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SELECTIVE SOLVENTS FOR EXTRACTION OF TRITERPENES FROM *BETULA PENDULA* OUTER BARK

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The volume of birch plywood production in Latvia is illustrated by the 208 000 m^3 of plywood sold in 2011 and about 562 000 m^3 of processed birch veneer blocks. Wood residues such as bark, veneer shorts, cut off ends and others are used as a fuel. It would be more expedient to increase the birch wood utilisation degree by involving also birch outer bark in the processing cycle. It makes up 2% of the veneer blocks' mass. At the J.S.C. "Latvijas Finieris", about 6000 t per year of graded and milled outer birch bark could be produced for further processing. Birch outer bark consists of triterpenes, suberine, lignin and sugars, the content of which is ~35%, ~45%, ~9% and ~6%, respectively. The content of lupane type pentacyclic triterpenes in Latvian silver birch (*Betula pendula* Roth.) and downy birch (*Betula pubescens* Ehrh.) outer bark is among the highest ones for the species. The main components of the triterpene mixture are betulin, lupeol and betulinic acid (Figure 1), which are characterised by a poor solubility in organic solvents.

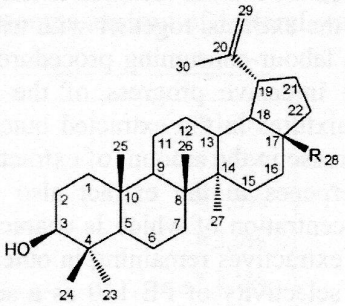
Birch outer bark and its extracts have been used in folk medicine for ages. Nowadays, interest in triterpene derivatives, especially those of betulin, is increasing, since they are promising biologically active substances for treatment of cancer, HIV, obesity, the herpes virus and other diseases.

In the most popular methods for triterpene extraction from outer birch bark, polar solvents such as ethanol and isopropanol or acetone are used. Unfortunately, these solvents, along with triterpenes, dissolve polyphenols, tannins, carbohydrates and other components of outer birch bark. The subsequent purification of extractives by recrystallisation is connected with the additional consumption of the solvent and losses of both the end product and the solvent. These operations are also labour-consuming.

Table 1. Results of 3-h intense extraction of outer birch bark.

Solvents	Yield of extractives		Compositions of crystals	
	Joint extract-ives	Crystali-sed extract-ives	Betulin	Lupeol
	% o.d. outer birch bark		% o.d. crystals	
Ethanol*	33.34	-	64.76	8.73
Cyclohexane	13.18	6.07	90.85	5.02
Petroleum ether 90-100°C	5.20	1.80	90.11	8.11
Petroleum ether 100-140°C	10.63	6.27	91.60	6.15

*The composition of the joint extract is given



R= CH_3 Lupeol
R = CH_2OH Betulin
R = COOH Betulinic acid

Figure 1. Chemical structure lupane type pentacyclic triterpenes*

To obtain extractives with the maximum content of triterpenes, selective solvents should be chosen, which dissolve triterpenes, but minimise the solubility of the undesired admixtures and, according to the directive of the European Medicines Agency regarding the solvent residues in preparations, would be permissible for obtaining pharmaceutical raw materials.

Based on the available information, we have chosen cyclohexane (CH) and petroleum ethers with a boiling temperature of 100-140°C (PE 140) for the experiments. The triterpenes' solubility in such solvents is improved by elevating temperature, but at the same time, the poor solubility of admixtures is maintained.

Outer birch bark was milled, pelletised and crushed to prepare particles measuring 0.4-1.0 mm, used for the extraction. In the Soxhlet apparatus, the yield of extractives after 33 h of extraction with hydrocarbons was modest, namely, 28.4% and 17.4% on the o.d. birch bark mass basis for CH and PE 140, respectively. Such a result showed the inefficiency of the stationary maceration type extraction. Therefore, we have devised an intensive mass exchange extraction apparatus with the possibility to shorten the extraction time and to obtain partially purified crystalline triterpenes with a high content of betulin.

Table 2. Progress of the intense extraction of triterpenes with petroleum ether at 100-140°C and the accumulation of tannins in leftover extractives in the bulk of leached bark

Duration of extraction, h	Leftover extractives, % o.d. bark	Triterpenes in leftover extractives		Amount of tannins*, % o.d. extractives
		Betulin	Lupeol	
		% o.d. extractives		
0	30.5	75.80	6.90	9.56
1	23.6	50.51	4.63	19.50
2	15.8	38.12	2.19	20.37
3	13.5	22.51	0.99	23.85
4	12.1	21.14	0.79	24.18
5	10.2	11.54	0.56	25.03
6	10.0	11.27	Trace	28.11
7	9.3	8.82	Trace	29.84

* Tannins calculated as tannic acid

The apparatus makes it possible, in a short period of time, to isolate a considerable amount of extractives (Table 1). The extracts were analysed by gas chromatography. Two parallel aliquots of each extract were analysed by triple injections. The gas chromatography analysis results shown in Table 1 are average values, obtained from aliquots' injections and separate extracts' aliquots, for which the mutual variation is less than 5%. The intensity of the extraction with ethanol is very high, but the extracts, together with triterpenes, contain also coloured admixtures, and freeing from them is a labour-consuming procedure, connected with losses of crystalline triterpenes and the solvent. The intensive progress of the extraction with PE 140 and the gradual accumulation of the admixtures in the extracted outer bark are shown in Table 2. It can be seen that, along with the decrease in the amount of extractives in the extracted outer bark, the concentration of the remaining triterpenes in the extract also decreases. At the same time, the content of polyphenols, the concentration of which is characterised by the quantitatively determined amount of tannic acid in the extractives remaining in outer bark, also increases. The data shown in Table 2 readily illustrate the selectivity of PE 140 as a solvent relative to pentacyclic triterpenes. Taking into account the directives of the European Medicines Agency, the recommended extractives for birch outer bark triterpenes' extraction could be carbohydrate solvents with boiling temperature in the range of 100-180°C, because their selectivity is appropriate and they do not contain aromatic carbohydrates, which are more difficult to purify from the crystalline product.

Keywords: *birch outer bark, triterpenes, extraction, hydrocarbons.*

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