

Effect of Accelerated Extraction and Sonication on the Antioxidant Capacity of Wood and Bark Extracts of Wet-hearted Silver Fir (*Abies Alba* Mill.)

Viljem Vek

`viljem.vek@bf.uni-lj.si`

University of Ljubljana

Tamás Hofmann

University of Sopron

Eszter Visiné Rajczi

University of Sopron

Urša Osolnik

University of Ljubljana

Ida Poljanšek

University of Ljubljana

Primož Oven

University of Ljubljana

Research Article

Keywords: wet-hearted silver fir, knotwood, bark, extraction, polyphenols, antioxidant capacity

Posted Date: March 12th, 2024

DOI: <https://doi.org/10.21203/rs.3.rs-4046321/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at European Journal of Wood and Wood Products on June 14th, 2024. See the published version at <https://doi.org/10.1007/s00107-024-02102-1>.

Abstract

The aim of this study was to compare the antioxidant capacity of hydrophilic extracts of different tissues of wet-hearted silver fir (*Abies alba* Mill.) logs. Wet heart is a wood defect of silver fir and lowers the value of wood. The exploitation of extractives could increase utilization and financial returns. The bark, sapwood, heartwood and knotwood were investigated by using two green extraction methods: ultrasound assisted extraction (US) and accelerated solvent extraction (ASE), with aqueous ethanol as solvent. Total extractive content (TEC), total polyphenol content (TPC) and antioxidant capacities (FRAP, DPPH, ABTS) from the extracts were determined and compared. The highest contents of total hydrophilic extractives were measured in knotwood (23.07%, ASE) and bark (10.31%, ASE), and the lowest values were determined for sapwood (2.00%, ASE) and heartwood (3.56%, ASE). The ASE method resulted in significantly higher TPC (0.65-10.58 %) than the US (0.46-9.19 %) method. Nevertheless, the simplicity of instrumentation and costs can make US also a potential candidate for future extraction and utilization. The highest antioxidant capacities were measured in knotwood (FRAP: 159.75 mg AAE/g, ABTS: 316.15 mg TE/g, DPPH: 189.23 mg TE/g) and bark (FRAP: 159.75 mg AAE/g, ABTS: 126.81 mg TE/g, DPPH: 74.52 mg TE/ g) extracts, prepared with ASE, which complements well the existing literature data on silver fir extractives. The knotwood and bark of wet-hearted silver fir is an abundant source of antioxidant polyphenols, whereas sapwood and heartwood are poor in these extractives, making them unlikely candidates for future extractions of natural antioxidants.

1. Introduction

The European Green Deal strategy (<https://commission.europa.eu>) encompasses the concepts of green transition and a circular economy, which give high priority to recycling and the sustainable use of raw materials, especially those of natural origin. In this context, the development of new side-streams and their treatment as new and important value chains is a challenge for all industries. It is well known that the side-streams of the forestry and wood processing industries hold great untapped potential (Verkasalo et al. 2019). Forest and wood processing residues have already been shown to be rich and environmentally friendly feedstock for various bioproducts (Holmbom 2011; Oleson and Schwartz 2016; Ragauskas et al. 2006). Nevertheless, wood residues and bark are mostly used in wood processing plants only as a source of energy. Although the amount of non-structural constituents in wood and bark from trees (generally referred to simply as extractives) is relatively small (about 5-15%, w/w), some of these compounds, e.g., stilbenes, flavonoids and condensed tannins, have been reported to possess antifungal, antimicrobial and antioxidant properties and they therefore have great potential for various applications (Belt et al. 2017; Lu et al. 2016; Valette et al. 2017; Välimaa et al. 2007). According to forecasts, the global market for polyphenols will grow significantly by 2030 (grandviewresearch 2022). The high demand for plant polyphenols with antioxidant, anti-inflammatory and antimicrobial properties is evidence of the increasing use of these plant products in the field of dietary supplements and nutraceuticals, cosmetics, “green” preservatives etc.

One of the negative consequences of climate change is its effect on the distribution of tree species (Gričar et al. 2015; Humar et al. 2021; Vítková et al. 2017). The latest model projections for Central Europe show that tree species of major industrial importance, i.e., spruce and Scots pine, will be most affected, and they are predicted to migrate almost entirely from Central Europe to northern countries (with the exception of the colder Alpine regions) (Dyderski et al. 2018). According to a study by Dyderski et al. (2018), silver fir is identified as a "climate winner", together with some autochthonous broadleaf species and also alien tree species. Silver fir will thus be one of the conifers that will gain new potential distribution areas in Europe in the future due to climate changes. This could give silver fir even greater economic importance for forest owners and the wood processing industry (Hanewinkel et al. 2013; Humar et al. 2022). Silver fir is already an economically valuable coniferous species in Europe (Dobrowolska et al. 2017). At the same time, silver fir logs and their wood often experience a sharp decrease in value due to the formation of so-called wet heart or wet wood, in the place of the normal heartwood (Torelli et al. 2005; Torelli et al. 2006). Wet heart of silver fir is described as wood tissue with compact, increased moisture content at the site of the heartwood (Torelli 1990). Wet heart is subject to mechanical anomalies involving cracking and ring shakes (Shigo 1984; Torelli et al. 2007; Torelli et al. 2006). In fact, the frequency and intensity of wet heart formation in silver fir critically increases with tree age, dimensions and frequent injuries, e.g., from broken/dropped branches. Wet heart in mature silver trees is therefore actually a normal and age-related phenomenon (Torelli et al. 2005). It is clear that not much attention is paid to the presence of wet heart in the existing literature on the extractive compounds of silver fir, even though this is a major quality defect and therefore reduces the value of silver fir logs. The utilization of the less valuable wood and bark biomass of silver fir remains a major challenge but the extraction of valuable bioproducts such as nanocellulose and bioactive extracts from tree biomass continues to be intensively researched (Brennan et al. 2020b; Fernandez-Costas et al. 2017; Oleson and Schwartz 2016; Žepič et al. 2022).

Extracts from low-grade tissues of silver fir, i.e., bark and branch wood, have already been investigated for health-promoting properties, highlighting the antioxidant effect of the extracts (Drevenšek et al. 2016; Tavčar Benković et al. 2017). Silver fir samples were extracted using both conventional and advanced extraction methods, and extractives were analyzed using various *in vitro* antioxidant assays. Bark samples were extracted by maceration followed by purification by liquid-liquid extraction (Tavčar Benković et al. 2014), then using an accelerated extraction system (ASE) (Brennan et al. 2020b) or using standard procedures as demonstrated on Caucasian fir bark samples (Ozgenc et al. 2017). Hamada et al. (2018) used a Soxhlet apparatus for the extraction of sapwood and heartwood samples of silver fir. The wood of silver fir branches was also conventionally extracted at higher temperature and analyzed by Tavčar Benković et al. (2017) but, due to the patent process, detailed information on the extraction process is not available. Since conifer knotwood is generally known to be a potential source of bioactive polyphenols (Holmbom 2011), silver fir knotwood has also been studied most extensively, with ASE and Soxhlet often used for extraction (Kebbi-Benkeder et al. 2017; Willför et al. 2004). Stemwood and knotwood of Siberian fir (*Abies sibirica*) were extracted using ASE and the extracts were subsequently compared for their antioxidant properties; however, with respect to the sampling procedure, it is unclear

whether sapwood or heartwood, or even a mixture of both, was extracted and analyzed (Ul'yanovskii et al. 2022). In terms of the solvents used for extraction, silver fir extractives have been obtained with water or various less and more polar organic solvents, e.g., hexane, toluene, aqueous acetone and aqueous ethanol (Hamada et al. 2018; Kebbi-Benkeder et al. 2015; Tavčar Benković et al. 2014; Willför et al. 2004).

The main objective of this study was to investigate the biorefinery potential of sapwood (SW), heartwood (HW), as well as bark (B) and knotwood (KW) extracts originating from lower value, wet-hearted silver fir logs, by comparing the chemical composition of the extracts. Two extraction methods were applied and compared: sonication (US) and accelerated solvent extraction (ASE), in order to assess their potential for future use. An ethanol-water mixture was used as solvent for environmental and future application reasons. The chemical composition of the tissues was assessed and compared by measuring total extractive content (TEC), Folin-Ciocalteu total polyphenol content (TPC) and by the complex evaluation of the antioxidant capacity by FRAP (Ferric reducing antioxidant power), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) and DPPH (2,2-diphenyl-1-picrylhydrazyl) assays. The results contribute to a better understanding of the utilization of wet-hearted silver fir wood and to the elaboration of novel and cost-effective green extraction methods for the utilization of the extractives.

2. Materials And Methods

1.1 Chemicals

Folin–Ciocalteu phenol reagent, gallic acid monohydrate, gallic acid (certified reference material), L-ascorbic acid, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox, an analogue of vitamin E), 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+ solution), 2,2-diphenyl-1-picrylhydrazyl (DPPH solution), 2,4,6-tripyridyl-S-triazine (TPTZ solution), potassium persulfate, iron(III)-chloride, acetic acid, sodium acetate, hydrochloric acid, sodium carbonate, potassium hydrogen phosphate and potassium dihydrogen phosphate were purchased from Merck (Sigma-Aldrich Chemie, Taufkirchen, Germany). Water, methanol and absolute ethanol were from J.T. Baker (Phillipsburg, NJ, USA). All chemicals used are commercially available, all were of pure/analytical grade and were used without further purification.

1.2 Material

The study was conducted on silver fir (*Abies alba* Mill.), i.e., a coniferous tree that plays an important role in the wood processing industry in Central Europe (Bianchi et al. 2014b; Butnaru et al. 2022; Dobrowolska et al. 2017). Wood and bark samples from a number of trees were included in the investigation. Trees averaged 32.24 (SD, 2.48) meters in height and 125.83 (SD, 42.02) years old. Age was measured by counting the annual rings of the cross-section at the base of the silver fir (at about 0.2 m height). Since the study was conducted on mature silver firs, they were naturally characterized by the presence of the

above-mentioned wet heart at the heartwood site. The presence of wet heart significantly reduces the value of silver fir logs. Nevertheless, an important goal of the research activity was to ensure the homogeneity of the silver fir wood and bark samples as much as possible. The samples were therefore cut from sample discs taken at different tree heights and homogenized. The silver firs were harvested from two locations: Kočevska Reka forest (45°34'31.5" N 14°46'27.8" E) and Kuželič forest, Grivac (45°28'11.7" N 14°50'43.8" E), Slovenia. The function of the tissues in a tree stem was considered in planning the study and selecting the sample groups, so wet heart, defined as defective or abnormal woody tissue, was not treated separately for the purposes of the present study. Samples of sapwood (SW), heartwood (HW), knotwood (KW) and bark (B) were thus sawn from each of the sampled discs in the manner previously described (Vek et al. 2021) (Fig. 1). SW and HW on the silver fir sample discs were distinguished with an iodine-starch test by applying iodine solution directly to the surface of the silver fir disc with a brush. In relation to the wood of knots, i.e., the base part of a branch imbedded in a tree stem, only the dark-colored central part of the knot was sampled, leaving out the adjacent light-colored tissues of the knot (Fig. 1). All collected silver fir samples were first oven-dried overnight at 50 °C, then ground using a cutting mill (Retsch SM 2000), and finally freeze-dried to a constant weight in a Telstar LyoQuest CC1930 freeze dryer at 0.045 mbar and - 85 °C. Finally, for the study of antioxidant properties, several homogenized samples of SW, HW, KW and B of silver fir were prepared by mixing the mass aliquots of each sample per tissue studied. The thus-prepared disintegrated samples of silver fir SW, HW, KW and B were stored in polypropylene bottles, which were air-tight sealed. The samples were kept in a cool, dark place until extraction.

Fig. 1 Schematic presentation of research activities. Sapwood (SW), heartwood (HW), knotwood (KW) and bark (B) of wet-hearted silver fir (*Abies alba* Mill.) were extracted by two extraction methods (sonication, ASE 350 system), and extracts were analyzed gravimetrically and colorimetrically for extractant content (soluble extractive content - TEC and total phenolic content - TPC) and antioxidant capacity (FRAP, ABTS, DPPH tests)

1.2.1 Extraction

The ground samples were first freeze-dried overnight. Extractions were performed in a Thermo Dionex system ASE 350 (Thermo Fisher Scientific Inc., Waltham, US) and in an Elma Transsonic T570 ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany) (Hofmann et al. 2015a; Vek et al. 2021) (Fig. 1). The ASE extractions were performed under nitrogen, and the extracts were filtered through a cellulose filter, i.e., "on-line" during extraction. All wood and bark extracts obtained by ASE and sonication were prepared to achieve a sample to solvent ratio of 1:100 (w/v). As a solvent, 80% aqueous ethanol (v/v) was used. Further information on ASE and sonication of silver fir wood and bark samples can be found in Table 1.

Both extraction techniques can be also referred to as "green" due to the short extraction time and low solvent and energy consumption. Since water and ethanol are known to be environmentally friendly solvents because of their chemical properties and accessibility, e.g., ethanol can be produced by

fermentation from renewable sources (Tekin et al. 2018), and to avoid the use of hazardous non-polar organic solvents, 80% aqueous ethanol (v/v) was chosen as the extraction solvent in the present study.

Table 1 Parameters of the extraction process

	System ASE ¹ 350	Sonication
sample amount	1.0 g	0.3 g
temperature	120 °C	25 °C
no. static cycles	4	3
cycle time	5 min	10 min
pressure	1500 psi	ambient
cell/tube volume	10 mL SST ² cell	50 mL "Falcon"
extract volume	100 mL	30 mL

¹ASE, Accelerated Solvent Extraction; ²SST, Stainless Steel;

Before gravimetric analysis, all silver fir extracts were centrifuged twice at 10000 rpm for 15 minutes in an Eppendorf 5810R centrifuge. The extracts were transferred to 2-mL polypropylene reaction tubes with safety caps and centrifuged again three times at 12000 rpm for 10 min in an Eppendorf MiniSpin Mini centrifuge. Prior to the analyses, all reaction tubes containing the extracts were stored in a refrigerator at 4 °C.

1.2.2 Content of extractives and polyphenols in wood and bark samples

The total extractive content (TEC) and total phenol content (TPC) were measured according to the protocol described in our previous research reports (Vek et al. 2019; Vek et al. 2020). The TEC was determined by drying 10 mL of extract to constant weight in an oven at 105 °C, and results were expressed as percentage TEC per dry weight of sample (% dw).

The TPC was measured by the Folin-Ciocalteu method (Albert et al. 2003; Singleton and Rossi 1965). Extracts were mixed with 10-fold diluted Folin-Ciocalteu phenol reagent (aq) and 0.7 M sodium carbonate solution (aq). The reaction mixtures were incubated for two hours and the absorbance was then measured at 765 nm in a Perkin-Elmer Lambda UV–VIS spectrophotometer. Gallic acid was used as a reference (Vek et al. 2019); results are expressed as the percentage of TPC per dry weight of a silver fir sample (% dw).

1.2.3 Antioxidant properties

The antioxidant capacity of silver fir wood and bark extracts was measured according to the protocols described by Hofmann et al. (2015b). Colorimetric analyses of antioxidant capacities were performed using a Hitachi U-1500 spectrophotometer (Hitachi Ltd., Tokyo, Japan). Measurements of silver fir extracts were performed in two replicates. The final results are given as mean with standard deviation.

1.2.3.1 FRAP assay

The FRAP (ferric reducing antioxidant power) assay was run following the method of Benzie and Strain (1996) at 593 nm, applying 5 min reaction time and using ascorbic acid as standard. Results were expressed as mass equivalents of ascorbic acid per dry weight of silver fir sample (mg AAE/g dw) (Hofmann et al. 2015b).

1.2.3.2 ABTS assay

The ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) test was performed according to the protocol described by Stratil et al. (2007). After 10 min incubation at room temperature, the absorbance was determined at 734 nm. Results were expressed in milligrams of Trolox equivalents per gram of dry sample (mg TE / g dw) (Hofmann et al. 2015b).

1.2.3.3 DPPH assay

The DPPH radical neutralization assay was performed by measuring the radical scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to a modified protocol of Sharma and Bhat (2009). A methanolic solution of DPPH with a concentration of 80 $\mu\text{M}/\text{l}$ was used for determination. DPPH reagent (2800 μl) was mixed with the sample (200 μl) and the mixture was incubated in the dark for 30 minutes at room temperature. The decrease in absorbance at 515 nm was measured. The DPPH radical scavenging capacity was expressed in milligrams of Trolox equivalents per gram of dry sample (mg TE / g dw) (Hofmann et al. 2015b).

1.3 Statistics and graphic material

Statistical significance was tested with basic statistical analysis using Statgraphics software. Before testing the differences between the means of the measurements using ANOVA, the results were tested for normal distribution. To determine which means were significantly different from the others, Fisher's least significant difference (LSD) procedure was performed at a 95.0% confidence level. All structural formulas were generated using ChemDraw software from PerkinElmer.

3. Results And Discussion

1.1 Total extractives content

According to the results, SW and HW contained significantly lower amounts of total extractives than B and KW ($p < 0.001$). On average, the two extractions of the silver fir samples yielded 2.22% of TEC and 0.55% TPC for SW, 3.80% of TEC and 1.07% TPC for HW, 24.65% of TEC and 9.89% TPC for KW and 11.82% of TEC and 3.22% TPC for B. Our results on extraction yields presented in Table 2 agree relatively well with previously published results on the chemistry of silver fir extracts, but there are some differences due to different sampling methods and extraction procedures (Brennan et al. 2020a; Hamada et al. 2018; Kebbi-Benkeder et al. 2017; Tavčar Benković et al. 2017; Willför et al. 2004). The following is a literature review of extractives obtained to date from various tissues of silver fir. It has been reported that the position of the bark on the silver fir can have a significant effect on extraction yield. Brennan et al. (2020a) demonstrated that ASE extraction of bark samples taken at different heights from a silver fir can yield TEC between 15% and even more than 30% (by weight). Treatment of Caucasian fir (*Abies nordmanniana*) bark with sodium hydroxide solution using the standard procedure (TAPPI; T 212 om-98) yielded 17.01% TEC (Ozgenc et al. 2017). In addition to the aforementioned longitudinal variation of water/ethanol soluble extractives in silver fir bark, radial variability in the content of total extractives was also observed. According to the results presented, Soxhlet extraction of sapwood and heartwood with a toluene/ethanol mixture yielded an average of 1.4% and 2.3% soluble extractives, respectively (Hamada et al. 2018). According to Willför et al. (2004) the total amount of hydrophilic extractives obtained with 95% acetone (v/v, aq) extraction was 0.7, 2.1 and 13-15% (dw) in SW, HW and KW, respectively. These amounts are lower than our measurements; however, their samples were washed with hexane (ASE) at a higher temperature and pressure prior to extraction with acetone, which probably also removed some of the nonstructural compounds that are soluble in acetone. Another possible explanation is that methanol and acetone are better suited for the extraction of polyphenols from fir bark samples.

Secoisolariciresinol, lariciresinol and oligolignans have been reported to be abundant in silver fir KW samples (Willför et al. 2004). This was also confirmed in our preliminary study on the chemical composition of silver fir KW extracts (Vek et al. 2021). Significant longitudinal variation in extractives content has also been demonstrated for silver fir knotwood (Kebbi-Benkeder et al. 2017). In this case, the knotwood tissues were extracted sequentially in a Soxhlet apparatus using hexane and acetone as solvents. Soxhlet extraction of the samples from KW gave on average about 0.5% - < 2% lipophilic and 17% - 28% acetone soluble extractives. In addition to the dominant lignans, viz. secoisolariciresinol, hydroxymatairesinol and lariciresinol, the authors also found traces of matairesinol and pinioresinol in the KW extracts of silver fir (Kebbi-Benkeder et al. 2017). Similar results were also presented by Gérardin et al. (2023), whose KW samples contained 21.64% ethanol-soluble compounds. Ul'yanovskii et al. (2022) found that successive ASE extractions of KW of Siberian fir (*Abies sibirica*) with hexane and acetone (95%, aq) yielded 17.0% (dw) of TEC, and the amounts of TEC were significantly higher in the knotwood than in the stemwood (3.0%, dw). The TEC in KW of Siberian fir was significantly lower than the TEC in our KW samples (Table 2). In addition, the stemwood of Siberian fir contained comparable amounts of TEC with our stemwood samples (Table 2) but it is not clear whether either SW or HW or even a mixture of both was extracted in that case. In our study, the stemwood tissues were separated and analyzed separately. Butnaru et al. (2022) demonstrated that Soxhlet extraction of silver fir bark, needles and cones with an ethanol/toluene mixture yielded an average of 16.57-17.33%, 28.33-29.30% and 17.90-

19.18% extractives, respectively. The bark was further described as an inexpensive and important source of phenols and flavonoids, containing about 95 mg GAE /g TPC and 130-175 mg QE /g flavonoids. Compared with the results of the present study (28.4-32.1 mg GAE /g TPC), the higher results for TPC in the B sample (Butnaru et al. 2022) could be explained by the composition of the solvent mixture and by the fact that the Folin-Ciocalteu reagent can also react with compounds that are soluble in less polar solvents than toluene, e.g., resin acids (Harju and Venäläinen 2006; Karppanen et al. 2007). Although the wood and bark of silver fir have been studied extensively for the chemical composition of extractives, this review of recent research reports clearly shows that only certain tissues of the fir trunk have been studied with the chosen extraction technique and solvent composition. The present report is the first comparative study to include all categories of silver fir stem tissues in one study, i.e., stemwood, knotwood and bark samples were extracted together and analyzed in the same manner.

Table 2 Chemical composition of wood and bark extracts of silver fir (*Abies alba* Mill.) with total extractive content (TEC) and total phenolic content (TPC).

Silver fir	ASE extraction				Sonication			
sample	TEC, % dw		TPC, % dw		TEC, % dw		TPC, % dw	
	avg	SD	avg	SD	avg	SD	avg	SD
Sapwood	2.00	0.57 E	0.65	0.13 d	2.43	0.54 E	0.46	0.11 d
Heartwood	3.56	0.63 E	1.18	0.22 d	4.05	0.77 E	0.96	0.20 d
Knotwood	26.23	2.65 A	10.58	1.07 a	23.07	2.97 B	9.19	1.19 b
Bark	12.41	1.69 C	3.21	0.59 c	10.31	1.79 D	2.84	0.85 c

Results are expressed as the mean of the measurements (avg) with standard deviation (SD). a - e Different letters within the same column indicate significant differences at a p<0.05 level.

The highest extractive contents were measured in KW samples. The extractives content in KW samples was more than 11 and 6 times higher than in SW and HW, respectively. Compared to SW and HW, much higher TEC and TPC were also measured in the B samples (Table 3). KW samples of silver fir were also richest in polyphenols, with ratios of TPC between KW and other samples even higher than the ratios of TEC (Table 3). Comparison of ASE and sonication showed that there was not much difference in the extraction yield of the studied samples. However, significantly more TEC and TPC were extracted from KW with ASE than with sonication (Table 2). ASE also proved to be more efficient than sonication in extracting hydrophilic compounds (TEC) from B samples. In the case of the B samples, the extraction technique had no significant effect on the TPC (Table 2). In terms of stem tissue, interestingly, higher TEC was obtained with sonication than with ASE from both SW and HW, but the significance here was not confirmed by statistical analysis (Table 2).

Table 3 Ratios in the contents of extractives among the studied tissues of silver fir (*Abies alba* Mill.)

Ratio (w/w)	TEC	TPC
Knotwood/Sapwood	11.12	17.83
Knotwood/Heartwood	6.48	9.21
Knotwood/Bark	2.09	3.07

TEC, total extractive content; TPC total phenol content

Table 4 Percentage of total phenolic compounds in hydrophilic crude extracts (% dw) for wood and bark of silver fir. Sapwood, heartwood and knotwood of bark samples were extracted by accelerated solvent extraction (ASE 350 system) and with sonication.

Silver fir	ASE 350	Sonication
sample	% (TPC/TEC)	% (TPC/TEC)
Sapwood	32.66	18.72
Heartwood	33.28	23.80
Knotwood	40.35	39.83
Bark	26.91	27.58

TEC, total extractive content; TPC total phenol content

According to Table 4, ASE was more efficient for the extraction of polyphenols using aqueous ethanol solution compared to US. This difference between extraction methods in TPC/TEC (% dw) was particularly evident for SW and HW samples. It has been described that the advantage of ASE extraction, commonly defined as subcritical application, is that organic solvents are heated above their boiling point during extraction but are maintained in a liquid state under high pressure. This increases the penetration of the solvent into the sample and enhances the desorption of the analyte, resulting in higher overall extraction performance with lower solvent consumption (Thurbide and Hughes 2000). This may explain the higher efficiency of ASE in extracting phenolic compounds from cell walls, especially from HW, compared with sonication (Table 4). For the extracts of KW and B, the difference between the TPC/TEC for ASE and the TPC/TEC for sonication was much smaller. KW samples, i.e., dark colored and impregnated wood tissue from branches embedded in the tree trunk, were confirmed to be rich in phenolic extractives. More than forty percent of the dried ethanol extract KW was ascribed to polyphenols (Table 4). A lower percentage of TCP/TEC was measured in HW, SW and B samples, about one-third from TEC (Table 4). The results are in good agreement with the report of Tavčar Benković et al. (2017), who found that a dried aqueous extract of silver fir branchwood consists of 25% TPC (GAE).

1.2 Antioxidant capacity of silver fir wood and bark extracts

The antioxidant potential of the extracts was assessed using three different assays, all of which have different selectivity and reaction mechanisms (Prior et al. 2005; Shalaby and Shanab 2013; Sharma and Bhat 2009), so the results provide a complex insight into the comprehensive antioxidant power of the samples. The highest antioxidant capacities were measured with all three assays on KW extracts (FRAP: 159.75 mg AAE/g, ABTS: 316.15 mg TE /g, DPPH: 189.23 mg TE /g). In the case of Siberian fir, excellent antioxidant properties have also been found for KW samples (Ul'yanovskii et al. (2022)). Lower antioxidant capacities were found for the B extracts (FRAP: 159.75 mg AAE/g, ABTS: 126.81 mg TE /g, DPPH: 74.52 mg TE /g). Butnaru et al. (2022) previously reported that ethanol/toluene extracts of silver fir bark were good DPPH radical scavengers but these results were only evaluated on the basis of a visible discoloration of the DPPH solutions. Water-soluble compounds of silver fir bark were found to be potent natural antioxidants tested by DPPH assay and a cell-based *in vitro* assay on primary human peripheral blood mononuclear cells (Tavčar Benković et al. 2014). According to the DPPH assay, silver fir bark extracts exhibited a significantly higher (91%) antioxidant activity than that of maritime pine bark extract (Tavčar Benković et al. 2014). The high application potential of silver fir bark extracts in nutrition, health and medicine is attributed to the at least 13 natural antioxidants, phenolic acids (gallic acid, homovanillic acid, protocatechuic acid, p-hydroxybenzoic acid, vanillic and p-coumaric acid), flavonoids (catechin, epicatechin and catechin tetramethyl ether) and lignans (taxiresinol, 7-(2-methyl-3,4-dihydroxytetrahydropyran-5-yloxy)-taxiresinol, secoisolariciresinol and lariciresinol) (Tavčar Benković et al. 2014).

Using FRAP and DPPH assay, the weakest antioxidant power was measured in SW (FRAP: 6.80 mg AAE/g, DPPH: 4.57 mg TE /g) and HW (FRAP: 12.35 mg AAE/g, DPPH: 10.19 mg TE /g). In the case of ABTS assay, a relatively high antioxidant capacity was measured for SW (13.80 mg TE /g) and HW (31.50 mg TE /g) extracts compared to B extracts. However, it has already been demonstrated that, in addition to knotwood and bark extracts, silver fir wood also possesses significant antioxidant activity. The latter has been demonstrated by both *in vitro* and *in vivo* tests (Tavčar Benković et al. 2017; Vek et al. 2021). A comprehensive study of antioxidant activities *in vitro* and *in vivo* was conducted by Tavčar Benković et al. (2017) using a water extract of silver fir branchwood. They found that the branchwood extract exhibited higher antioxidant activity *in vitro* than selected reference antioxidants, i.e., ascorbic acid, resveratrol and butylated hydroxytoluene (BHT), and similar antioxidant activity to epigallocatechin gallate. Branch wood extractives were found to be particularly effective OH radical scavengers. The *in vivo* antioxidant activity of silver fir extracts was also confirmed by intracellular assay. The *in vitro* and *in vivo* antioxidant activities of branchwood extracts were explained by the presence of lignans (Tavčar Benković et al. 2017). The effects of the chosen extraction technique on the antioxidant capacity of the silver fir samples studied are shown in Fig. 2, Fig. 3 and Fig. 4. It can be clearly seen that, for the SW and HW samples, the extraction technique had no significant effect on the antioxidant activity. Both extraction methods also had similar effects on the antioxidant capacity of the KW and B samples, but

only when these extracts were analyzed with FRAP (Fig. 2). In contrast, the B extracts prepared by ASE extraction were found to be significantly better ABTS radical scavengers than the B extracts obtained by sonication (Fig. 3). In our study, the ASE extracts from KW proved to be the silver fir extracts with clearly the best DPPH radical scavenging activity (Fig. 4).

Fig. 2 Ferric reducing antioxidant power (FRAP) for sapwood (SW), heartwood (HW), knotwood (KW) and bark extracts (B) of silver fir prepared with different extraction methods (sonication and accelerated solvent extraction (ase350)). Results are expressed in ascorbic acid mass equivalents per gram of dry sample (mg AAE/g dw). a-c, Different letters on bars indicate a significant difference at the $p < 0.05$ level

Fig. 3 ABTS radical scavenging activity for extracts of sapwood (SW), heartwood (HW), knotwood (KW) and bark (B) of silver fir prepared with different extraction methods (sonication and accelerated solvent extraction (ase350)). Results are expressed in Trolox mass equivalents per gram of dry sample (mg TE/g dw). a-d, Different letters on the bars indicate a significant difference at the $p < 0.05$ level

Fig. 4 DPPH radical scavenging activity for extracts of sapwood (SW), heartwood (HW), knotwood (KW) and bark (B) of silver fir prepared with different extraction methods (sonication and accelerated solvent extraction (ase350)). Results are expressed in Trolox mass equivalents per gram of dry sample (mg TE /g dw). a-d, Different letters on bars indicate a significant difference at the $p < 0.05$ level

The antioxidant capacity of wood and bark extracts has been explained by the presence of different classes of phenolic compounds (Miranda et al. 2021; Pietarinen et al. 2006; Rosales-Castro et al. 2012; Willför et al. 2003). Previous reports have indicated that hydrophilic extracts of silver fir bark consist of simple phenolic acids, flavonoids, lignans and condensed tannins, viz., prodelphinidins (Bianchi et al. 2014a; Tavčar Benković et al. 2014; Vek et al. 2023), whereas the studied wood of silver fir contained mainly lignans, with a dominance of secoisolariciresinol (Kebbi-Benkeder et al. 2017; Vek et al. 2021; Willför et al. 2004). However, one of the objectives of the present study was to investigate the statistical relationships between the measured TEC and TPC of the extracts obtained by both ASE and sonication and the measured antioxidant capacities. The results of statistical analysis clearly showed a linear model to describe the significant relationships between the antioxidant capacities and the amount of extractives in the fir samples (Table 5). Since the p -values of the ANOVA are below 0.001 (Table 5), a significant relationship between the antioxidant capacities (measured by FRAP, ABTS and DPPH assay) and the content of silver fir polyphenols was confirmed at a confidence level of 95.0%. Extraction of the silver fir wood and bark samples, either with ASE or by sonication, revealed a strong relationship between the measured TEC and TPC ($p_{\text{ASE, sonication}} < 0.001$, $r_{\text{ASE}} > 0.980$, $r_{\text{sonication}} > 0.988$). Our results show that the amount of TEC and TPC in the wood and bark extracts obtained by ASE extraction significantly described the antioxidant capacity (ANOVA, $p_{\text{ASE}} < 0.001$). These relationships were statistically confirmed for all antioxidant tests performed (Table 5). Similar results were also characteristic of the silver fir extracts prepared with sonication (Table 5). This may mean that the quality of the extracts obtained with ASE and sonication is similar, which to some extent confirms the presence of polyphenols in the silver fir extracts, as previously reported (Kebbi-Benkeder et al. 2017; Tavčar Benković et al. 2014;

Vek et al. 2021; Vek et al. 2023; Willför et al. 2004). To obtain more precise information on which isolates and phenolic compounds of silver fir are responsible for the antioxidant activity of the extracts, further research activities using appropriate sample-prep techniques for purification and the correct analytical tools for compound identification are needed. These samples should also be tested with other biological tests to determine the possible bioactivity of silver fir extractives.

Table 5 Relationships (*R*-squared/coefficient of determination) between total extractive (TEC) and total phenolic content (TPC) of silver fir samples (*Abies alba* Mill.) and antioxidant capacity measured by three colorimetric assays, i.e., FRAP, ABTS and DPPH. A statistically significant relationship between extractives content and antioxidant capacity at a confidence level of 95.0% is represented by p-values of less than 0.05. The relationships (*p*, *R*²) are presented separately for accelerated solvent extraction (ASE 350 system) and sonication.

Antioxidant	ASE extraction				Sonication			
	TEC		TPC		TEC		TPC	
capacity; assay	<i>p</i>	<i>R</i> ² [%]	<i>p</i>	<i>R</i> ² [%]	<i>p</i>	<i>R</i> ² [%]	<i>p</i>	<i>R</i> ² [%]
FRAP	<0,001	90,52	<0,001	96,77	<0,001	95,00	<0,001	97,87
ABTS	<0,001	84,57	<0,001	78,82	<0,001	81,94	<0,001	85,56
DPPH	<0,001	97,83	<0,001	96,59	<0,001	96,48	<0,001	95,67

TEC, total extractive content; TPC total phenol content; FRAP, ferric reducing antioxidant power assay; ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assay; DPPH, 2,2-diphenyl-1-picrylhydrazyl assay.

4. Conclusion

This study investigated the antioxidant polyphenol content of wet-hearted silver fir stem tissues, including sapwood, heartwood, bark and knotwood, to find possible applications of the tissues of wet-hearted, thus lower grade wood logs. Accelerated solvent extraction and ultrasonic extraction techniques were compared using 80% aqueous ethanol (v/v) as a solvent. The highest contents of ethanol-soluble extractives were measured in knotwood and bark, which well supports the existing literature on extractives of silver fir. Although sonication proved to be an efficient, low-cost and fast extraction method, the present study revealed that, because of the higher temperature and pressure, the accelerated solvent extraction technique proved to be more efficient, resulting in significantly higher polyphenol contents. The results were also confirmed by the combined evaluation of multiple antioxidant capacity assays (FRAP, ABTS and DPPH). The highest antioxidant concentrations were found in knotwood and bark, whereas sapwood and heartwood yielded significantly lower levels of polyphenols. According to the results, the knotwood and bark tissues of lower grade wet-hearted silver fir logs can be potential sources of natural antioxidants for future applications.

Declarations

Acknowledgements

The authors thank the Slovenian Research and Innovation Agency (ARIS) for supporting project L4-2623 (Production of knotwood and bark extracts with high content of polyphenols from un-derutilized silver fir biomass; ArsAlbi), project J2-1723 (Catalytic lignin conversion to bio-based polymer blocks with CO₂ utilisation; CaLiBration), project V4-2017 (Improving the competitiveness of the Slovenian forest-wood chain in the context of climate change and the transition to a low-carbon society), and program group P4-0015 (Wood and lignocellulosic composites). Special thanks are due to the EU Erasmus+ programme for financial support to staff mobility for teaching and training activities STA(T) at HU-SOPRON01 in 2023 (2022-1-SI01-KA131, Erasmus STA contract number STA-2022-2023- 2597). This article was made within the framework of the project TKP2021-NKTA-43, which has been implemented with support provided by the Ministry of Culture and Innovation of Hungary from the National Research, Development and Innovation Fund, financed under the TKP2021-NKTA funding scheme. The authors sincerely thank Ars Pharmae d.o.o., Marko Domazet, Urška Zaloker and Ante Zaloker, and Aleš Marolt and Kočevski les d.o.o. for providing the silver fir material. Many thanks also to Samo Grbec, Anže Lopatič and Peter Hrovatič for their help and support in the field and in the laboratory and to Mr. Martin Cregeen for the linguistic editing.

References

1. Albert L, Hofmann T, Németh ZI, Rétfalvi T, Koloszá J, Varga S, Csepregi I (2003) Radial variation of total phenol content in beech (*Fagus sylvatica*) wood with and without red heartwood Holz als Roh- und Werkstoff 61:227-230 doi:10.1007/s00107-003-0381-x
2. Belt T, Hanninen T, Rautkari L (2017) Antioxidant activity of Scots pine heartwood and knot extractives and implications for resistance to brown rot Holzforschung 71:527-534 doi:10.1515/hf-2016-0232
3. Benzie IFF, Strain JJ (1996) The Ferric Reducing Ability of Plasma (FRAP) as a Measure of "Antioxidant Power": The FRAP Assay Analytical Biochemistry 239:70-76 doi:https://doi.org/10.1006/abio.1996.0292
4. Bianchi S, Gloess AN, Krosłakova I, Mayer I, Pichelin F (2014a) Analysis of the structure of condensed tannins in water extracts from bark tissues of Norway spruce (*Picea abies* [Karst.]) and Silver fir (*Abies alba* [Mill.]) using MALDI-TOF mass spectrometry Industrial Crops and Products 61:430-437 doi:https://doi.org/10.1016/j.indcrop.2014.07.038
5. Bianchi S, Gloess AN, Krosłakova I, Mayer I, Pichelin F (2014b) Analysis of the structure of condensed tannins in water extracts from bark tissues of Norway spruce (*Picea abies*) and Silver fir (*Abies alba* Mill.) using MALDI-TOF mass spectrometry Industrial Crops and Products 61:430-437 doi:10.1016/j.indcrop.2014.07.038

6. Brennan M, Fritsch C, Cosgun S, Dumarcay S, Colin F, Gerardin P (2020a) Quantitative and qualitative composition of bark polyphenols changes longitudinally with bark maturity in *Abies alba* Mill Ann For Sci 77:14 doi:10.1007/s13595-019-0916-x
7. Brennan M, Fritsch C, Cosgun S, Dumarcay S, Colin F, Gerardin P (2020b) Yield and compositions of bark phenolic extractives from three commercially significant softwoods show intra- and inter-specific variation Plant Physiology and Biochemistry 155:346-356 doi:10.1016/j.plaphy.2020.07.033
8. Butnaru E, Pamfil D, Stoleru E, Brebu M (2022) Characterization of bark, needles and cones from silver fir (*Abies alba* mill.) towards valorization of biomass forestry residues Biomass and Bioenergy 159:106413 doi:https://doi.org/10.1016/j.biombioe.2022.106413
9. Dobrowolska D, Boncina A, Klumpp R (2017) Ecology and silviculture of silver fir (*Abies alba*): A review Journal of Forest Research 22:326-335 doi:10.1080/13416979.2017.1386021
10. Drevenšek G, Lunder M, Benković ET, Štrukelj B, Kreft S (2016) Cardioprotective effects of silver fir (*Abies alba*) extract in ischemic-reperfused isolated rat hearts Food Nutr Res 60:7 doi:10.3402/fnr.v60.29623
11. Dyderski MK, Paž S, Frelich LE, Jagodziński AM (2018) How much does climate change threaten European forest tree species distributions? Global Change Biology 24:1150-1163 doi:https://doi.org/10.1111/gcb.13925
12. Fernandez-Costas C, Palanti S, Charpentier JP, Sanroman MA, Moldes D (2017) A Sustainable Treatment for Wood Preservation: Enzymatic Grafting of Wood Extractives ACS Sustainable Chemistry & Engineering 5:7557-7567 doi:10.1021/acssuschemeng.7b00714
13. Gérardin P, Hentges D, Gérardin P, Vinchelin P, Dumarçay S, Audoin C, Gérardin-Charbonnier C (2023) Knotwood and Branchwood Polyphenolic Extractives of Silver Fir, Spruce and Douglas Fir and Their Antioxidant, Antifungal and Antibacterial Properties Molecules 28:13 doi:10.3390/molecules28176391
14. Gričar J, Prislan P, De Luis M, Gryc V, Hacurova J, Vavrčik H, Čufar K (2015) Plasticity in variation of xylem and phloem cell characteristics of Norway spruce under different local conditions Frontiers in Plant Science 6 doi:10.3389/fpls.2015.00730
15. Hamada J, Petrissans A, Ruelle J, Mothe F, Colin F, Petrissans M, Gerardin P (2018) Thermal stability of *Abies alba* wood according to its radial position and forest management Eur J Wood Wood Prod 76:1669-1676 doi:10.1007/s00107-018-1353-5
16. Hanewinkel M, Cullmann DA, Schelhaas M-J, Nabuurs G-J, Zimmermann NE (2013) Climate change may cause severe loss in the economic value of European forest land Nature Climate Change 3:203-207 doi:10.1038/nclimate1687
17. Harju AM, Venäläinen M (2006) Measuring the decay resistance of Scots pine heartwood indirectly by the Folin-Ciocalteu assay Canadian Journal of Forest Research 36:1797-1804
18. Hofmann T, Nebenhaj E, Albert L (2015a) The high-performance liquid chromatography/multistage electrospray mass spectrometric investigation and extraction optimization of beech (*Fagus sylvatica*) bark polyphenols J Chromatogr A 1393:96-105 doi:10.1016/j.chroma.2015.03.030

19. Hofmann T, Nebehaj E, Stefanovits-Bányai É, Albert L (2015b) Antioxidant capacity and total phenol content of beech (*Fagus sylvatica*) bark extracts Industrial Crops and Products 77:375-381
doi:<http://dx.doi.org/10.1016/j.indcrop.2015.09.008>
20. Holmbom B (2011) Extraction and utilisation of non-structural wood and bark components. In: Alén R (ed) Biorefining of forest resources. vol book 20. Paper Engineers' Association/Paperi ja Puu Oy, Helsinki, pp 178-224
21. Humar M, Balzano A, Grbec S, Gričar J, Kržišnik D, Lesar B, Vek V (2021) Investigation of the material resistance and moisture performance of pubescent oak (*Quercus pubescens*) Holzforschung 75:22-36 doi:[doi:10.1515/hf-2020-0045](https://doi.org/10.1515/hf-2020-0045)
22. Humar M et al. (2022) Durability and Moisture Dynamics of Douglas-Fir Wood From Slovenia Frontiers in Plant Science 13 doi:[10.3389/fpls.2022.860734](https://doi.org/10.3389/fpls.2022.860734)
23. Karppanen O, Venäläinen M, Harju AM, Willför S, Pietarinen S, Laakso T, Kainulainen P (2007) Knotwood as a window to the indirect measurement of the decay resistance of Scots pine heartwood Holzforschung 61:600-604 doi:[10.1515/hf2007.091](https://doi.org/10.1515/hf2007.091)
24. Kebbi-Benkeder Z, Colin F, Dumarcay S, Gerardin P (2015) Quantification and characterization of knotwood extractives of 12 European softwood and hardwood species Ann For Sci 72:277-284 doi:[10.1007/s13595-014-0428-7](https://doi.org/10.1007/s13595-014-0428-7)
25. Kebbi-Benkeder Z, Manso R, Gérardin P, Dumarçay S, Chopard B, Colin F (2017) Knot extractives: a model for analysing the eco-physiological factors that control the within and between-tree variability Trees 31:1619-1633 doi:[10.1007/s00468-017-1573-z](https://doi.org/10.1007/s00468-017-1573-z)
26. Lu JR, Venalainen M, Julkunen-Tiitto R, Harju AM (2016) Stilbene impregnation retards brown-rot decay of Scots pine sapwood Holzforschung 70:261-266 doi:[10.1515/hf-2014-0251](https://doi.org/10.1515/hf-2014-0251)
27. Miranda I, Ferreira J, Cardoso S, Pereira H (2021) Composition and antioxidant properties of extracts from Douglas fir bark %J Holzforschung 75:677-687 doi:[10.1515/hf-2020-0097](https://doi.org/10.1515/hf-2020-0097)
28. Oleson KR, Schwartz DT (2016) Extractives in Douglas-fir forestry residue and considerations for biofuel production Phytochemistry Reviews 15:985-1008 doi:[10.1007/s11101-015-9444-y](https://doi.org/10.1007/s11101-015-9444-y)
29. Ozgenc O, Durmaz S, Kustas S (2017) Chemical Analysis of Tree Barks using ATR-FTIR Spectroscopy and Conventional Techniques BioResources 12:9143-9151 doi:[10.15376/biores.12.4.9143-9151](https://doi.org/10.15376/biores.12.4.9143-9151)
30. Pietarinen S, Willför S, Ahotupa M, Hemming J, Holmbom B (2006) Knotwood and bark extracts: strong antioxidants from waste materials Journal of Wood Science / J Wood Sci 52:436-444 doi:[10.1007/s10086-005-0780-1](https://doi.org/10.1007/s10086-005-0780-1)
31. Prior RL, Wu XL, Schaich K (2005) Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements J Agric Food Chem 53:4290-4302 doi:[10.1021/jf0502698](https://doi.org/10.1021/jf0502698)
32. Ragauskas AJ et al. (2006) The path forward for biofuels and biomaterials Science 311:484-489 doi:[10.1126/science.1114736](https://doi.org/10.1126/science.1114736)

33. Rosales-Castro M, Gonzalez-Laredo RF, Rocha-Guzman NE, Gallegos-Infante JA, Rivas-Arreola MJ, Karchesy JJ (2012) Antioxidant activity of fractions from *Quercus sideroxylla* bark and identification of proanthocyanidins by HPLC-DAD and HPLC-MS *Holzforschung* 66:577-584
34. Shalaby EA, Shanab SMM (2013) Antioxidant compounds, assays of determination and mode of action *African Journal of Pharmacy and Pharmacology* 7:528-539 doi:10.5897/AJPP2013. 3474
35. Sharma OP, Bhat TK (2009) DPPH antioxidant assay revisited *Food Chem* 113:1202-1205 doi:https://doi.org/10.1016/j.foodchem.2008.08.008
36. Shigo AL (1984) Development and characteristics of discoloured wood *IAWA Bulletin* 5:99
37. Singleton VL, Rossi JA, Jr. (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents *American Journal of Enology and Viticulture* 16:144-158
38. Stratil P, Klejdus B, Kubáň V (2007) Determination of phenolic compounds and their antioxidant activity in fruits and cereals *Talanta* 71:1741-1751 doi:https://doi.org/10.1016/j.talanta.2006.08.012
39. Tavčar Benković E, Grohar T, Žigon D, Švajger U, Janeš D, Kreft S, Štrukelj B (2014) Chemical composition of the silver fir (*Abies alba*) bark extract Abigenol® and its antioxidant activity *Industrial crops and products* 52:23-28 doi:https://doi.org/10.1016/j.indcrop.2013.10.005
40. Tavčar Benković E, Žigon D, Mihailović V, Petelinc T, Jamnik P, Kreft S (2017) Identification, in vitro and in vivo antioxidant activity, and gastrointestinal stability of lignans from silver fir (*Abies alba*) wood extract *J Wood Chem Technol*:1-11 doi:10.1080/02773813.2017.1340958
41. Tekin K, Hao N, Karagoz S, Ragauskas AJ (2018) Ethanol: A Promising Green Solvent for the Deconstruction of Lignocellulose *ChemSusChem* 11:3559-3575 doi:https://doi.org/10.1002/cssc.201801291
42. Thurbide KB, Hughes DM (2000) A rapid method for determining the extractives content of wood pulp *Ind Eng Chem Res* 39:3112-3115 doi:10.1021/ie0003178
43. Torelli N (1990) Les&skorja (Wood&bark). Slovar strokovnih izrazov. Univerza v Ljubljani, Biotehniška fakulteta, Oddelek za lesarstvo, Ljubljana
44. Torelli N, Gorišek Ž, Oven P, Merela M (2005) Mokro srce pri jelki (*Abies alba*). Wetheart in silver fir (*Abies alba* Mill.) *Les wood* 57:4-16
45. Torelli N, Sinjur I, Gorišek Ž (2007) Biologija mokrega srca pri navadni jelki (*Abies alba*) in njegove lastnosti (Biology of wet heart in silver fir (*Abies alba* Mill.) and its propereties) *Gozdarski vestnik* 65:443-460
46. Torelli N, Trajkovic J, Sertic V (2006) Influence of phenolic compounds in heartwood of Silver fir (*Abies alba*) on the equilibrium moisture content *Holz Als Roh-und Werkst* 64:341-342
47. Ul'yanovskii NV, Onuchina AA, Faleva AV, Gorbova NS, Kosyakov DS (2022) Comprehensive Characterization of Chemical Composition and Antioxidant Activity of Lignan-Rich Coniferous Knotwood Extractives *Antioxidants* 11:2338 doi:10.3390/antiox11122338
48. Valette N, Perrot T, Sormani R, Gelhaye E, Morel-Rouhier M (2017) Antifungal activities of wood extractives *Fungal Biology Reviews* 31:113-123 doi:http://dx.doi.org/10.1016/j.fbr.2017.01.002

49. Välimaa A-L, Honkalampi-Hämäläinen U, Pietarinen S, Willför S, Holmbom B, von Wright A (2007) Antimicrobial and cytotoxic knotwood extracts and related pure compounds and their effects on food-associated microorganisms International journal of food microbiology 115:235-243 doi:10.1016/j.ijfoodmicro.2006.10.031
50. Vek V, Keržič E, Poljanšek I, Eklund P, Humar M, Oven P (2021) Wood Extractives of Silver Fir and Their Antioxidant and Antifungal Properties Molecules 26:6412 doi:10.3390/molecules26216412
51. Vek V, Poljanšek I, Oven P (2019) Efficiency of three conventional methods for extraction of dihydrorobinetin and robinetin from wood of black locust Eur J Wood Wood Prod 77:891-901 doi:10.1007/s00107-019-01430-x
52. Vek V, Poljanšek I, Oven P (2020) Variability in content of hydrophilic extractives and individual phenolic compounds in black locust stem Eur J Wood Wood Prod 78:501–511 doi:10.1007/s00107-020-01523-y
53. Vek V, Šmidovnik T, Humar M, Poljanšek I, Oven P (2023) Comparison of the Content of Extractives in the Bark of the Trunk and the Bark of the Branches of Silver Fir (*Abies alba*) Molecules 28:225 doi:10.3390/molecules28010225
54. Verkasalo E, Leppälä J, Muhonen T, Korpinen R, Möttönen V, Kurppa S (2019) Novel industrial ecosystems and value chains to utilize side-streams of wood products industries – Finnish approach Pro Ligno Journal 15:157-165 doi:http://www.proligno.ro/ro/articles/2019/4/VERKASALO.pdf
55. Vítková M, Müllerová J, Sádlo J, Pergl J, Pyšek P (2017) Black locust (*Robinia pseudoacacia*) beloved and despised: A story of an invasive tree in Central Europe Forest Ecology and Management 384:287-302 doi:http://dx.doi.org/10.1016/j.foreco.2016.10.057
56. Willför S, Nisula L, Hemming J, Reunanen M, Holmbom B (2004) Bioactive phenolic substances in industrially important tree species. Part 2: Knots and stemwood of fir species Holzforschung 58:650-659 doi:https://doi.org/10.1515/HF.2004.119
57. Willför SM et al. (2003) Antioxidant activity of knotwood extractives and phenolic compounds of selected tree species Journal of Agricultural and Food Chemistry / J Agric Food Chem 51:7600-7606 doi:10.1021/jf030445h
58. Žepič V, Oven P, Čop M, Vek V, Janković B, Poljanšek I (2022) Physical, Rheological and Mechanical Properties of Alkali Activated Hydrogels Based on Nanofibrillated Cellulose Journal of Natural Fibers:1-13 doi:10.1080/15440478.2022.2123879

Figures

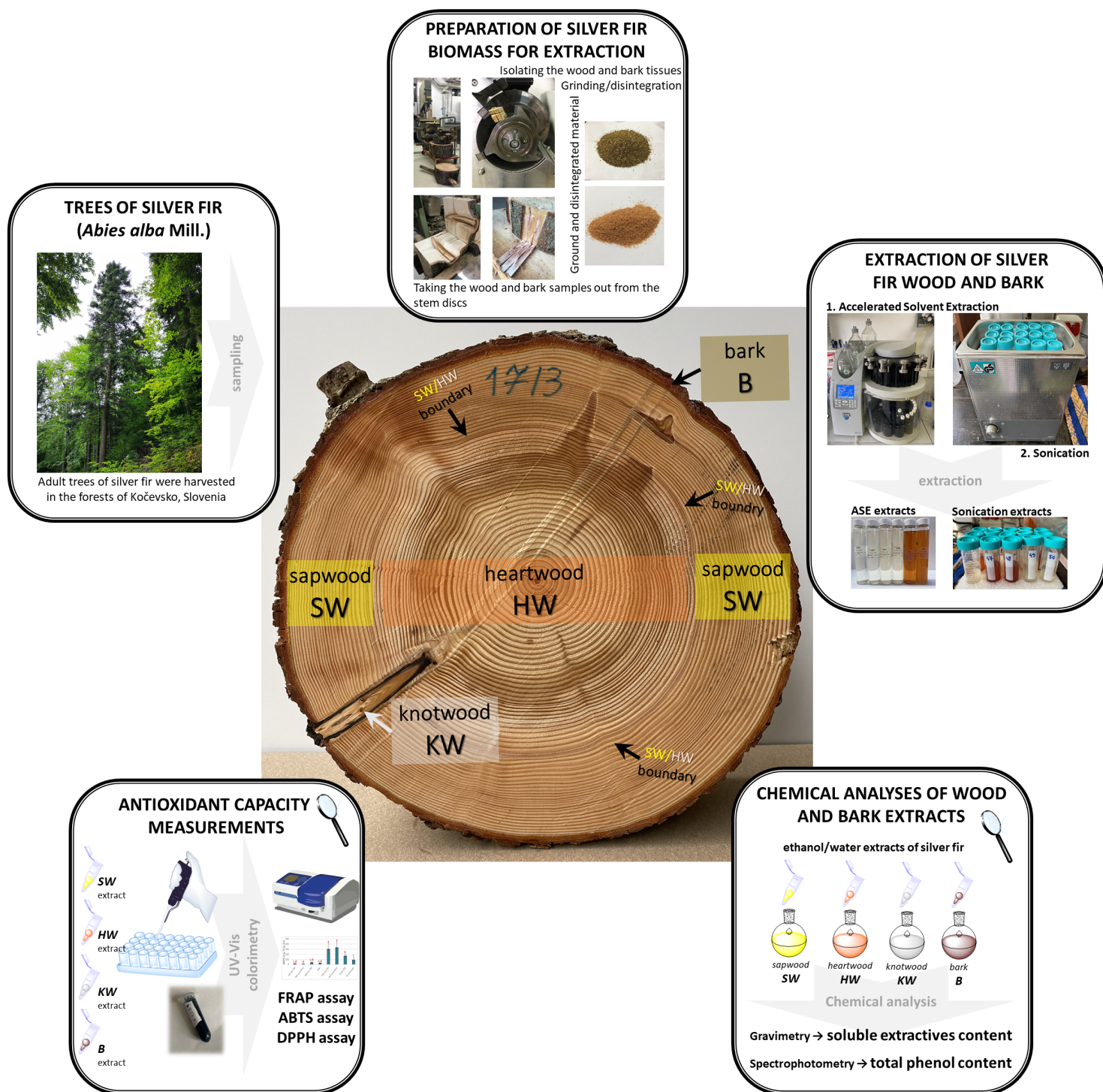


Figure 1

Schematic presentation of research activities. Sapwood (SW), heartwood (HW), knotwood (KW) and bark (B) of wet-hearted silver fir (*Abies alba* Mill.) were extracted by two extraction methods (sonication, ASE 350 system), and extracts were analyzed gravimetrically and colorimetrically for extractant content (soluble extractive content - TEC and total phenolic content - TPC) and antioxidant capacity (FRAP, ABTS, DPPH tests)

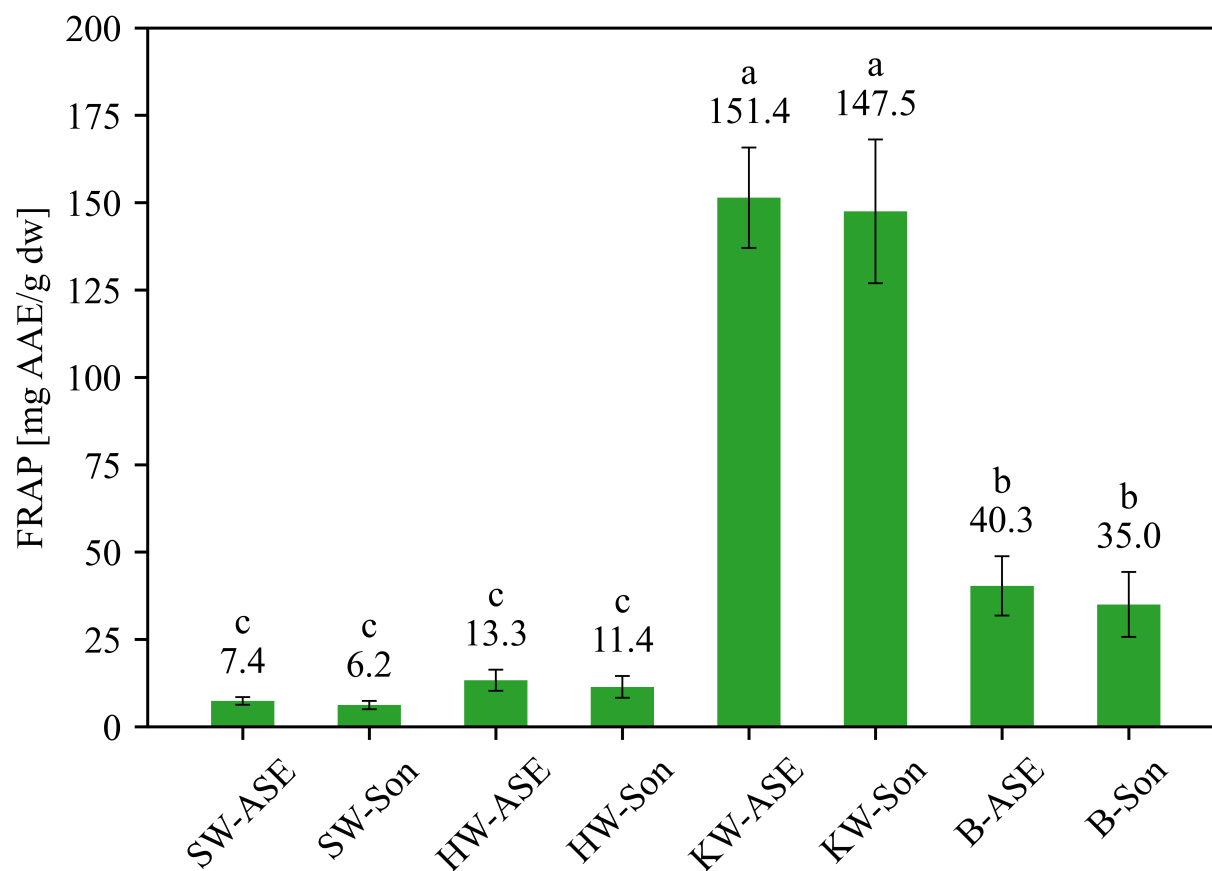


Figure 2

Ferric reducing antioxidant power (FRAP) for sapwood (SW), heartwood (HW), knotwood (KW) and bark extracts (B) of silver fir prepared with different extraction methods (sonication and accelerated solvent extraction (ase350)). Results are expressed in ascorbic acid mass equivalents per gram of dry sample (mg AAE/g dw). a-c, Different letters on bars indicate a significant difference at the $p < 0.05$ level

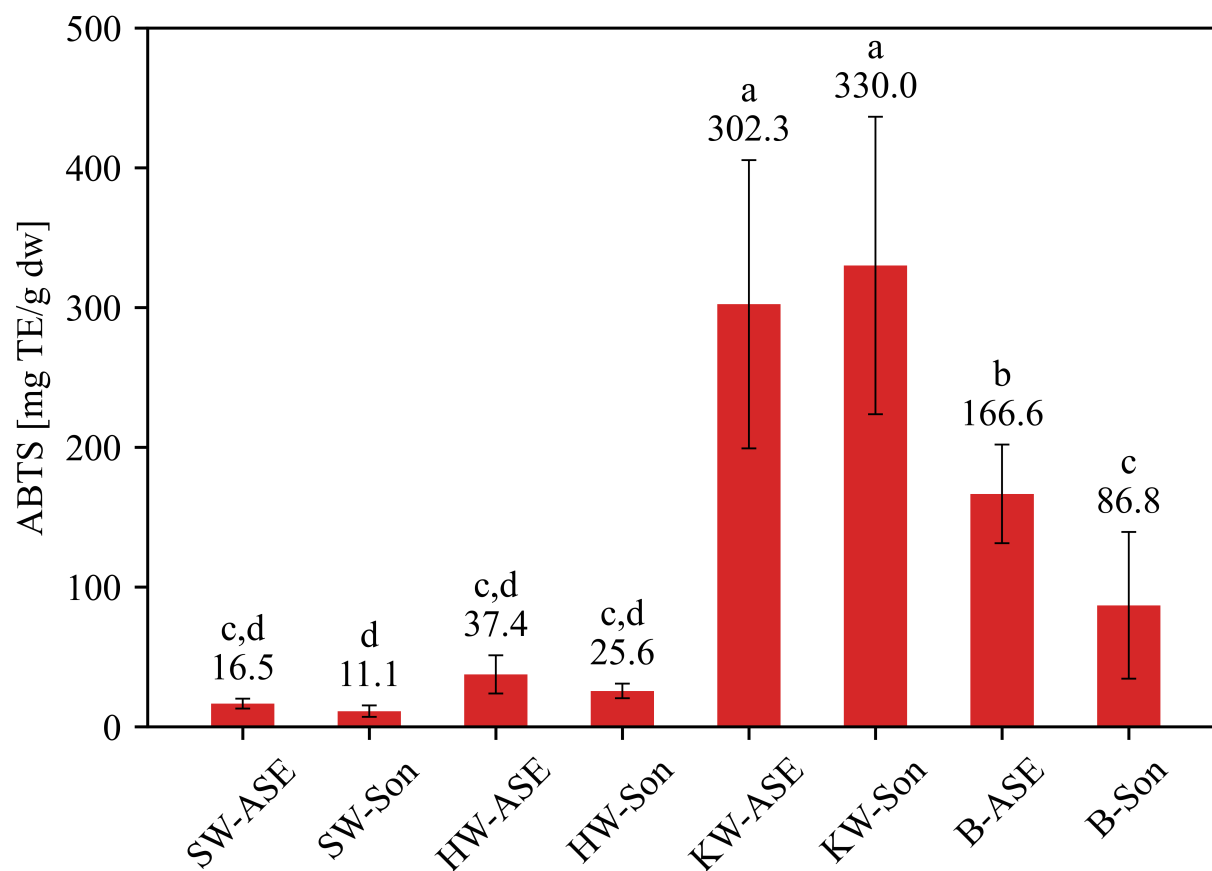


Figure 3

ABTS radical scavenging activity for extracts of sapwood (SW), heartwood (HW), knotwood (KW) and bark (B) of silver fir prepared with different extraction methods (sonication and accelerated solvent extraction (ase350)). Results are expressed in Trolox mass equivalents per gram of dry sample (mg TE/g dw). a-d, Different letters on the bars indicate a significant difference at the $p<0.05$ level

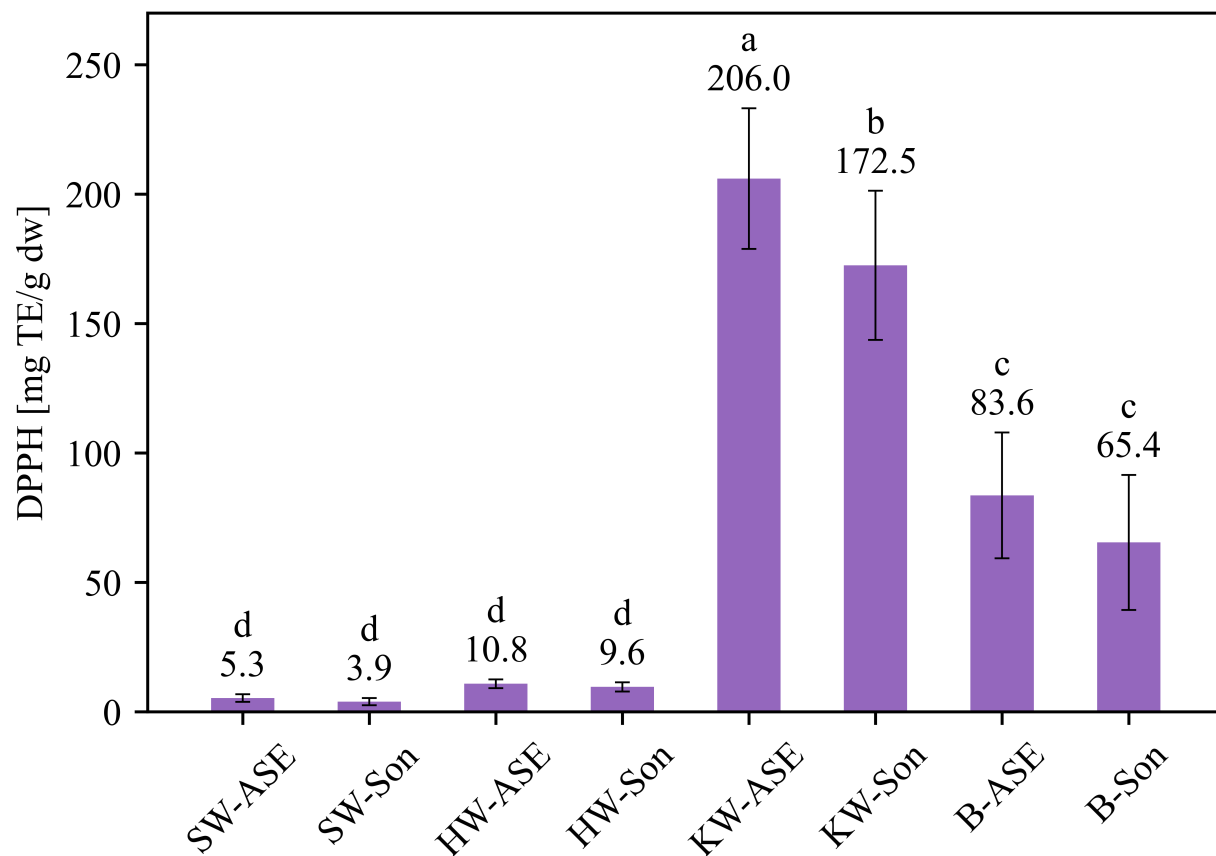


Figure 4

DPPH radical scavenging activity for extracts of sapwood (SW), heartwood (HW), knotwood (KW) and bark (B) of silver fir prepared with different extraction methods (sonication and accelerated solvent extraction (ase350)). Results are expressed in Trolox mass equivalents per gram of dry sample (mg TE /g dw). a-d, Different letters on bars indicate a significant difference at the $p < 0.05$ level