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Investigation of selective extraction of phenolic compounds and of saccharides from *Picea abies* bark using organosolv solvents

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Abstract:

Enormous quantities of softwood and hardwood tree bark are generated yearly in the pulp and woodworking industry. Chemical compounds which can be found in bark extracts have important biological properties and therefore a high potential for using in the production of special formulations. In the present study, extraction of *Picea abies* bark with organosolv solvents was investigated to achieve a high yield of desirable compounds. For the extraction four different solvent ratios of ethanol and water were applied. The temperature range of extraction was set between 40 and 100 °C and stirring velocity of 400 rpm. Extractions were performed with a solid/liquid ratio of 1:20. Yields of phenolics, flavonoids and tannins were determined using gallic acid for total phenolic content (TPC), quercetin for total flavonoid content (TFC) and tannic acid for total tannins content (TTC) as calibration compounds. Qualitative analysis of phenolic constituents was performed after their derivatization, by gas chromatography coupled with mass spectrometry identification. The total yield of bark extract of 14.75 wt.% was achieved by using an ethanol/water ratio of 50% (v/v) and a temperature of 100 °C. Gas chromatography results show 9 aromatic carboxylic acids as phenolic acids and quercetin and (+)-catechin as flavonoid compounds. Highest concentrations of TPC = 3.21 mg gallic acid equivalent/g, TCF = 0.62 mg quercetin equivalent/g and TTC = 0.84 tannic acid equivalent/g were determined at the same process conditions by photometric method. This study determined the conditions for organosolv extraction of useful compounds and suggests that some of extractives, e.g., taxifolin and rhamnetin presented in the spruce bark extracts, have antiviral (SARS-CoV-2) effects. Therefore, this warrants further evaluation of the antiviral properties of spruce bark constituents.

Keywords: Phenolic compounds, organosolv extraction, spruce bark, *Picea abies*, GC-MS identification, Total phenolic content, Total flavonoid content, Total tannins content, Saccharide's content, antiviral properties.

1. Introduction

Enormous quantities of tree bark of softwood (coniferous trees) and hardwood (deciduous trees) are generated yearly in the pulp, paper, and woodworking industry. Chemical compounds which can be found in bark extracts have important biological properties and therefore a high potential for using in the production of special formulations. Bio-based phenolic compounds found in spruce bark are known as value-added products with antioxidant capacity. These bioactive compounds have further antibacterial, anti-inflammatory, antiviral, antimalarial, antimutagenic, antitumor, cytotoxic, fungicidal, insecticidal, pharmacokinetic activities and properties. Therefore, using these extractive

compounds requires adequate separation technology. Over the last few decades, the focus of research within the lignocellulosic biorefinery was set on by-product side streams in agricultural and forestry waste. It is essential for the industry and the economic growth in the future to establish “green” solutions for the refinery and processing of fossil fuels, fossil raw materials and their corresponding products [1]. In this context the valorization of process waste streams remains the objective. Especially bark is currently regarded as one of the most abundant raw materials [2]. Spruce and pine are usually used in the pulp, paper, and woodworking industry. At present, most of the bark waste will be subjected to combustion for heat or electricity. Previous works have identified different aromatic compounds such as lignans, tannins or stilbenes [3]. A comprehensive review of extractives properties and valorization of softwood bark through extraction of utilizable chemicals was carried out in the same study [3].

The extraction and purification of these desired compounds is the key tasks. Organosolv solvents are the preferred process media for biorefining lignocellulosic feedstocks, such as wood [4]. Thus, it is worthwhile to also look to valorize the bark extract by means of organosolv media. The extraction of hydrophobic structures, such as phenolics and flavonoids are the major focus because they have a great application potential due of its antioxidant capacity [5]. Pressurized or unpressurized hot-water extraction is a well-known method to obtain tannins out of bark [6]. The yield of bark extract in this process is around 17 – 30 wt.% due to the temperature range between 100 and 180 °C using pure distilled water [6,7]. In the range of 60 °C to 90 °C the hot water extraction was enhanced with a dosage of 2 wt.% of sodium bisulfite and 0.5 wt.% sodium carbonate to obtain 14.62 mmol phenolic compounds per gram [8]. Due to these extraction conditions high concentrations of non-cellulose carbohydrates such as starch, pectins and hemicelluloses are quantified in the crude extracts. This improved hydrolysis of the glycosidic bonds was observed especially in the temperature range above 100 °C (Autoclav) [9]. The total phenolic content in extracts of hot water extractions were 2.8 and 5.2 g per kg dry bark in the temperature range between 30 °C and 150 °C [10]. Mozdyniewicz et al showed 14.7 wt.% and 16.3 wt.% of total solid yields in hot water extraction of *Picea abies* bark at 120 °C without detailed data [11].

Bark extraction yield is enhanced using ethanol as solvent. Total extraction yields of ethanol and ethanol/water mixtures differ between 6 wt.% [12] and 35 wt.% [13]. Ethanol extracts from spruce bark total phenolic content differ between 10 mg [14] and 45 mg [15] per gram of a crude extract. In previous works of Sladkova et al, 7.61 wt.% of total extract yield was achieved with a temperature of 100 °C and 96.6 % ethanol as solvent [16]. In addition, aromatic compounds can also be extracted out of spruce bark with hexane and acetone at 90 °C in concentrations of 0.7 and 2.8 mg per gram dry bark (as described by Krogell et al [17]. Further experiments were done by Burcova et al, who showed 6.7 wt.% yield after a Soxhlet extraction using 96 % (v/v) ethanol as solvent and 5.4 wt.% total yield using n-hexane as solvent [18]. Kreps et al achieved the isolation of phenolic compounds by the extraction with n-hexane at 60, 80 and 120 °C with yields of 3.8 wt%, 4.1 wt.% and 5.1 wt.% referred to dry bark [19].

In this study, we present a systematic view on the extraction process of spruce bark and demonstrate the impact of the temperature and solvent polarity by different ethanol/water ratios. The concentration of hydrolyzed saccharide monomers compared to the amount of phenolic compound is also shown. The concentration of total phenolic content dependent on solvent polarity, temperature and time is shown to propose an improved biorefinery concept.

Bio-based phenolic compounds found in spruce bark are known as value-added products with beneficial properties. These bioactive compounds have antibacterial, anti-inflammatory, antiviral, antioxidant, antimalarial, antimutagenic, antitumor, cytotoxic, fungicidal, insecticidal, pharmacokinetic activities and properties. Here we would like to highlight the antiviral properties of spruce bark. For example, taxifolin and rhamnetin have proposed effects against coronavirus 2 (SARS-CoV-2 or COVID). In this study [29] were computationally screened over of 606 million compounds for binding at the recently solved crystal structure of the main protease (M^{pro}) of SARS-CoV-2. A screening of such a vast chemical space for SARS-CoV-2 M^{pro} inhibitors has not been reported before. After evaluation of potential off-target binding, a list of 12 purchasable compounds with binding affinity to the target protease was reported. To quickly advise ongoing therapeutic intervention for patients, were evaluated approved antiviral drugs and other protease inhibitors to provide a list of nine compounds for drug repurposing. Furthermore, were identified the natural compounds (–)-taxifolin and rhamnetin as potential inhibitors of M^{pro} . Rhamnetin is already commercially available in pharmacies.

2. Results and Discussion

2.1. Characterization of bark extract

Some important properties of the bark composition are shown in Table 1.

Table 1. Bark composition (wt.% of dm bark)

Constituent	Content in wt. %
Acid insoluble Lignin	36.71
Acid soluble Lignin	3.57
Glucose	29.85
Mannose	9.87
Arabinose	3.33
Galactose	2.76
Xylose	4.57
Ash	2.89

Spruce bark was characterized by NREL analysis dry matter (dm) [30], ash [31], carbohydrates and lignin [32] and calculated relatively to dry matter. The sum of all constituents contains 93 wt. %. The determination of the extractives was performed twice, and the determined values were not given in the characterization of bark composition (Table 1). The results of the extractive compounds yield are shown in Table 2.

Table 2. Extraction yield of spruce bark in % w/w of dry bark

Temp (°C)	EtOH = 0 %	EtOH = 25 %	EtOH = 50 %	EtOH = 75 %
40	5.48 (± 0.12)	7.52 (± 0.11)	10.41 (± 0.21)	10.13 (± 0.18)
60	5.99 (± 0.15)	9.73 (± 0.22)	11.50 (± 0.31)	10.48 (± 0.16)
80	9.49 (± 0.27)	9.66 (± 0.19)	11.67 (± 0.28)	11.84 (± 0.13)
100	10.58 (± 0.23)	14.49 (± 0.48)	14.75 (± 0.34)	11.94 (± 0.17)

Temperature and solvent ratio were selected out as parameters for the extraction. The effect of the extraction parameters on the yields of obtained soluble extracts was described as gravimetric yields. All data are shown in Table 2. Results showed that the solvent ratio of ethanol/water of 50% (v/v) gives the highest crude extract total yield of 14.75 wt. %. Hot water extraction without ethanol showed a 10.58 wt.% yield at 100 °C. Le Normand et al 2014 showed that the saccharides hydrolysis of the non-cellulose polysaccharides starts above 100 °C and increases rapidly at 140 °C and 160 °C [6]. That observation can be explained by low hydrolysis reactions within the carbohydrate backbone in the bark material. Crude extracts by ethanol/water extraction with ethanol content of 75 % (v/v) show constantly yields of more than 10 wt. %. Similar yields were described by Neiva et al [20], who showed 9.48 wt.% with ethanol extraction and 14.88 wt.% by hot-water extraction. The high yield of ethanol extraction even at lower temperatures is influenced by the co-extraction of nonpolar compounds such as fatty acids or resin acids. For this a preliminary extraction with n-hexane was suggested by Valimaa et al [21]. The yield of total extracts was expressed as soluble extracts compared to inserted bark material. At 40 °C and 60 °C the higher amount of ethanol significantly increased the extraction yield.

Ethanol is required for extracting high molecular stilbenes such as piceasides or astringines as detailed described by Gabaston et al [22]. Co et al [13] showed that ethanol and water as solvents lead to confident yields of aromatic compounds. The effect of temperature on extraction remains still a challenge. Even with low amounts of water in the solvent, a certain concentration of carbohydrates is co-extracted because of hydrolysis of the glycosidic bonds. Valimaa et al [21] used an ion exchange resin and high purity water to purify the crude extracts. In other experimental works, crude extracts were dried and dissolved in ethyl acetate as described by St. Pierre [23]. In the present study, a subsequent further treatment of the obtained extracts was not carried out due to direct quantification of saccharide monomers in the liquid phase. The results of determination of saccharides are given as the average of 3 measurements with a standard deviation ≤ 5% and are given in Table 3.

Table 3. Carbohydrate monomers content in % (w/w) in the freeze-dried extracts

No	Ethanol / water % (v/v)	Temperature (°C)	Glu	Xyl	Man	Ara	Gal	Sum
1	0	40	0.39	0.14	0.24	0.15	0.19	1.11
2	0	60	0.47	0.17	0.33	0.17	0.23	1.37
3	0	80	0.49	0.16	0.34	0.18	0.24	1.41
4	0	100	1.80	0.30	0.79	0.27	0.37	2.81
5	25	40	0.32	0.13	0.25	0.17	0.19	1.06
6	25	60	0.31	0.14	0.24	0.16	0.20	1.05
7	25	80	0.38	0.19	0.29	0.19	0.23	1.28
8	25	100	0.41	0.21	0.32	0.21	0.25	1.40
9	50	40	0.18	0.11	0.14	0.12	0.12	0.67
10	50	60	0.21	0.13	0.16	0.13	0.12	0.75
11	50	80	0.23	0.14	0.19	0.11	0.14	0.81
12	50	100	0.26	0.14	0.20	0.15	0.16	0.91
13	75	40	0.14	0.10	0.13	0.07	0.11	0.55
14	75	60	0.16	0.11	0.15	0.11	0.14	0.67
15	75	80	0.17	0.10	0.16	0.12	0.13	0.68
16	75	100	0.19	0.11	0.17	0.13	0.14	0.74

Pure water extraction at 100 °C shows the highest content of carbohydrate monomers. In all obtained extracts, glucose is the predominant saccharide detected in the samples showing concentrations between 0.14 wt. % and 1.08 wt. % (Table 3). Mannose was also detected as the most abundant saccharide in softwood hemicelluloses till concentrations of 0.79 wt. %. Here are several effects to consider. The gap of saccharide concentrations especially in the hot-water extractions between 80 and 100 °C is probably due to hydrolysis reactions of hemicellulose and starch layers within the bark material. In this temperature range, the hemicellulosic fraction is easily hydrolyzed due to its amorphous structure and exposition to the polar solvent. Pectins and higher molecular non-cellulosic polysaccharides are more stable and were quantified at larger concentrations using extraction temperatures of 140 and 160 °C as reported by Le Normand et al [6]. In our described extraction experiments, we did not use such high temperatures to avoid an exceed amount of carbohydrates in the crude extracts.

2.2. Characterization of total content of phenolics, flavonoids and tannins

Total phenolic content is measured as gallic acid equivalent (GAE). Total flavonoid content is determined as quercetin equivalents (QE). Total tannins content is determined as tannic acid (TA). All experimental results were determined as the average of 2 measurements and are shown in Table 4.

Table 4. Concentrations of total phenolic content, total flavonoid content, and total tannins content

Temp (°C)	Parameter	EtOH = 0 % (v/v)	EtOH = 25 % (v/v)	EtOH = 50 % (v/v)	EtOH = 75 % (v/v)
40	TPC*	0.13 (± 0.02)	0.51 (± 0.04)	1.28 (± 0.08)	1.45 (± 0.10)
	TFC*	< 0.01	< 0.01	0.24 (± 0.03)	0.22 (± 0.04)
	TTC***	< 0.01	< 0.01	0.18 (± 0.02)	0.24 (± 0.03)
60	TPC*	0.1 (± 0.01)	0.66 (± 0.05)	2.19 (± 0.17)	1.82 (± 0.14)
	TFC**	< 0.01	< 0.01	0.28 (± 0.03)	0.35 (± 0.04)
	TTC***	< 0.01	0.03 (± 0.01)	0.34 (± 0.03)	0.41 (± 0.05)
80	TPC**	0.17 (± 0.02)	0.91 (± 0.07)	2.76 (± 0.19)	2.72 (± 0.17)
	TFC**	< 0.01	0.12 (± 0.02)	0.51 (± 0.4)	0.53 (± 0.05)
	TTC***	0.09 (± 0.01)	0.14 (± 0.01)	0.39 (± 0.03)	0.64 (± 0.05)
100	TPC*	0.31 (± 0.02)	1.02 (± 0.08)	3.21 (± 0.22)	3.08 (± 0.19)
	TFC**	0.11 (± 0.01)	0.15 (± 0.01)	0.62 (± 0.04)	0.57 (± 0.06)
	TTC***	0.19 (± 0.02)	0.26 (± 0.02)	0.84 (± 0.06)	0.92 (± 0.08)

* TPC – total phenolic content, calibration standard - gallic acid (GAE), expressed as mg of GAE on g of dry bark,

** TFC – total flavonoids content, calibration standard – quercetin (QE), expressed as mg of QE on g of dry bark.

*** TTC – total tannins content, calibration standard – tannic acid (TA), expressed as mg of TA on g of dry bark.

The data obtained show that concentrations of total phenolic content and total flavonoid content are dependent on the ethanol/water ratio in the solvent. Without addition of ethanol, phenolic contents are between 0.13 and 0.31 g per gram of dry bark. Significantly higher concentrations of phenolic and flavonoid compounds were determined in the extracts of 50 and 75 % ethanol/water ratio. By increasing the temperature, the concentrations of the target compounds enriched in the crude extracts. This may be contrary to the results of Le Normand et al [9]. In this previous study hot-water extractions were used to isolate condensed tannins from spruce bark. The disadvantage of pure water extractions with respect to biorefinery processing is the required high temperature above 100 °C and the corresponding impurification of the crude extracts by pectins and hemicelluloses. Ghitescu et al showed in their previous works that 50 % and 70 % ethanol are required to obtain high concentrations of phenolic compounds [12]. Sladkova et al carried out the extraction experiments with pure 96 % ethanol at 100 °C and proposed this as the optimum process [16]. Legault et al described that increasing ethanol content will lead to higher total yields of bark residuals [24]. Therefore, most promising results were achieved using 50 wt.% and 75 wt.% ethanol for the quantification of bioactive aromatic compounds.

2.3. GC-MS characterization of bark extract

One of the key tasks in bark extraction and purification is the identification of the target compounds. For this, GC-MS was used for compound profiling of the crude extract. Through silylation all types of hydrophilic and lipophilic extracted compounds are volatile and identified from mass spectra. In the present study, BSTFA was used in excess to enhance a complete silylation of the phenolic compounds. The GC oven program as well as detection and injection conditions were used compared to previous works on phenolic compounds as described by Proestos et al [25] and Zhong et al [26]. The Total Ion Current (TIC) chromatogram of the experiment (ethanol/water = 50:50, temperature = 100°C) is shown below as a representative example of our study (Figure 1). All phenolic compounds were identified as trimethylsilyl (TMS) derivatives and listed in table 5.

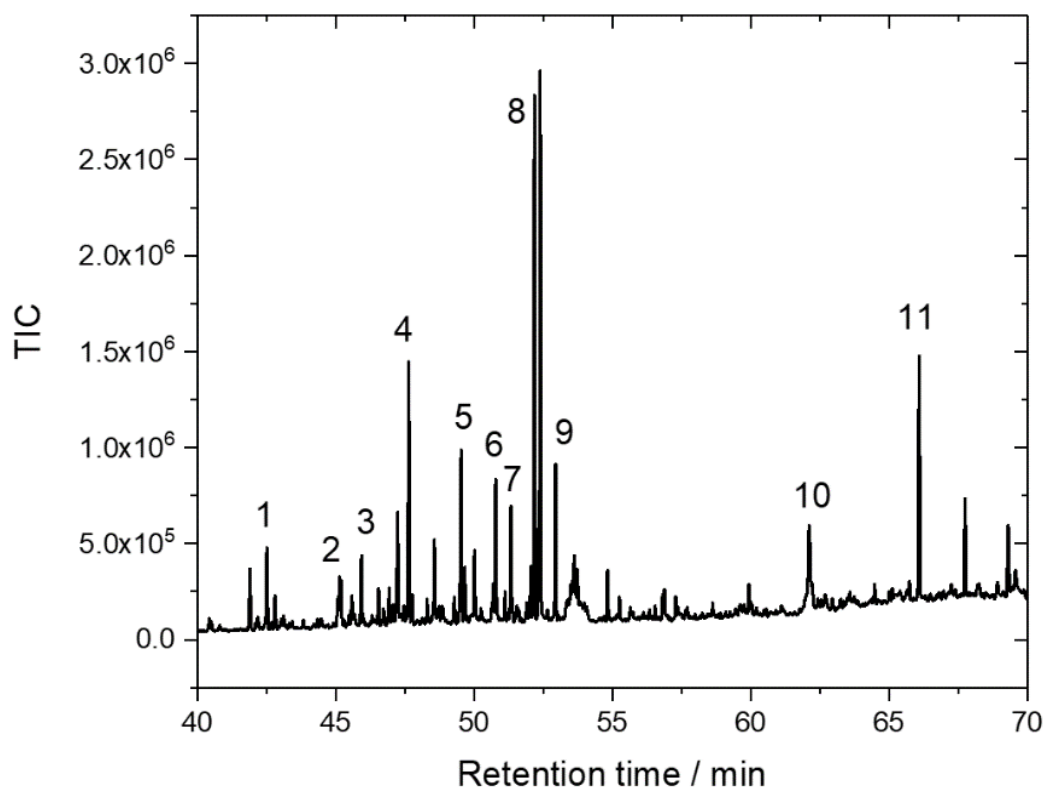


Figure 1. GC-MS analysis of phenolic compounds

(1 = o-Hydroxybenzoic acid, 2 = Ferulic acid, 3 = Syringaldehyde, 4 = Vanillic acid, 5 = p-Hydroxybenzoic acid, 6 = Protocatechoic acid, 7 = p-Coumaric acid, 8 = Gallic acid, 9 = Cinnamic acid, 10 = Quercetine, 11 = (+)-Catechine)

Table 5: Phenolic compounds identified as trimethylsilyl derivatives

N.	Rt * (min)	Compound	Target ions (m/z)
1	42.51	o-Hydroxybenzoic acid	267, 73, 223
2	45.08	Ferulic acid	338, 73, 323
3	45.97	Syringaldehyde	224, 239, 254
4	47.64	Vanillic acid	73, 267, 297
5	49.52	p-Hydroxybenzoic acid	267, 73, 223
6	50.79	Protocatechoic acid	73, 193, 370
7	51.42	p-Coumaric acid	73, 219, 293
8	52.20	Gallic acid	73, 281, 458
9	52.93	Cinnamic acid	205, 131, 161
10	62.04	Quercetine	73, 147, 193
11	66.15	(+) - Catechine	368, 73, 355

* Rt = Retention time

As predominant phenolic compounds, aromatic carboxylic acids showed good resolution of the peaks and revealed an identification of >90 wt. % similarity in the mass spectra library. In most of the mass spectra the base peak of the histograms was $m/z = 73$ due to the trimethylsilyl substituent. This was reported by Rohloff et al [27]. Especially ferulic acid, o-hydroxybenzoic acid and gallic acid are typical phenolic compounds of *Picea abies*. The GC-MS data obtained show a variation of aromatic compounds with different substituents only in the extraction experiments using water/ethanol = 50:50. Whereas experiments with ethanol/water ratio of 25 % (v/v) and without ethanol predominantly showed hydroxyl carboxylic acids and saccharide monomers. By ethanol/water ratio of 75 % (v/v), more lipophilic compounds, such as fatty acids and resin acids, were detected in the extracts. Lazar et al [14] did the extraction of *Picea abies* bark with ethanol/water ratio of 70 % (v/v) and identified aromatic carboxylic acids as predominant compounds by HPLC-UV. As a possible explanation, no lipophilic pre-extraction of the bark material was previously conducted. Especially in the case of the ethanol/water ratio of 75 % (v/v), the occurrence of palmitic acid, stearic acid, abietic and pimaric acid was determined by GC-MS measurements. For the processing of lignocellulosic waste in biorefineries, this is one of the promising findings. The processing should be run by use of the right polarity of the solvent to avoid on the one hand a too high degree of hydrolysis and on the other hand the impurification by lipophilic compounds such as resin or fatty acids.

3. Materials and Methods

3.1. Materials and Reagents

Spruce bark was purchased from SAPPI (Gratkorn, Austria). After drying at 90 °C the bark was milled with a cutting mill and separated using a 1 mm sieve. Ethanol, anhydrous pyridine, aluminium chloride, and sodium acetate were obtained from Carl Roth (Karlsruhe, Germany). Bis(trimethylsilyl)-trifluoroacetamide (BSTFA), Folin-Ciocalteu reagent, quercetin and gallic acid were purchased from Sigma (Taufkirchen, Germany). Glucose, mannose, galactose, arabinose, and xylose were provided from Alfa Aesar (Karlsruhe, Germany).

3.2. Extraction Method

Organosolv extraction was performed in a PARR 4560 Autoclave with a volume of 450 ml (produced by PARR Germany Frankfurt/Main, Germany). The set-up includes the monitoring of time, temperature, pressure, heating, and stirring velocity. Extractions were conducted with a solid/liquid ratio of 1:20. In each case 5 g of spruce bark was examined with differently defined solvent mixtures of ethanol / water. The ethanol/water ratios were chosen with 0 % (v/v), 25 % (v/v), 50 % (v/v) and 75 % (v/v), respectively. Stirring control was kept at 400 rpm. The extraction time was 2 hours for all experiments and temperature was set to 40, 60, 80 and 100 °C. Every experiment was conducted twice. After extraction, the crude suspension was filtered through a funnel with porosity 3. Residual bark was dried at 90 °C for 1.5 hours and its amount determined by gravimetric analysis.

3.3. Determination of Extractives Yield

The yield of extractives (YE, %) in each experiment was determined by drying the bark samples at 105 °C to a constant weight. The results are expressed based on the dry matter weighed before and after extraction, as shown in Equation 1:

$$YE (\%) = 100 \times (m_i - m_j)/m_i, \quad (1)$$

where m_i is the dry mass (g) of the bark before extraction and m_j is the mass (g) of the bark after extraction and drying.

3.4. Determination of Chemical Composition

Determination of ash content, acid insoluble lignin, acid soluble lignin and saccharides was done according to NREL/TP-510-42618 [32]. 300 mg of milled bark was suspended in 3 ml of 72 wt.% sulphuric acid at 30 °C in a heater bath. After storage time of 1 hour 84 ml of distilled water was added into the sample tubes and heated at 121 °C in an autoclave for 1 hour. After cooling down the suspension was filtered over porous crucibles. Determination of acid soluble lignin was expressed as absorbance at 215 nm wavelength. Acid insoluble lignin (Klason Lignin) was determined gravimetrically after drying of the crucibles at 105 °C (24 hours). Concentrations of carbohydrate monomers were quantified by High Performance Anion Exchange Chromatography.

3.5. Determination of Total Phenolics Content (TPC)

1 ml of extract and 5 ml of Folin-Ciocalteu reagent were added into a 25 ml flask and dissolved with 0.1 M sodium hydroxide. 10 ml of distilled water were dosed, and prepared samples were stored for 5 - 8 min for dissolution. 5 ml of 20 wt.% Na_2CO_3 solution were added into the 50 ml flask containing the sample solution and filled up with distilled water. After that, sample solutions were stored for 2 hours at room temperature with mixing every 20 minutes. Total phenolic content was determined as absorbance at 760 nm wavelength in polystyrene cuvettes. All UV/VIS measurements were performed twice on a Thermo Scientific Multiscan Go Spectrophotometer. 10 mg of gallic acid was dissolved in a 50 ml flask in distilled water and used as a calibration substance.

3.6. Determination of Total Flavonoid Content (TFC)

5 ml of extracts and 15 ml of 96 wt.% ethanol was added into a 50 ml flask. After 10 min of dissolution time, 1 ml of aluminium (III) chloride-ethanol ratio of 10 w% (w/v) and 1 ml of 0.1 M sodium acetate solution was added to the sample. The flask was filled up with distilled water. The sample solution was stored for 2 hours at room temperature. Total flavonoid content was determined in two parallel measurements as absorbance at 415 nm wavelength in polystyrene cuvettes. Quercetin was used as calibration substance using 5 mg as standard solution with different dilutions for the calibration curve.

3.7. Determination of Total Tannin Content (TTC)

To 1 ml of extract, 0.5 ml of Folin-Ciocalteu reagent was added into a 25 ml flask. 1 ml of 20 wt. % was dosed and filled up with distilled water. After 2 hours of incubation time the absorbance of the solution was measured at 760 nm in two parallel measurements. TTC was determined using a calibration curve obtained with tannic acid (TA). Results were expressed in milligrams of tannic acid per gram of dry bark.

3.8. Determination of Saccharide's content

All liquid samples were diluted 1:100 and subjected to saccharides quantification. Saccharides contained in extracts were quantified by high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). All measurements were performed on a Dionex 5000+ (ThermoFisher Scientific, Waltham, MA) equipped with a CarboPac20 Column (particle size = 6 μm , pore size $\leq 10\text{\AA}$, crosslinking = 55%, ion exchange capacity = 65 μeq) and a VWD detector. 1.5 mM NaOH was used as solvent A and 200 mM NaOH was used as solvent B. Flow rate was set isocratically with 0.8 ml/min using a gradient of A/B = 80:20. Quantification of saccharides was achieved by a 5-point

calibration. Saccharide's concentrations were quantified 3 times each sample and the determined standard deviation was below 5%.

3.9. GC-MS determination of phenolic compounds

200 µl of extract was pipetted into a 1.5 ml GC vial and subjected to lyophilization at -54 °C for 48 hours. 900 µl of anhydrous pyridine was added to the sample and kept under 70 °C for 1 hour. The sample was cooled down to room temperature and 100 µl of BSTFA was added for derivatization and heated at 70 °C for 1 hour. After cooling down, samples were separated by gas chromatography and identified by mass spectrometry. GC-MS measurements were conducted on a Shimadzu QP2010 gas chromatograph coupled with a Shimadzu QP2020 Dual Stage Mass Spectrometer. A HP5-MS column (60 m length x 0.25 mm inner diameter x 0.25 µm film thickness; J&W Scientific, Folsom, CA, USA) was used. The injection port was operated under the following conditions: split injection, constant column flow: 1.2 mL/min with helium carrier gas, purge flow: 3.0 mL/min, split ratio: 1:10, total flow: 15.2 mL/min; injector temperature: 250 °C constant. Column temperature gradient profile: 50 °C (15 min), followed by 5 °C min⁻¹ to 300 °C (5 min). The mass spectrometric detector was operated in EI-mode (70 eV ionization energy at 1.13 x 10⁻⁷ Pa). Ion source temperature: 200 °C, transfer line: 250 °C. Data was acquired in Scan mode ranging from 45 to 500 m/z. 1 µL aliquots were injected by an AOC 6000 Autosampler. The NIST/Wiley 2014 database was used for compound identification.

4. Conclusions

The use of Organosolv media is of great relevance in the context of the extraction of phenolic bioactive components in the development of lignocellulosic feedstock biorefineries. In this study the extraction of phenols (flavonoids, tannins) from the bark of *Picea abies* was carried out. Organosolv media (ethanol, water) not only extract polyphenols, but also saccharides. Therefore, the choice of the ethanol / water ratio and the choice of suitable extraction conditions minimize the undesired saccharide extraction. Tests with an ethanol / water ratio of 50% (v/v) showed the highest content of phenolic components with 3.21 mg / g dry bark. In the GC-MS analysis, 9 phenols and 2 flavonoids were detected in the crude extract of the experiments with an ethanol / water ratio of 50% (v/v). The pure water extraction at 100 °C showed the highest content of carbohydrate monomers with 2.81 wt. % of the extract. The best conditions for the isolation of hydrophobic tree bark extracts were found at an ethanol / water ratio of 50% and a temperature of 100 °C (14.75 wt. %). In our previous study [3], we report the presence of taxifolin in the spruce bark extracts. Another study [29], in which over 606 million compounds were computationally screened for binding the recently solved crystal structure of the main protease (M^{pro}) of SARS-CoV-2, suggested that taxifolin and rhamnetin have anti-coronavirus 2 (SARS-CoV-2 or COVID) effects. Therefore, together this warrants further evaluation of the antiviral properties of spruce bark constituents.

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Abbreviations: Ara, Arabinose; BSTFA, Bis(trimethylsilyl)-trifluoro-acetamide; dm, dry matter; EtOH, Ethanol; GAE, Gallic Acid Equivalent; Gal, Galactose; GC, Gas chromatography; Glu, Glucose; HPAEC, High Performance Anion Exchange Chromatography; Man, Mannose; MS, Mass spectrometry; NREL, National Renewable Energy Laboratory of the U.S. Department of Energy; PAD, Pulsed Amperometric Detection; QE, Quercetin Equivalent; RT, Retention time; S/L ratio, solid/liquid ratio, TFC, Total Flavonoid Content; TPC, Total Phenolic Content; TTC, Total Tannin Content; wt., weight; Xyl, Xylose.

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