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TOTAL PHENOLIC CONTENT IN SPRUCE BARK

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Abstract

Tree bark is a renewable raw material. It is composed of major compounds such as cellulose, hemicellulose or lignin and minor compounds as extractives and ash. A promising source of many products with valuable substances of tree bark are extractives, especially phenolic compounds. These bioactive phenolic compounds are the major constituent of spruce bark. They have antithrombotic, antioxidant, and anti-inflammatory properties. The potential applications of phenolic compounds have the extensive use. In summary, phenols demonstrated favorable effect particularly on human health like protective effect on cardiovascular disease, thrombosis, and tumorigenesis. Phenols could be used in a variety of fields, from pharmaceutical to chemical industries.

The work was focused on isolation and identification of extractives from the bark of Norway spruce. The raw material was treated by supercritical fluid extraction with carbon dioxide. Supercritical fluid extraction was carried out as static extraction for 50 min. at 7 388 PSI with pure CO_2 at 50 - 90 ^{0}C with 50 - 85% ethanol as co-solvent. Yields were determined by weighting. The work was also focused on the determination of total phenolic content (TPC). TPC was measured using the Folin–Ciocalteu assay and the TPC was expressed as gallic acid equivalent (GAE).

The highest yield of extractives (6.4%) was reached extracting: temperature 88 ^{0}C, pressure 7388 psi, the concentration of co-solvent ethanol 51 %. For the characterization extractives from the spruce bark was used gas chromatography coupled with mass spectrometry. The main identified compounds in bark were methyl dehydroabietate acid, and derivatives of isopymaric acid and hexadecanoic acid methyl ester.

Exploitation of barks extractives should be in the future one of the most important sources of valuable substances - materials, bioactive chemical, adhesives, and at least a source of upgraded food.

***Key words**: Tree bark, Extractives, Phenolic compounds.*

1. Introduction

In recent years, the interest in plant raw products has risen enormously. Many plant-derived substances such as phenolic compounds have attracted increasing interest. These compounds are powerful antioxidants and secondary plant metabolites, which are important determinants of nutritional and sensory quality of vegetables, fruits and higher plants [1, 2]. Natural phenols have been reported to have excellent properties as food preservatives. Moreover, they show photoprotective properties in terms of absorption of UV radiation and preventing photodamage to molecular structures of the human skin. Epidemiological data also have shown that phenolic compounds indicate health benefits to the human body. It is due to their antioxidant effect [1, 2, and 3]. Antioxidant activity of phenolic compounds appears to be closely related with the prevention of degenerative illnesses such as the different types of cancer, neurological diseases, cardiovascular diseases, oxidative stress dysfunctions, prevention of obesity, protection against a number of pathological disturbances, such as atherosclerosis, brain disfunction. Polyphenols have many industrial applications. They may be used as natural colourants in the production of paints, dyes or in cosmetics. [1, 3, 4, 5, and 6].

The term 'phenolic' or 'polyphenol' may be defined chemically as a substance which possesses an aromatic ring bearing one or more hydroxy substituents, including functional derivatives (esters, methyl ethers, glycosides, etc.) [7]. The main group of polyphenols with antioxidant activity are: flavanoids, phenolic acids, tannins, lignans and stilbenes [1]. Phenolic compounds are one of the most widely occurring groups of phytochemicals. They play an important role in reproduction, growth, colour and sensory characteristics of plants, providing protection against predators and pathogens [8].

The key point in getting extractives with desired properties is to find a suitable method of extraction and experimental conditions of isolation. The total

phenolic content from spruce bark with different method extraction such as accelerated solvent extraction, microwave-assisted extraction or extraction with deep eutectic solvents was determined in works [9, 10, and 11]. Supercritical fluid extraction (SCE) with carbon dioxide is considered an alternative method for the extraction of a large class of bioactive compounds (volatile oils, triterpenoids, triterpenes, phenolic compounds, lipids). SCE is the process of separating one component (the extractant) from another (the matrix) using supercritical fluids. A supercritical fluid can be any substance at a temperature and pressure above its critical point. It can diffuse through solids like a gas, and dissolve materials like a liquid. Carbon dioxide is besides water the most commonly used supercritical fluid for extraction of raw plants. Carbon dioxide is readily available, inexpensive, nontoxic, nonflammable and environmentally acceptable. Conditions for supercritical CO_2 are above the critical temperature 31 ^{0}C and critical pressure of 31.3 MPa [12 - 17]. The work of Talmaciu *et al.*, [18], investigated the method to extract polyphenolic substances from spruce bark, namely extraction by ethanol, extraction supported by ultrasound (UAE) and SCE. They found that total phenolic compounds retrieved from spruce bark are depended on the extraction techniques. At extraction by UAE was the content of phenolic compounds 33.48 mg GAE g^{-1}, and the using supercritical carbon dioxide, the value reached 122.41 mg GAE g^{-1} [18]. According to the work of Co *et al.*, [19], supercritical extraction provides extracts with higher content. This may be due to the use of the critical point of fluid to increase extraction yield of total polyphenolic substances [20]. In the Table 1, of the summary of the recent works published on this topic, is shown.

The aim of this study was the isolation and determination of total phenolic content in spruce bark. Tree bark, especially spruce bark is a rich source of phenolic compounds which have been identified as a strong antioxidant [2]. In this work, phenolic compounds were isolated by using supercritical extraction. The isolation procedure is important because the extraction conditions determine the quality and the yield of the individual constituents. The total phenolic content was determined by using Folin-Ciocalteu assay.

2. Materials and Methods

2.1 Plant materials

The sample of Spruce bark (*Picea Abies*) was provided as waste product from the wood processing company Bio Energo, Slovak Republic. The spruce bark was dried at room temperature 24 ^{0}C. The samples of bark were milled and separated to 1 mm fraction by using a knife mill. The humidity of the material (10.93% ± 0.01) was determined by drying ~1 g of spruce bark at 105 ^{0}C for 6 hours until complete water removal according to ISO 3130:1975. The samples were extracted using the supercritical extractor SFT-150.

2.2 Chemicals

Chemicals and reagents were obtained from the following commercial sources: carbon dioxide (Messer Tatragas, Slovak Republic), ethanol 96.6% (Centralchem, Slovak Republic), gallic acid (Alfa Aesar, USA), Folin-Ciocalteu reagent (VWR International, USA), sodium carbonate (Centralchem, Slovak Republic), pyridine (Centralchem, Slovak Republic), N, N-dimethylformamide dimethyl acetal (Sigma Aldrich, Germany)

2.3 Extraction with supercritical carbon dioxide

Extractions were performed using supercritical extractor SFT-150. All bark samples (40 g) were loaded into the vessel of the extractor. Then ethanol as co-solvent was added to the sample. The samples were extracted using in a static mode with pure CO_2 and with ethanol about concentration 51% and 85%, (V = 200 mL), temperature 52 ^{0}C and 88 ^{0}C and a constant pressure 51 MPa (7388 PSI) for 50 min.

2.4 Yield of extractives

The yield of extractives (Y, %) was determined after each experiment by drying the samples at 105 ^{0}C to a constant weight. The results are expressed on the basis of the dry matter before and after extraction as shown in Equation 1:

$$Y\ (\%) = 100 \times (m_1 - m_2)/m_1 \qquad (1)$$

Where:
Y is the yield of extractives (%), m_1 is the mass (g) of the bark before extraction, and m_2 is the mass (g) of the bark after extraction and drying.

2.5 GC/MS analysis

GC/MS analysis was performed on a gas chromatograph (Agilent 7890 GC) coupled with mass detector (Agilent 5975C) which ran electron ionization equipped with a capillary column (HP-5MS, 30 m × 250 µm i.d.,

Table 1. Summary of the works published on the SCE extraction of bioactive compounds in the period 2007 -2009

Plant material	Compounds of interest	Related functional activities	Ref.
Pinus sp.	Flavanoids	Antioxidant activity	[21]
Ramulus cinnamoni	Volatile oil	Antioxidant activity	[22]
Cardamon	Fatty acids, tocopherols, volatile oils	Antioxidant activity	[23]
Braccharis dracunculifolia	Phenolic compounds	Antioxidant activity	[24]

0.25 µm film thickness; Agilent). Helium was used as carrier gas at a rate of 2 mL/min. Chromatograph oven temperature program was 120 ^{0}C held for 2 min, then the heating of 10 ^{0}C/min^{-1} to 300 ^{0}C. The final temperature was held for 10 min. Recording and evaluation of data were performed by using ChemStation software E 02/01/1177 and identification of compounds using electronic libraries NIST and Wiley.

2.6 Derivatization

Chemical derivatization of an analyte improves sensitivity and detectability. [25] From the published derivatization techniques is shown as the preferred alkylation (methylation) with DMF-DMA (N, N-dimethylformamide dimethylacetal). Pyridine was used as the solvent for derivatization. 50 mg dried sample was used as a mixture (1 : 1) of 0.5 mL pyridine and 0.5 mL of DMF-DMA. Derivatization was carried out at 75 ^{0}C for 15 min.

2.7 Total phenolic estimation

Total phenolic content (TPC) of spruce bark extracts was determined spectrophotometrically by the Folin–Ciocalteu method [26, 27]. This method is based on the colourimetric oxidation/reduction reaction of phenols with the Folin-Ciocalteu reagent. For all analyses, 0.5 mL of diluted extract (distilled water for control sample) was mixed with 0.5 mL Folin-Ciocalteu reagent. After 3 minutes 1.5 mL of Na_2CO_3 (20 g/L) was added and made up to 10 mL volume with water. After stirring, the mixture was incubated at room temperature for 120 min in a dark place. The absorbance of the solution was recorded at 765 nm using a UV-VIS spectrophotometer. The total phenolic content (TPC) in extracts was quantified using a calibration curve obtained with gallic acid (GAE) and expressed in mg GAE per 1mL of extract.

3. Results and Discussion

3.1 Extraction of phenolic compounds

The results that show the effect of concentration of ethanol and temperature of extraction on the yield of extractives at a constant pressure 7388 PSI are depicted in Table 2. Both parameters as the temperature of extraction and concentration of co-solvent have the influence to the process efficiency. Extracting samples at 51% concentration of co-solvent produced higher yields compared to at a higher concentration of co-solvent 85%. The yield of extractives from the spruce bark was between 2.99 % and 6.41 %. A lower concentration of co-solvent was found as favourable parameter for the higher extraction efficiency. The concentration of co-solvent enhances the solubility of analytes in the solvent and increases the interactions of target analytes with solvent. The highest yield of extractives (6.41%) was reached with the following conditions: a temperature of 88 ^{0}C, a concentration of ethanol 51%, a pressure 7388 PSI (51 MPa) at 50 min.

Temperature is one of the most critical parameters for SCE. High temperature used during the process affects properties of a solvent. Increased temperature causes an increase in solubility of analytes and an increase in diffusion rates. Increasing temperature was found to be favourable for the extraction efficiency, but only for 88 ^{0}C and 51% concentration of ethanol extraction. At temperature 88 ^{0}C and 85% concentration of ethanol, the yields were lower. High temperatures might affect thermo-labile compounds that are subject to disintegration and hydrolytic degradation. The process parameters for SCE and the results are shown in Table 2.

3.2 Total phenolic content

Characterization and identification of biological activity represent a key tool determining the choice of acceptable methods. In recently published work was identified 237 bioactive compounds in the bark of softwood. The substances in the softwood bark extracts and their physical properties were identified in the recent published study (Jablonsky, M. *et al.,* [28]). Extractive compounds identified from softwood barks contain hundreds of substances (28), some on which have cytotoxic (properties of 25 identified substances), antioxidant (26 substances), fungicidal (20 substances), antibacterial (42 substances) effects, and are repellents (9 substances), antifeedants (2 substances), or increase the activity of pheromones or act themselves as pheromones (10 substances). The polyphenolic compounds such as: astringin, catechin, epicatechin, ellagic acid, ferulic acid, gallic acid, hydroxymatairesinol, isolariciresinol, isorhapontigenin, isorhapontin, lariciresinol, lariciresinol-9-p-coumarate, methylthymol, p-coumaric acid, piceatannol, piceid, podocarpic acid, quercetin, resveratrol, sesquipinsapol B, sinapic acid, tannic acid, taxifolin, vanillic acid, and vladinol D were identified in the work (Jablonsky *et al.,* [28]).

Table 2. Process parameters for extraction with SCE and yields of extractives from spruce bark

Extraction temperature (^{0}C)	Extraction pressure (PSI, MPa)	Concentration of ethanol (% v/v)	Yield of extractives (%)	Total phenolic content (mg GAE/ml)
52	7388 PSI (51 MPa)	51	6.09	24.16
52	7388 PSI (51 MPa)	85	5.31	29.48
88	7388 PSI (51 MPa)	51	6.41	19.14
88	7388 PSI (51 MPa)	85	2.99	20.13

In our work were found these phenolic compounds: guaiacol, 2-Methoxy-4-vinylphenol, trans-isoeugenol, epicubenol, tau-muurolol, alpha-cadinol, oplopanone. The compounds of extract analysed by GC/MS are shown in Table 3. The Tab.2 presents total phenolic content in extracts expressed as mg GAE/mL. The total extracted polyphenolics, as assessed by Folin-Ciocalteu assay, varied between 29.48 and 19.14 mg GAE per 1mL of extract. The maximum yield reached 29.48 mg GAE/ml at temperature 52 ^{0}C, 85% concentration of ethanol. As results shown, lower temperature produced higher concentrations of polyphenols in extracts.

Table 3. Main compounds of extracts identified by GC/MS (7388 PSI, 52 ^{0}C, 51%)

RT (min.)	Hit Name	Mol Weight	CAS Number
3.914	Urea, tetramethyl-	116.095	000632-22-4
4.67	Guaiacol	124.052	000090-05-1
5.471	2-Hydroxybornane	154.136	010385-78-1
5.705	.alpha.-Terpineol	154.136	000098-55-5
6.022	Benzofuran. 2.3-dihydro-	120.058	000496-16-2
6.582	2.5-Bornanedione	166.099	004230-32-4
7.201	2-Methoxy-4-vinylphenol	150.068	007786-61-0
7.564	6-Methylenespiro[4.5]decane	150.141	019144-01-5
8.191	8-Methyltricyclo[5.2.1.0$^{2.6}$]decane	150.141	1000215-29-3
8.781	Cyclopropane. nonyl-	168.188	074663-85-7
8.917	11-Azatricyclo[4.4.1.0$^{1.6}$]undecane	151.136	005735-21-7
9.189	trans-Isoeugenol	164.084	005932-68-3
9.377	Oxirane. [(dodecyloxy)methyl]-	242.225	002461-18-9
9.446	1-Tridecene	182.203	002437-56-1
10.027	1-Heptadecanamine. N.N-dimethyl-	283.324	002437-56-2
10.269	(+)-gamma-cadinene	204.188	002437-56-3
10.375	1.1.4.5.6-Pentamethyl-2.3-dihydro-1H-indene	188.157	002437-56-4
10.783	.alpha.-Calacorene	200.157	002437-56-5
11.184	7-Hexadecene. (Z)-	224.25	002437-56-6
11.576	Ledene	204.188	002437-56-7
12.332	Epicubenol	222.198	002437-56-8
12.582	.tau.-Muurolol	222.198	002437-56-9
12.831	.alpha.-Cadinol	222.198	002437-56-10
13.042	1-Heptadecene	238.266	002437-56-11
13.186	Naphthalene. 1.6-dimethyl-4-(1-methylethyl)-	198.141	002437-56-12
13.647	1-Tetradecanamine. N.N-dimethyl-	241.277	002437-56-13
14.456	Oplopanone	238.193	002437-56-14
15.393	(1aR.4aS.8aS)-4a.8.8-Trimethyl-1.1a.4.4a.5.6.7.8-octahydrocyclopropa[d] naphthalene-2-carbaldehyde	218.167	002437-56-15
18.136	Tridecanoic acid. 4.8.12-trimethyl-. methyl ester	270.256	002437-56-17
18.332	Cembrene	272.25	002437-56-18
18.566	1-Cyclohexene-1-ethanol. 2.6.6-trimethyl-	168.151	002437-56-19
18.997	Limonene Dioxide	168.115	002437-56-20
19.126	As-Indacene. 1.2.3.6.7.8-hexahydro-1.1.6.6-tetramethyl-4-(1-methylethyl)-	256.219	002437-56-21
19.254	Cobalt. (.eta.5-2.4-cyclopentadiene-1-yl)[(1.2.3.3a.7a-.eta.)-1H-inden-1-yl]-	239.027	002437-56-22
19.322	1H-Naphtho[2.1-b]pyran. 3-ethenyldodecahydro-3.4a.7.7.10a-pentamethyl-. [3R-(3.alpha..4a.beta..6a.alpha..10a.beta..10b.alpha.)]-	290.261	002437-56-23
19.662	18-norabieta-8.11.13-triene	256.219	002437-56-24
19.927	Isolongifolol	222.198	002437-56-25
20.244	1-Naphthalenepropanol. .alpha.-ethenyldecahydro-.alpha..5.5.8a-tetramethyl-2-methylene-. [1S-[1.alpha.(R*).4a.beta..8a.alpha.]]-	290.261	002437-56-26
20.433	Azacyclotridecan-2-one. 1-(3-aminopropyl)-	254.236	002437-56-27
20.516	Sclareolide	250.193	002437-56-28
21.083	Alloaromadendrene oxide-(1)	220.183	002437-56-29
21.234	Dehydroabietic acid	300.209	002437-56-30

4. Conclusions

- Norway spruce is the most important fibre source for pulp and paper production in Slovak Republic. The pulp industries generate large amounts of bark which are burned for energy production and don't have any other utilization.

- This paper provides information on phenolic compounds found in the bark. These compounds represent a huge potential. These bioactive compounds may be a good source of compounds with several applications in the food industry, like food ingredients and nutraceuticals, in the cosmetics, and in pharmaceutical industries.

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