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BIOMASS FROM THE WOOD PROCESSING INDUSTRY AS A SOURCE OF PHENOLIC COMPOUNDS FOR VARIOUS CHEMICAL APPLICATIONS

Martin Štosel – Aleš Ház – Richard Nadányi

ABSTRACT

European forests, which cover approximately 1,040 million hectares in Europe, are crucial sources of renewable biomass. In the Slovak Republic, where forests constitute 41.3% of the land area, broadleaf forests are the dominant type; however, the processing of coniferous wood, particularly spruce (*Picea abies*), is highly prevalent. Spruce wood is extensively used in the papermaking and construction industries due to its rapid growth and increased wood mass production. This study aims to extract phenolic compounds from spruce bark, a byproduct of the wood industry, using supercritical CO2 extraction—a method known for its environmental safety and efficiency. The bark was manually collected, air-dried, and ground to a fraction size of 1-1.5 mm. To enhance the extraction of phenolic compounds, ethanol and ethyl acetate (1:1) were used as co-solvents. Design of experiment (DoE) was used to optimize the extraction conditions, varying temperatures from 40 °C to 140 °C and pressures from 80 to 480 bars. The results indicate that temperature has a significant impact on the extraction yield, with an indirectly proportional relationship observed. The optimal extraction conditions were identified at a temperature of 47.7 °C and a pressure of 80 bars, achieving the highest yield. Subsequently, an analysis was performed using gas chromatography coupled with mass spectrometry (GC-MS), which identified 27 terpenes, 11 resin acids, 4 phenols, 4 phytosterols, and 17 other compounds. The total phenolic content (TPC) was determined using the Folin-Ciocalteu method as Gallic Acid Equivalent (GAE), ranging from 44.376 to 648.752 mg GAE/100 g of dry bark. The antioxidant activity was determined to be in the range from 27.269 to 284.642 mg GAE/g of sample.

Keywords: bark; extraction; phenolic compounds; supercritical extraction; industry utilization.

INTRODUCTION

Forests cover approximately 3.87 billion hectares of the Earth's surface, with Europe accounting for more than a quarter of that, around 1.04 billion hectares. The European Union and its member states represent 5% of the global forest area, roughly 158 million hectares, which make up 37.7% of the EU's land area. Between 1990 and 2010, the EU's forested area grew by 11 million hectares (Nègre, 2022). A similar trend can be observed in Slovakia, where forest coverage has increased by 1% since 1990, and forests now comprise 41.3% of the country's territory (Výročná Správa, 2021, 2022). Coniferous forests make up the largest portion of EU forests at 42%, followed by broadleaf forests at 40% and mixed forests at 18%

(Négre, 2023). In Slovakia, however, broadleaf forests dominate, covering 64.25% of the total forested area (Výročná Správa 2021, 2022). Despite the prevalence of broadleaf forests, coniferous wood processing is widespread in Slovakia, particularly in industries such as paper production and construction, where coniferous wood is favored as a building material. Conifers also benefit from a faster growth rate and higher wood mass yield compared to the slower-growing hardwoods of broadleaf trees. Their rapid growth and widespread distribution across Europe make coniferous forests an excellent source of renewable raw materials.

European forests provide a significant source of renewable biomass, with nearly half of the renewable resources used for energy production coming from wood. Of the wood harvested, approximately 42% is utilized in the energy sector, 24% is used by sawmills, 17% is used in the paper industry, and 12% is allocated for the production of wooden boards (Nègre, 2022). The industrial processing of wood also produces a significant number of by-products, including bark, leaves, needles, and fruits or cones, which are predominantly used for energy generation today.

However, numerous studies indicate that by-products from the forestry, wood, and paper industries contain valuable compounds, particularly phenolic substances. These compounds are widely recognized for their beneficial properties, including antioxidant or scavenging of free radicals, antiviral (Tirado-Kulieva *et al.,* 2022), antibacterial, antifungal (Burčová *et al.,* 2018), cardioprotective and neuroprotective (Freyssin *et al.,* 2020; Rege *et al.,* 2014), anti-inflammatory (Ali Redha, 2021; Lesjak *et al.,* 2011), and especially anticancer (Ali Redha, 2021; Burlacu and Tanase, 2021) effects. As secondary metabolites in plants, phenolic compounds have garnered significant attention in research due to their diverse bioactive properties.

Due to their beneficial effects, these substances have a broad range of applications in industries such as pharmaceuticals, cosmetics, and food, where they are used to extend the shelf life of products (Silva *et al.,* 2021). There is also ongoing research into incorporating phenolic compounds as additives in polymers, mainly for their antioxidant properties (Samper *et al.,* 2013). Furthermore, these phytochemicals are utilized in agriculture for plant protection, particularly as a defense against harmful insects (Neis *et al.,* 2019).

These properties are mainly due to their chemical structure, which includes at least one benzene ring substituted with one or more hydroxyl groups. The hydrogens in the hydroxyls are easily donated in radical reactions, allowing these compounds to act as effective radical scavengers (de la Rosa *et al.,* 2019).

Supercritical CO2 extraction is recognized as a safe and environmentally friendly alternative to traditional extraction methods, which often rely on large amounts of organic solvents. While this technique is primarily used for the isolation of lipophilic compounds, adding a cosolvent like ethanol can shift the polarity, allowing a wider range of substances to be extracted. The use of cosolvents enables this method to isolate natural phytochemicals, including phenolic compounds, which are relatively abundant in plant biomass (Bukhanko *et al.,* 2020; Ghoreishi *et al.,* 2016).

The aim of this study is to highlight the potential of utilizing waste biomass from the wood-processing industry, specifically bark, as a valuable source for extracting bioactive compounds and their subsequent applications across various industrial sectors.

MATERIALS AND METHODS

Spruce (*Picea abies*) bark was manually collected in May 2023 from four trees in western Slovakia near the city of Nitra, air-dried at laboratory temperature, and then ground

to a particle size of 1 to 1.5 mm. The prepared sample was stored in a dark, dry environment until further use.

The moisture content of the spruce bark was measured using the gravimetric method by drying the sample at 105 °C until it reached a constant weight according Tappi T201 cm-03 (2003). Approximately 1 g of the sample was used for this determination, resulting in a moisture content of 9.39%.

Extraction by supercritical carbon dioxide

The extraction of natural substances from spruce bark was performed using the SFT-150 SFE SYSTEM laboratory equipment from Supercritical Fluid Technology, Inc. Pure CO_2 (> 99%, Messer) was used as the extraction agent, with pure ethanol (> 94%, Centralchem) and pure ethyl acetate (> 99.7%, Centralchem) serving as cosolvents in a 1:1 ratio in a batch extraction process.

Two factors and five levels design of experiment (DoE) was used to determine the dependence of extraction yield from the extraction conditions, namely temperature and pressure. The temperatures range from 40 °C to 140 °C and pressures from 80 to 480 bars. For each experiment, approximately 20 g of spruce bark was weighed and extracted. The flow rate of the extraction mixture was maintained at 2 ml/min during the discharge phase of the extraction. The extraction was carried out in dynamic mode for 60 minutes, and the collected extract was stored in a vial with a septum. The vial was cooled during the extraction using ice bath and stored in a freezer for further analysis.

The extracted spruce bark samples were then lyophilized for 24 hours using a Lyovac GT2 (Leybold-Heraeus) lyophilizer. The extraction yield was calculated by comparing the weight of the absolutely dry fresh sample to the weight of the extracted and lyophilized bark.

Gas chromatography

Gas chromatography and mass spectrometry (GC-MS) analysis was conducted using an Agilent 7890 GC gas chromatograph coupled with an Agilent 5975C mass detector, operating in electron ionization mode. The system utilized a capillary column (HP-5MS, 30 m × 250 μm i.d., 0.25 μm film thickness; Agilent) for the separation and analysis of the extracted compounds. The temperature program for the chromatograph oven started at 80 °C, held for 2 minutes, then increased at a rate of 10 °C/min to 260 °C, followed by heating rate of 5 °C/min to 300 °C. The final temperature was maintained for 8 minutes. The minimum concentration of the extract in the solvent was set to 10 mg/mL, and the injection volume was 1 μL.

Determination of Total Phenolic Content

The total phenolic content (TPC) in the spruce bark extracts was determined using UV-Vis spectroscopy, which involves previous redox reactions of Folin-Ciocalteu's reagent with phenolic compounds. To prepare the stock solution, 0.25 g of the extract was placed in a 10 mL flask, which was then filled with ethanol. The reaction mixture was prepared by combining 0.25 mL of the stock solution with 0.25 mL of Folin-Ciocalteu's reagent and 1.25 mL of a 20% Na_2CO_3 solution in a 10 mL volumetric flask, which was subsequently filled with distilled water. After thorough agitation, the mixture was allowed to stand for 1 hour at ambient temperature. The absorbance of the resulting solution was measured against blanks using 0.5 cm cells at a wavelength of 765 nm. The concentration of phenolic compounds was expressed as milligrams of gallic acid equivalents (GAE) per 100 g of dry bark, using a linear calibration curve. All measurements were conducted in triplicate for each individual sample (Jablonsky *et al.,* 2020).

Determination of Antioxidant Activity

The determination of antioxidant activity (AOA) in the extracts was carried out based on the free radical scavenging ability of 2,2-diphenyl-1-picrylhydrazyl (DPPH) using a modified method from (Brand-Williams et al., 1995). Samples with a concentration of 350 µg/mL were prepared using the same solvent mixture (ethanol and ethyl acetate in a 1:1 v/v ratio) employed during the extraction process. A DPPH solution was then prepared at a concentration of 120 µg/mL. The prepared solutions were mixed in a 1:1 ratio and measured at a wavelength of 517 nm using the Epoch 2 microplate reader from BioTek USA.

RESULTS AND DISCUSSION

The DoE aimed to optimize extraction conditions, specifically temperature and pressure, to achieve the highest possible extraction yield. In total, 13 extractions were conducted over a temperature range of 40 to 140 °C and a pressure range of 80 to 480 bars. The extracts were collected in cooled vials to minimize the undesirable evaporation of volatile compounds and the solvents used (ethanol and ethyl acetate in a 1:1 v/v ratio). Following the completion of all experiments, we assessed the optimal extraction conditions. The mathematical model developed for this work indicated that extraction temperature significantly affects yield during the supercritical extraction of spruce bark using carbon dioxide. The optimal conditions for achieving the highest extraction yield were determined to be 47.7 °C and 80 bars. These conditions will be used for further extraction to validate the results of the planned experiment. Notably, the results demonstrated an inverse relationship between temperature and extraction yield.

Based on the analysis of extracts from spruce bark using GC-MS, a total of 63 compounds were identified: 27 terpenes, 11 resin acids, 4 phenolic compounds, 4 phytosterols, and 17 other substances (Tab 1). This distribution indicates that predominantly lipophilic groups of phytochemicals were extracted. This fact can be explained by the extraction with supercritical carbon dioxide, which primarily obtains non-polar and lipophilic natural molecules from plant matrices (Bukhanko *et al.,* 2020; Ghoreishi *et al.,* 2016)**.** A change in polarity can be achieved by the addition of co-solvents such as ethanol and ethyl acetate, which in our case proved ineffective compared to the use of a mixture of ethanol and water. However, our extracts demonstrate higher antioxidant activity as well as total phenolic content compared to comparable extractions using 10%, 20%, and 40% ethanol as co-solvents.

The evaluation of total phenolic content in individual extract samples from the planned experiment was performed using the Folin-Ciocalteu method, and the results were expressed as mg GAE per 100 g of dry bark. The highest total phenolic content was 648.752 mg GAE/100 g of dry bark, corresponding to extraction conditions of 90 °C and 40 bars, while the lowest TPC value of 44.376 mg GAE/100 g of dry bark was observed at the highest temperature of 140 °C and a pressure of 80 bars.

The results of the AOA evaluation, measured as the DPPH radical scavenging ability, ranged up to 284.642 mg GAE/g of the sample under extraction conditions of 40 °C and 90 bars. In contrast, the lowest AOA value 27,269 mg GAE/g of sample was found under extraction conditions of 90 °C and 480 bars.

The results of both the TPC and AOA assessments suggest that these values are significantly influenced by pressure and increased temperature, which may cause thermal degradation of the molecules in the extract.

Tab.1 Table of identified compounds in spruce bark extracts by GC-MS.

Compound number (#)	RT (min)	Hit Name		Mol Weight (amu)	CAS Number
1	3,411	α-pinene		136,125	007785-26-4
2	3,93	β-pinene		136,125	018172-67-3
3	4,566	Limonene		136,125	005989-27-5
4	5,702	cis-Verbenol	(1;14;15;16)	152,12	1845-30-3
5	5,429	β-Camphor	(7)	152,12	13854-85-8
6	5,812	Fenchol	(7)	154,136	001632-73-1
7	5,929	α-Campholenal		152,12	004501-58-0
8	6,169	Pinocarveol		152,12	000547-61-5
9	6,228	trans-Verbenol		152,12	1820-09-3
10	6,351	Pyranone	(14)	144,042	028564-83-2
11	6,468	Pinocarvone		150,104	030460-92-5
12	6,552	endo-Borneol		154,136	000507-70-0
13	6,682	Verbenyl ethyl ether	(9;15)	180,151	080581-06-2
14	6,688	Pinocamphone		152,12	547-60-4
15	6,844	Octanoic acid, ethyl ester	(7;13;14;15)	172,146	000106-32-1
16	6,889	L-α-Terpineol		154,136	010482-56-1
17	6,961	2-Pínén-10-ol		152,12	019894-97-4
18	7,156	L-Verbenone		150,104	001196-01-6
19	7,597	Coumaran	(14;15)	120,058	000496-16-2
20	8,136	Bornyl acetate		196,146	000076-49-3
21	8,648	2-Methoxy-4-vinylphenol	(n.i. in 7;8;13)	150,068	007786-61-0
22	9,518	Decanoic acid, ethyl ester		200,178	000110-38-3
23	9,57	β-Elemene		204,188	000515-13-9
24	10,822	β-Selinene		204,188	017066-67-0
25	10,92	α-Selinene		204,188	000473-13-2
26	11,127	γ-Cadinene	(11)	204,188	39029-41-9
27	11,731	Citronellyl valerate	(7;8;9;11)	240,209	7540-53-6
28	12,841	Benzenepropanol, 4-hydroxy-3-methoxy-		182,094	002305-13-7
29	13,749	Oplopanone		238,193	001911-78-0
30	13,925	Coniferyl	(1;14;15)	180,079	32811-40-8
31	15,709	Thunbergen		272,25	001898-13-1
32	15,988	Cambrene A	(3;7;9;14;16)	272,25	031570-39-5
33	16,345	Manoyl oxide		290,261	000596-84-9
34	16,43	α-Pinacene	(n.i. in 7;8)	272,25	064363-64-0
35	16,923	Thunbergol		290,261	025269-17-4
36	17,189	Sclareolide		250,193	000564-20-5
37	17,475	Sclareol		308,272	515-03-7
38	17,695	Prasterone	(3)	288,209	53-43-0
39	17,702	trans-β-Ionone	(13)	192,151	000079-77-6
40	17,708	Longifolene	(11)	204,188	000475-20-7
41	17,793	Abienol	(n.i. in 9)	290,269	1616-86-0
42	18,221	1-Propene, 1,2-bis(4-methoxyphenyl)-	(n.i. in 3;7;13;16)	254,131	020802-02-2
43	18,552	Copalol	(n.i. in 13)	290,261	021738-29-4
44	18,63	Copalic acid methyl ester	(1)	304,24	024470-48-2
45	18,863	Dehydroabietic aldehyde		284,214	013601-88-2
46	19,116	Retinoic acid	(15)	300,209	000302-79-4
47	19,246	(3E,5E,7E)-6-Methyl-8-(2,6,6-trimethyl-1-cyclohexenyl)-3,5,7-octatrien-2-one	(7;11;13)	258,198	017974-57-1
48	19,415	Methyl dehydroabietate		314,225	001235-74-1
49	19,461	Squalene	(1)	410,391	111-02-4
50	19,681	Dehydroabietyl alcohol		286,23	003772-55-2
51	19,973	Retinol	(1;3;11;14;16)	286,229	69-26-8
52	20,492	1-Octadecene		252,282	000112-88-9
53	20,583	Hexadecanoic acid, octyl ester	(13;14;15)	368,365	16955-58-3
54	20,772	Methyl 7,13,15-abietatatrienoate		300,209	54850-32-7
55	20,992	α-levantenolide	(7;14;15;16)	318,219	30987-48-5
56	21,044	15-Hydroxydehydroabietic acid, methyl ester	(n.i. in 14;15;16)	330,219	029461-23-2
57	22,336	Stearic acid, octyl ester	(15;16)	396,396	109-36-4
58	23,153	Lignoceric acid, ethyl ester	(14;15)	396,396	24634-95-5
59	27,722	Campesterol	(n.i. in 1;13)	400,371	474-62-4
60	28,001	o-o-dimethyl-Pinoresinol	(n.i. in 1;8)	386,173	526-06-7
61	28,696	γ-Sitosterol		414,386	000083-47-6
62	30,377	γ-Sitostenone	(n.i. in 7)	412,371	84924-96-9
63	32,979	Dehydroabietic acid		300,209	001740-19-8

* (The numbers 1-13 indicate in which sample the respective molecules were identified or not identified.).

CONCLUSION

In this study, a two-factor and five level DoE was implemented to optimize the extraction conditions for supercritical carbon dioxide extraction, using ethanol and ethyl

ester as cosolvents in a 1:1 volumetric ratio. The evaluation of the results revealed that the optimal conditions for achieving the highest extraction yield were a temperature of 47 °C and a pressure of 80 bar.

The total phenolic content of the extracts was determined using the Folin-Ciocalteu method, with results ranging from 44,376 to 648,752 to mg GAE/100 g of dry spruce bark. Additionally, the antioxidant activity of the prepared extracts was assessed using the DPPH method. It was observed that the extract prepared at 40 °C and 90 bar exhibited the highest antioxidant activity (284.641 mg GAE/g of sample), whereas the lowest activity (27.269 mg GAE/g of sample) was obtained at the highest tested pressure of 480 bar combined with a temperature of 90 °C.

These results indicate that both the antioxidant activity and the total phenolic content of the extracts are significantly influenced by the extraction conditions, particularly temperature and pressure. Furthermore, elevated temperatures may lead to the thermal degradation of bioactive compounds present in the spruce bark sample.

The extracts were further evaluated through GC-MS, which identified 27 terpenes, 11 resin acids, 4 phenolic compounds, 4 phytosterols, and 17 other substances. Based on this distribution, it is clear that the supercritical extraction using carbon dioxide and the cosolvent ethanol and ethyl acetate in a 1:1 ratio did not succeed in increasing the polarity of the extraction medium to such an extent that the extraction of phenolic compounds would occur in larger quantities. However, looking at the total phenolic content, there appears to be a relatively high occurrence of phenolic compounds in the prepared extracts. The Folin-Ciocalteu method is an approximate determination of total phenolic content because the reagent reacts with hydroxyl groups. These groups may also be present in compounds such as terpenes, resin acids, and other substances, potentially skewing the results.

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AUTHORS' ADDRESSES

Ing. Martin Štosel[1]
doc. Ing. Aleš Ház, PhD.[1]
Ing. Richard Nadányi, PhD.[1,2]
[1]Slovak University of Technology in Bratislava, Faculty of Chemical and Food Technology, Institute of Natural and Synthetic Polymers, Department of Wood, Pulp and Paper; Radlinského 9, 812 37 Bratislava;
[2]Christian Doppler Laboratory for Next-Generation Wood-Based Biocomposite, Institute of Chemical, Environmental and Bioscience Engineering (ICEBE), TU Wien, Getreidemarkt 9, 1060 Vienna, Austria
martin.stosel@stuba.sk
ales.haz@stuba.sk
richard.nadanyi@stuba.sk; richard.nadanyi@tuwien.ac.at