).

				% of total		
#	RT/min	Compound Name	Formula	Needles	Branch bark	Branch wood
1	1.5058	Dimethyl ether	C_2H_6O		3.41	< 1
2	5.1241	Hexanal	$C_6H_{12}O$			1.96
3	7.4187	Tricyclene	$C_{10}H_{16}$	2.40		
4	7.6176	α-Pinene	$C_{10}H_{16}$	8.64	12.03	43.18
5	7.8521	Camphene	$C_{10}H_{16}$	11.30	< 1	
6	8.2207	Sabinene	$C_{10}H_{16}$	1.98	1.28	1.15
7	8.2700	β-Pinene	$C_{10}H_{16}$	3.63	7.32	18.98
8	8.4569	β-Myrcene	$C_{10}H_{16}$	4.58	3.03	2.00
9	8.7336	3-Carene	$C_{10}H_{16}$	4.53	21.56	5.66
10	8.9882	D-Limonene (needles) orPseudo-limonene (bark/wood)	$C_{10}H_{16}$	11.77	5.92	6.76
11	9.0290	Eucalyptol	$C_{10}H_{18}O$	8.26		
12	9.7222	Terpinolene	$C_{10}H_{16}$	< 1	3.96	
13	10.4123	(+)-2-Bornanone	$C_{10}H_{16}O$	3.33		
14	10.4559	Camphene hydrate	$C_{10}H_{18}O$	2.13		
15	10.6393	endo-Borneol	$C_{10}H_{18}O$	4.18		
16	10.7481	Terpinen-4-ol	$C_{10}H_{18}O$	< 1	1.46	
17	10.8786	L-α-Terpineol	$C_{10}H_{18}O$	1.76	< 1	
18	11.8610	Bornyl acetate	$C_{12}H_{20}O_2$	11.08		
19	12.5262	α-Longipinene	$C_{15}H_{24}$	< 1	2.55	1.01
20	12.9866	Guaiyl acetate	$C_{17}H_{28}O_2$		< 1	1.17
21	13.0609	Longifolene	$C_{15}H_{24}$	< 1	5.59	2.04
22	13.1572	Caryophyllene	$C_{15}H_{24}$		1.00	
23	13.6081	γ-Cadinene or isomer	$C_{15}H_{24}$	< 1	1.31	1.06
24	13.6516	2-Tridecanone	$C_{13}H_{26}O$	< 1	7.79	
25	13.6792	Copaene or isomer	$C_{15}H_{24}$			8.45
26	13.8008	α-Muurolene	$C_{15}H_{24}$	1.23		
27	13.8471	Himachalene-1,4-diene	$C_{15}H_{24}$			1.01
28	13.8504	Isoitalicene	$C_{15}H_{24}$		1.57	
29	13.9870	delta-Cadinene	$C_{15}H_{24}$			2.07
30	13.9904	β-Cadinene	$C_{15}H_{24}$	2.33	1.84	
31	17.0928	Cembrene or isomer	$C_{20}H_{32}$		2.35	1.12
		SUM (%)		**84.14**	**83.97**	**97.62**

also screened by LC-DAD/MS to detect higher molecular weight phenolic compounds and to compare identification data with GC-MS results. The LC chromatograms of hand-picked fractions, MS/MS data, and tentative component identifications are presented in Supplementary Fig. S2 and Supplementary Table S5. From the branch wood fraction, only hydroxymatairesinol isomers were identified by LC-DAD/MS, consistent with the GC-MS data, which revealed that 83 % of wood extractives consisted of hydroxymatairesinol isomers. Needles contained stilbene glucosides such as piceid and isorhapontin, as well as astringin and an isorhapontin isomer not detected by GC-MS. Additionally, needles contained small phenolic compounds and flavonoid aglycones, glycosides, and hydroxycinnamic acid derivatives, of which catechin was the only one detected by GC-MS. Similar to GC analyses, piceid and isorhapontin were identified from branch bark, whereas five dimeric stilbenes and the flavonoid eriodictyol were detected exclusively by LC-MS.

3.7. Volatile organic compounds in the hand-picked fractions

Logging residues are known to release volatile organic compounds (VOCs) during decomposition (Prinz et al., 2024). Monoterpenes, such as limonene, are utilized in various applications, and the growing demand has led to a search for novel sources (Sun et al., 2020). Therefore, we investigated how VOC profiles differ among the pure hand-picked fractions of needles, branch bark, and branch wood, measured using headspace analysis and GC-MS (Table 3). Furthermore, only limited information is available in the literature on the VOC composition of branch wood and branch bark fractions.

Only the most intense peaks were included in the identifications, and peak percentages were calculated as normalized amounts. These values represent the relative proportions of selected compounds, not exact concentrations. Full VOC profiles are shown in Supplementary file (Supplementary Table S6 and Supplementary Figure S3). The needle, branch bark, and branch wood samples exhibited distinct profiles. Total peak area sum for branch wood was 97.6 %, higher than that of needles (84.1 %) and branch bark (84.0 %), indicating that most of the compounds in the branch wood sample exceeded 1 % of the total peak area and were tentatively identified. Branch bark and needle samples contained more compounds than branch wood and showed a variety of small peaks (<1 % area), which are detailed in Supplementary file (Supplementary Table S6 and Supplementary Figure S3).

Branch wood contained a significant amount of α-pinene and β-pinene (62.2 % of the total peak area). These compounds are also well known as by-products of the pulping process in crude turpentine. The highest concentrations of volatile compounds in branch wood were α-pinene, β-pinene, copaene, pseudo-limonene (D-limonene), and 3-carene (43.2 %, 19.0 %, 8.5 %, 6.8 %, and 5.7 %, respectively). Wajs and co-workers analyzed various sapwood and heartwood samples using a similar headspace solid-phase microextraction (HS-SPME) method and column as in the current study (Wajs et al., 2007, 2006) and their findings indicated high amounts of α-pinene and β-pinene. Additionally, the presence of 3-carene and limonene is consistent with previous analyses (Wajs et al., 2007, 2006). The copaene content detected in branch wood (8.5 %) was markedly higher than that in sapwood and heartwood, which have been reported to contain only 0.4–0.5 % (Wajs et al., 2007, 2006).

Needles contained nearly equal proportions of D-limonene (11.8 %), camphene (11.3 %), and bornyl acetate (11.1 %). D-limonene, or pseudo-limonene, was found in all three fractions, although its amount was highest in needles. In addition, α-pinene was present in all three fractions: needles (8.6 %), branch bark (12.0 %), and branch wood (43.2 %) making its proportion in branch wood about five times higher than in needles. Spruce needles contained the highest total number of compounds, for example, camphene and bornyl acetate were only identified in the needle fraction. Spruce needles have previously been reported with similar compound profiles (Isidorov et al., 2003; Mofikoya et al., 2020; Persson et al., 1996; Pohjola et al., 1989; Raber et al., 2021). Isidorov and co-authors used a similar methodology and found that sesquiterpene hydrocarbons (58.7 %) were particularly abundant in fresh spruce needle litter. In our data, however, monoterpene hydrocarbons were the most abundant group of identified VOCs. This is likely due to differences in needle biomass freshness or seasonal variation. For example, a substantial proportion of monoterpenes in green needles has been found to decrease after 4–6 months following litter fall (Ludley et al., 2009) but variations in handling and storage conditions can enormously affect the VOC profiles (Prinz et al., 2024). In addition, factors such as biotic and abiotic stressors can induce monoterpene and diterpene accumulation, while only modest effects have been observed on sesquiterpene levels (Martin et al., 2002).

The composition of volatiles in spruce branch bark differed from that in needles and branch wood. Like needles, branch bark contained a high proportion of monoterpenes (53.8 %), with the most abundant compounds being 3-carene (21.6 %), α-pinene (12.0 %), and β-pinene (7.3 %). These compounds have previously been reported in conifer bark, and their proportions have been found to increase particularly during attacks by plant pathogens (Croteau et al., 1987).

While VOC content in the extracts is likely reduced, water extracts of spruce bark have still been shown to contain terpenes and terpenoids (Tienaho et al., 2025). These compounds have demonstrated strong antimicrobial efficacy but only low antioxidant activity (Muilu-Mäkelä et al., 2022) and can consequently influence some of the observed activities in the logging residue extracts.

4. Conclusions

This study demonstrated that mechanical separation can produce needle fractions from spruce logging residues with yields and purity levels competitive with hand-picked reference and with beneficial antimicrobial and antioxidant properties. Although statistical differences were observed, their practical significance for industrial applications is minimal: the mechanical method achieved approximately 70–75 % of the yield of hand-picking, representing a major step toward cost-effective and scalable processing. These findings indicate that mechanization is a realistic alternative to manual sorting for managing raw material streams in bioproduct industries.

The observed antioxidant and antimicrobial bioactivities were positively correlated with the amount of stilbenes and flavonoids. Similarly extracted hand-picked fractions were investigated and results showed that the branch wood sample showed low bioactivities. The VOC analysis of the pure fractions showed that while the needle fraction contained a mixture of compounds, the branch wood mostly contained α- and β-pinene.

It is, however, important to acknowledge the limitations of the experimental design. Because sorting methods and collection seasons were partially linked, seasonal and processing effects could not be fully separated. Nevertheless, the results provide valuable insights into how sorting techniques influence fraction composition and bioactivity. Future controlled experiments, including hand-picked samples from both seasons, could strengthen these observations and clarify the impact of seasonal variation.

The findings support the concept that spruce logging residues can serve as a promising feedstock for bioproduct industries. Mechanical separation enables efficient recovery of valuable needle fractions, which may advance the development of biorefinery concepts. Future research should focus on process optimization, recovery of bioactive compounds, and economic assessment to integrate mechanized sorting into sustainable industrial production chains.

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CRediT authorship contribution statement

Jenni Tienaho: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Marina Fidelis:** Writing – review & editing, Writing – original draft, Validation, Methodology, Investigation, Formal analysis, Data curation. **Anni Perämäki:** Writing – review & editing, Writing – original draft, Visualization, Validation, Investigation, Formal analysis, Data curation. **Leif Hed:** Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation. **Hanna Brännström:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Magnus Rudolfsson:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Jaana Liimatainen:** Writing – review & editing, Writing – original draft, Validation, Investigation, Formal analysis, Data curation. **Marjo Haapakoski:** Writing – review & editing, Writing – original draft, Visualization, Validation. **Varpu Marjomäki:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation. **Risto Korpinen:** Writing – review & editing, Writing – original draft, Supervision, Resources, Project administration, Funding acquisition. **Tuula Jyske:** Writing – review & editing, Writing – original draft, Supervision, Funding acquisition. **Magnus Matisons:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Petri Kilpeläinen:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.2026.122696.

Data availability

Data will be made available on request.

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