Subcritical extraction of birch bark pentacyclic triterpenes

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Natural pentacyclic triterpenes were extracted from birch bark with subcritical solvents. The influence of the solvent nature, temperature, and the number of extraction cycles on the efficiency of the process was studied. Using methanol at 100 °C provides the highest triterpene extraction rate. Bark extracts prepared using various solvents were analyzed for chemical composition and antioxidant activity.

Key words: subcritical extraction, pressurized liquid extraction, birch bark, pentacyclic triterpenes, extractive compounds.

Extractive compounds, along with the major components of wood biomass (lignin, cellulose, hemicellulose) are an ecologically benign and renewable source of a wide range of valuable compounds with high biological activity. Pentacyclic triterpenes (PCTT) deserve particular attention as they possess hepatoprotective, antiviral, antitumor, and antibacterial properties.[1] Being secondary metabolites of plants, PCTT are widespread in nature. One of the major sources of PCTT is wood bark, mostly birch bark, which is a large scale waste product (12—17% of the processed biomass[2]) in wood processing and paper mill industry. The highest amount of triterpenes (up to 40%) is contained in the outer layer of the bark (smooth elm). Betulin (**1**) is the most abundant compound, its content being 10—35% depending on the birch species, place and conditions of growth, tree age and other factors.[3] Other compounds found in considerable quantities are: lupeol (**2**), betulinic acid (**3**), erithrodiol (**4**): 5.9—7.9%, 4.3—5.4% and ~2.8%, respectively. The minor components that can be detected are betulinic aldehyde, betulin caffeate, oleanolic acid, etc.[4] Regardless of the wide occurrence of the object of the study, the complete chemical composition of birch bark extractive compounds has not been established and the relevant publications[4,5] contain contradictory information.

Efficient and rapid extraction procedures of the target components are required to improve chemical processing of plant raw materials and develop better procedures for determination of the composition of these materials and final products.

Most of the known methods of PCTT isolation can be divided into two groups. The first group includes procedures using solvents at temperatures not higher than their boiling points. In this case, ethanol is the optimal solvent. The process is time-consuming and extraction extent
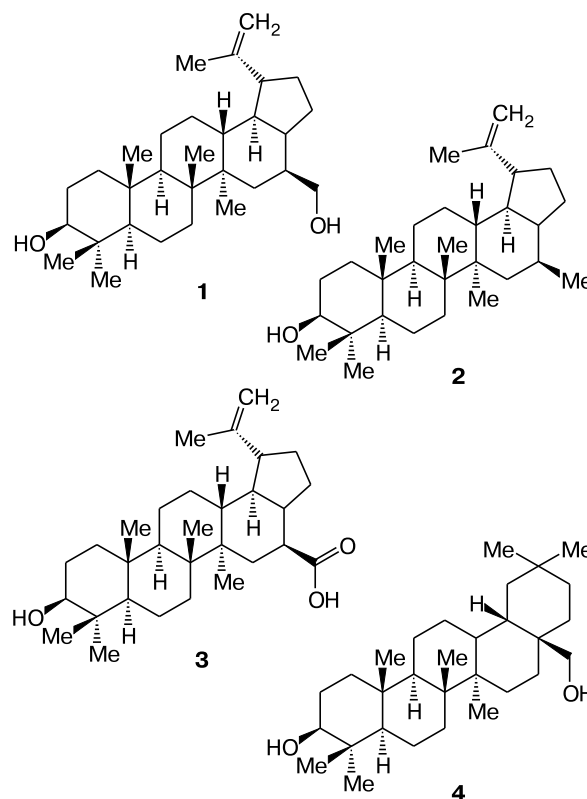
does not exceed 70% of the total amount of the target components.[6—8] An analytical variant uses exhaustive Soxhlet extraction with aliphatic alcohols, the extraction time can be greater than 10 h.[7] The methods of the second group employ preliminary partial destruction of the lignocellulosic material to increase the yield of PCTT. Such methods involve alkaline hydrolysis, ultrasonication, and steam explosion followed by extraction with refluxing aliphatic alcohols. In this case the extraction times are

3—4 h, sometimes more, while triterpene recovery can reach 95—97%.[9—11]

Using more harsh conditions can improve the process time and yield of PCTT and decrease consumption of the extragents. Supercritical fluid extraction (SCFE) and pressurized liquid extraction (PLE) are the most perspective methods of accelerated extraction of triterpenes.

Supercritical CO_2 was used[12,13] to extract PCTT from wood bark. Optimal yield was achieved when ethanol (about 10%) was used as the modifier, at 55—60 °C and 100—200 bar. Due to the low dissolving capacity of CO_2 towards PCTT, the achieved extraction rates were not high (15—25%), while the process time was several hours. The efficiency of supercritical extraction of triterpenes can probably be increased by using another solvent instead of carbon dioxide, but this will require sophisticated instrumentation.

Using PLE (extraction with subcritical solvents) often gives better results for biomass objects compared to SCFE, due to a wide choice of extragents with different polarity and significantly high temperatures. This technique has found extensive applications in analytical practice. There is a considerable amount of publications on isolation of flavonoids, phenols, carotenes, and other compounds from plant raw materials with organic solvents at elevated temperatures and pressures.[4—16] We previously used the method in sample preparation step for determination of PCTT in birch bark by LC-MS/MS[17] (the extraction medium was methanol at 100 °C and 100 bar). In spite of this, there are only scarce systematic studies of the influence of different extraction parameters on the processes of triterpene isolation under subcritical conditions. An exception is a work[18] devoted to liquid extraction under pressure and to studies of antioxidant properties of birch bark extractive compounds. The only compound determined in this work was betulin (**1**), while water and ethanol were tested as extraction media. It was found that betulin makes up about 26% of the weight of birch bark and the optimal extraction conditions were: ethanol, 120 °C, and 50 bar. Using water affords betulin in only insignificant yield even at high temperatures (up to 200 °C).

Considering the above stated, the aim of the current research is to study the influence of the subcritical extraction medium and extraction parameters on the isolation of various triterpenes from plant tissues, and to determine the chemical composition of the obtained extracts.

Experimental

The outer layer of birch bark (*Betula pendula* species) collected in 2015 in Arkhangelsk region was used as the test material for the study. The material was dried in a drying oven at 50 °C, ground to a particle size of 0.5—1 mm, thoroughly mixed and stored in a dessicator over silica gel in the dark.

The extraction efficiency was monitored by the content of four main PCTT compounds in the obtained extracts, that belong to the classes of triterpene alcohols (diol **1**, mono-ols **2** and **4**), and pentacyclic triterpene acids (**3**).

Water and eight organic solvents of different polarity and ability to specific (donor-acceptor) interactions were tested as the extraction media, namely: methanol (HPLC grade, Merck KGA, Germany), ethanol (96%, rectified grade, Russia), 2-propanol (99.9%, chromatography grade, Component-reactiv, Russia), acetonitrile (grade 0, Cryochrom, Russia), chloroform (99%, Component-reactiv, Russia), dichloromethane (99.8%, Lab-Scan, Poland), acetone (99.8%, Component-reactiv, Russia), hexane (grade 1, Cryochrom, Russia).

Commercially available PCTTs were used as reference standards: **1** (> 98%, Aldrich), **3** (> 97%, Anal. std., Fluka), **2** (> 94%, Anal. std., Fluka), and **4** (> 97%, Anal. std., Fluka). The compounds were used as recieved, without further purification.

Mobile phases for HPLC were prepared using acetonitrile (grade 0, Cryochrom, Russia), methanol (HPLC grade, Merck KGA, Germany), formic acid (> 96%, ACS Reagent, Sigma-Aldrich), ultrapure water with a specific resistivity of 18.2 MΩ cm, obtained on a Simplicity UV system (Millipore, France).

PLE extraction was performed on a ASE-350 (Dionex, USA) system operating at 100 bar. Bark sample (1 g) was mixed with 1 g of granulated diatomite filler and placed into a steel extraction cell (10 mL). Each extraction cycle included filling the cell with the solvent, heating up to the set temperature, maintaining the temperature for 10 min, washing with the solvent (60% from the cell volume), and displacement of the solvent with nitrogen to a receiver vessel.

Exhaustive Soxhlet extraction was performed using ethanol (200 mL) for 12 h. The sample (5 g) was mixed with 5 g of diatomite filler and placed to a cellulose extraction shell.

Ultrasonic extraction was performed in an Elmasonic (Elma, Germany) ultrasound bath at 50 °C for 3 h using ethanol (20 mL per 1 g of the sample).

Determination of PCTT in the extracts was performed by LC-MS/MS using multiple reaction monitoring (MRM) mode.[17] The system consisted of a triple quadrupole mass spectrometer LCMS-8030 equipped with an atmospheric pressure chemical ionization (APCI) ion source, and a LC-30 Nexera liquid chromatograph (Shimadzu, Japan), including two LC-30AD pumps, a degasser, a LC-30AC autosampler, and a CTO-30A column thermostat. The separation was performed on a reversed phase column Shim-pack XR-ODS II (3×30 mm, 2.2 μm, Shimadzu, Japan) at 40 °C. The eluent flow rate was 0.6 mL min^{-1}, the injection volume was 10 μL. APCI parameters were the following: ion source temperature 350 °C, heater and desolvation line temperature 250 °C, corona discharge voltage 3.5 kV; nebulizing and drying gas flows 4 and 15 L min^{-1} respectively.

The GC-MS studies were conducted on a GC-MS QP2010Plus system (Shimadzu, Japan). Chromatographic conditions: capillary column Rtx-5MS, column diameter 0.25 mm, column length 30 m, stationary phase thickness 0.25 μm. Sample injection with a split ratio of 1 : 50, injector temperature 280 °C, helium carrier gas. Gas flow regulation: constant flow rate, 1 mL min^{-1} column flow rate. Initial thermostat temperature 60 °C, a 5 min isotherm, temperature increase at a 20 °C

Birch bark extraction

Russ. Chem. Bull., Int. Ed., Vol. 66, No. 5, May, 2017 877

min^{-1} rate up to 200 °C, temperature increase at a rate of 10 °C min^{-1} up to 300 °C, a 20 min isotherm. Interface temperature 280 °C. Ion source temperature 250 °C, ionization energy 70 eV, detector voltage 0.8 kV. Mass detector operation mode: scan, scan range of *m/z* 50—600.

Antioxidant activity (AA) of the extracts was determined by a photochemical method (luminescence quenching) on a Photochem analyzer (Analytik Jena, Germany). Calibration of the instrument was done using Trolox.

^{1}H NMR spectra were recorded on a AVANCE III (Bruker, Germany) instrument operating at 600 MHz. Samples of 20 mg were dissolved in 0.8 mL of CDCl$_3$.

High resolution mass spectra were obtained on a Q Exactive Plus (Thermo Scientific, USA) mass spectrometer with an orbital ion trap mass analyzer operating with a 70000 FWHM resolution (APCI ionization, positive detection mode). Solutions (5 μL) of the studied samples were injected directly to the ion source using the solvent flow (acetonitrile, 300 μL min^{-1}) produced by LC-30AD pump (Shimadzu, Japan). The mass spectra were recorded in the *m/z* range of 50—750. The background signal from the solvent was subtracted. Optimal parameters of the ion source were used, which allowed maximal ion currents: capillary voltage 5 kV, drying, nebulizing, and curtain gas (N$_2$) flow rates 25, 5, and 2 rel. units respectively, desolvation line temperature 250 °C, nebulizing gas temperature 500 °C, radio frequency voltage on S lens 55 rel. units. The instrument was calibrated in the studied mass range by peaks of sodium formate clusters. The *m/z* determination error did not exceed 3 ppm. Empirical formula of the components of the extracts were determined by the exact ion masses.

Principal component analysis method using Marker View software (ABSciex, Canada) was employed to compare mass spectra of different extracts. Peak positions in the mass spectra were preliminary aligned at an allowed deviation of 3 ppm. Pareto type normalization was used.

Results and Discussion

Optimization of PCTT extraction conditions. Determination of PCTT in the obtained extracts using HPLC coupled with tandem mass spectrometry allowed one to minimize the matrix effect, resulting in increased analysis selectivity and validity of the data, compared to previously used spectrophotometric detection. An example chromatogram is given in Fig. 1.
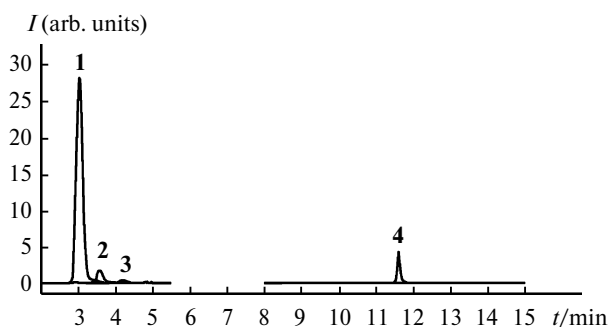

Fig. 1. HPLC-MS/MS chromatogram of a birch bark methanolic extract of compounds **1**—**4**.

Table 1. Recovery of PCTT (g kg^{-1} of birch bark) with ethanol using Soxhlet and ultrasonic extraction

Extraction method	3	1	4	2
Soxhlet	14.7	195	2.47	6.04
Ultrasonic	4.58	60.8	0.73	4.19

Soxhlet extraction and ultrasonic extraction with ethanol were used as the reference methods. The results of LC-MS/MS analysis of the extracts are presented in Table 1. Obviously, sonication under relatively mild conditions (50 °C) does not provide rapid isolation and acceptable recovery rates of the target components. In this case the yields of PCTT are 1.3—3 times lower compared to Soxhlet extraction. Increasing the sonication time to 12 h did not significantly enhance the extraction due to the very low solubility of triterpenes in ethanol. Preliminary experiments employing extraction under pressure (100 bar) at temperatures below the normal boiling points of the tested solvents proved very inefficient — the extraction rate of **1** with methanol (50 °C) was 196 mg kg^{-1} even after three subsequent extraction cycles, which is comparable to Soxhlet extraction.

The main factor that influences the isolation of PCTT from plant tissue is the temperature of the extraction medium. Its raising leads to increased solubility of extractive compounds. Another important effect of the elevated temperature is that the properties of both the solvent and the material change, allowing better penetration of the solvent into the plant tissue, as well as more rapid diffusion of the compounds to the solution. Working temperatures around 100—200 °C allowed to dramatically increase the extraction efficiency and reach high PCTT yields using most of the tested solvents (Table 2). The only exception was water, which, despite a significant decrease of its dielectric constant under subcritical conditions, would still dissolve only minute amounts of **1** and **3**.

The best solvents for PCTT extraction under subcritical conditions were aliphatic alcohols and acetone. The corresponding extraction rates were in accord to data[3] on their maximal content in bark and were much higher compared to using Soxhlet extraction. Acetonitrile, chloroform and dichloromethane showed somewhat moderate results at 100 °C, however raising the temperature to 200 °C provided complete extraction as well. Hexane, being a poor extraction medium at 100 °C for all compounds but non-polar **2**, exhibited a surprising increase in extraction efficiency at higher temperatures. At maximal temperature, the extraction rate for **1** is increased almost five times, while for compound **3** the difference is by an order. At 200 °C the amounts of isolated PCTT are comparable for all the tested solvents. It seems that under harsh conditions the dissolving power of different solvents is not a limiting factor for the studied extraction

Table 2. Recovery of PCTT (g kg^{-1} of birch bark) using subcritical organic solvents at 100, 150, and 200 °C ($p = 100$ bar, two extraction cycles)

Extractant	3			1			4			2		
	100	150	200	100	150	200	100	150	200	100	150	200
Water	—	—	0.01	—	—	0.43	—	—	—	—	—	—
Methanol	16.7	14.2	12.1	282	256	237	5.14	3.24	2.21	8.13	7.41	6.47
Ethanol	15.6	14.6	12.1	264	246	237	4.77	4.57	2.91	7.66	6.17	5.02
Isopropanol	15.9	15.0	13.1	252	244	241	5.14	4.97	4.51	8.31	8.10	7.44
Acetonitrile	9.05	13.0	15.6	206	233	268	3.62	4.42	5.41	7.81	8.17	8.56
Chloroform	12.0	15.7	13.0	236	261	245	4.18	4.78	4.30	7.32	7.72	7.55
Dichloromethane	9.45	13.9	11.3	190	242	226	3.39	4.75	3.39	7.62	8.27	8.07
Acetone	16.5	16.7	16.2	267	269	265	3.50	4.92	3.45	7.71	7.79	7.74
Hexane	1.28	9.92	13.0	40.1	197	226	1.60	1.98	3.87	8.08	8.57	8.73

times and used solvent volumes. Analysis of the number of PLE cycles on the yield of PCTT showed two extraction cycles to be enough for the maximal extraction at optimal temperatures. In this case the volumes of obtained extracts were about 30 mL.

It is noteworthy that alcohols exhibit anomalous behavior, namely, at higher temperatures the observed extraction rates of PCTT fall greatly. This effect is most pronounced in the case of methanol, which extracts compound **4** 2.3 times less at 200 °C than at 100 °C. Considering possible chemical reactions of triterpenes with alcohols at elevated temperatures (alkylation), we conducted model experiments: the studied PCTTs (pure compounds) were treated with methanol at 200 °C in the same PLE instrument and the same program used for extraction from plant tissue. Additional experiments were conducted in steel autoclaves at the same temperature for 6 h. NMR spectroscopy showed that the ^{1}H NMR spectra for PCTTs before and after subcritical treatment were identical. The absence of methylation of OH groups with methanol was further confirmed by the absence of ~3.5 ppm signals, related to methoxy group. The extremal extraction rate of PCTT at 100 °C can be explained by undesired swelling

of plant tissue in alcohols, which decreases their permeability, and decreased solubility of PCTT at increased temperatures. The former suggestion is confirmed by calculations of solubility parameters made by Hansen[19], according to which the maximal solubility of **1** in ethanol is reached[18] at 90 °C (close to our experimental value).

Analysis of the extracts. In order to determine the specific action of different extraction media and to develop pathways of use of the obtained extracts, and to create novel analytical methods for the determination of different target components, one should have the data on the chemical composition and properties of the extracted compounds isolated by PLE.

Table 3 summarizes the data on the total yield of compounds extracted by different solvents at optimal temperatures, as well as the values of their antioxidant activity (AA), which is an integral parameter of a whole range of valuable biologically active compounds (namely of phenolic nature). The total yield of the extractive compounds lies in a rather narrow range for most solvents (39—45%), while four most important triterpenoids compose 28—31% of the total amount (see Table 2). Non-polar hexane shows a significantly lower extraction efficiency,

Table 3. Yields of extractive compounds and antioxidant activity (AA) of birch bark extracts in Trolox units

| Extractant	T/°C	Yield of extractive compounds /g kg^{-1}			AA/g kg^{-1}	
		Total	PCTT	Other	For birch bark	For extractive compounds
Water	200	97.7	0.44	97.3	58.6	600
Methanol	100	448.0	312.00	136.0	118.0	265
Ethanol	100	408.0	292.00	116.0	115.0	283
Isopropanol	100	405.0	281.00	124.0	114.0	282
Acetonitrile	200	415.0	298.00	117.0	160.0	387
Chloroform	150	393.0	289.00	104.0	97.7	249
Dichloromethane	150	373.0	269.00	104.0	91.4	245
Acetone	150	445.0	298.00	147.0	123.0	278
Hexane	200	309.0	252.00	57.4	30.3	98
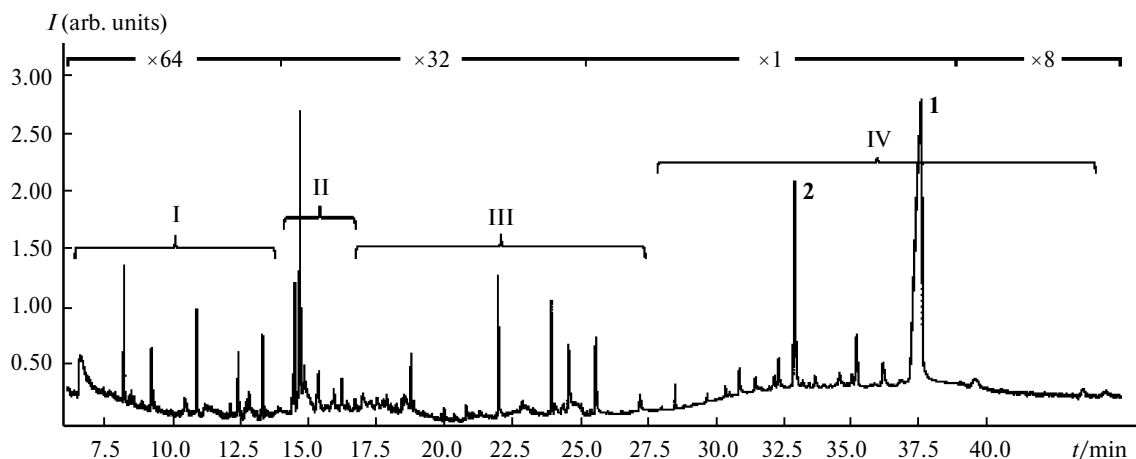
Fig. 2. GC-MS chromatogram of a methanolic extract of birch bark (hydrocarbons (I), sesquiterpenes (II), fatty alcohols and acids (III), sterodis and triterpenes (IV), including compounds **1** and **2**).

especially for non-PCTT compounds. These dependencies are reflected also in the AAs of the extracts.

Subcritical water deserves particular attention as a selective extraction medium of valuable secondary compounds with antioxidant properties. Using water allows one to isolate the maximal amount of polyphenols, and the resulting extracts exhibit AAs comparable to such a reference compound as Trolox. As about 10% (from the bark weight) of extractive compounds are extracted with water, it can be used for preliminary treatment of raw material followed by PCTT extraction with organic solvents and their purification.

Identification of individual components of the extracts with GC-MS (Fig. 2) showed that all organic solvents under optimal conditions give close patterns of compounds, mostly hydrocarbons (mainly C_{11}—C_{17}), sesquiterpenes (α-bergamotene, α-santalene etc.), fatty alcohols and acids (henicosanol, 1,2-dipalmitin, 1,3-dipalmitin, α-monostearin, 2-monopalmitin, etc.), and steroids (lanosterol, stigmasterol, β-sitosterol) and PCTT (Table 4).

Table 4. Content of the primary compound groups in birch bark extracts (in percents from the sum of hydrocarbons, sesquiterpenes, fatty alcohols and acids, steroids and PCTT)

Extractant	T/°C	Hydro-carbons	Sesqui-terpenes	Fatty alcohols and acids	Steroids and PCTT
Methanol	100	0.15	0.49	3.26	96.1
Ethanol	100	0.13	0.66	2.91	96.3
Isopropanol	100	0.10	0.84	2.96	96.1
Acetonitrile	200	0.24	0.39	2.03	97.3
Chloroform	150	0.13	0.92	1.67	97.3
Dichloro-methane	150	0.07	0.73	1.76	97.5
Acetone	150	1.17	0.70	3.76	94.4
Hexane	200	0.05	0.36	1.93	97.7

Considering the inherent limitations of gas chromatography, in particular its inconsistency for thermally labile, non-volatile and high molecular weight compounds, we performed an additional study of the extracts using high resolution mass spectrometry using APCI ionization. About 900 peaks of protonated molecules of individual compounds were reliably detected using this method. As in the case of GC-MS analysis, the molecular
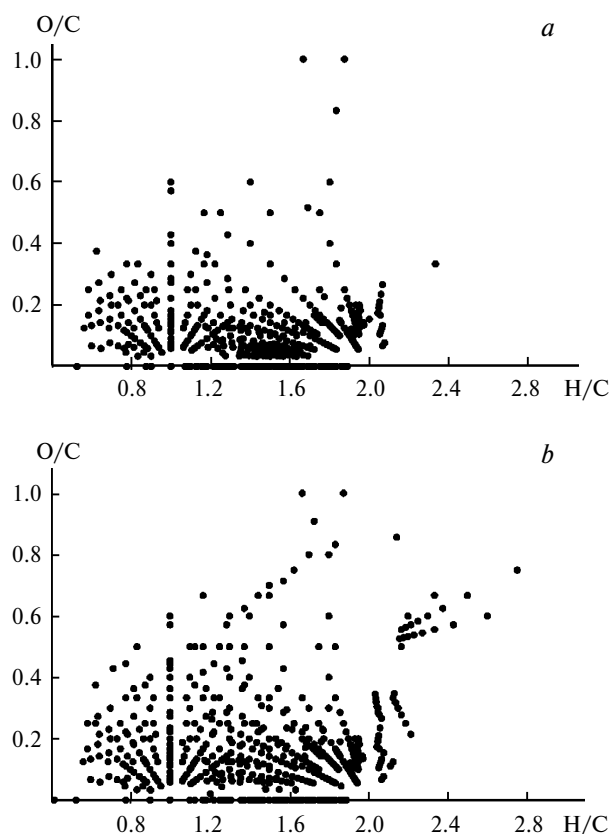

Fig. 3. Van Krevelen plots of a methanolic (*a*) and aqueous (*b*) birch bark extracts.

composition of extracts obtained using organic solvents were very similar. Van Krevelen plots[20] for the composition of the extractive compounds are presented in Fig. 3. Aqueous extracts, naturally, show significant differences (see Fig. 3, *b*): they contain many polar compounds with O to C atomic ratios (O/C) more than 0.4. These compounds can be polyphenols and their glycoside derivatives. In aqueous extracts the amount of hydrocarbons with H/C atomic ratios from 1 to 2 and O/C ratios equal 0 is small compared to even methanolic ones.

We used the chemometric approach (principal component analysis)[21] to outline little differences in the exceedingly complex mass spectra of the obtained extracts. Plots of scores and loadings given in Fig. 4 demonstrate two principal components (PC1 and PC2, covering a total of 66% of the observed differences) are enough for adequate data analysis. An expected grouping of solvents in accord with their properties is observed (see Fig. 4, *a*). The loadings plot (see Fig. 4, *b*) shows that aliphatic alcohols have the strongest specifity: ions with *m/z* 177.1273, 331.2841, and 359.3152 are present in the corresponding extracts. These ions relate to empirical formulae of

$C_{12}H_{16}O$, $C_{19}H_{38}O_4$, and $C_{21}H_{42}O_4$ respectively. The first formula suits alkyl-substituted phenols, while two others seemingly are esters of glycerol and fatty acids. The specifity of non-polar solvents (dichloromethane, hexane) is mainly exhibited in extraction of the most lipophilic PCTT (mono-ols) with a $C_{30}H_{48}O$ empirical formula, which give protonated molecules with a *m/z* 425.3776. Acetone has a significantly different, compared to the other solvents, PC2 component and shows great selectivity in extraction of a number of low molecular compounds, for example C_6H_8O and $C_7H_{10}O_2$ (*m/z* 97.0647 and 127.0754) that presumably have furan-like nature.

In the conclusions, extraction with subcritical solvents is the most rapid and efficient way to isolate PCTT from plant raw materials. Aliphatic alcohols, acetonitrile and chloroform exhibits the highest extraction power with respect to triterpenes. Using methanol as the extraction medium at 100 °C provides extraction of compounds **1—4** from birch bark in amounts of ~30% from the weight of the material, which is significantly higher compared to Soxhlet extraction and requires much less time and solvent volume.

Using GC-MS and APCI-HRMS allows one to determine the composition of subcritical extracts of birch bark. Under optimal conditions, organic solvents were demonstrated to extract similar patterns of extractive compounds, though certain selectivity towards some classes is observed.

Subcritical water is a poor solvent for PCTTs. However, its use results in selective extraction of phenols with high antioxidant activity.
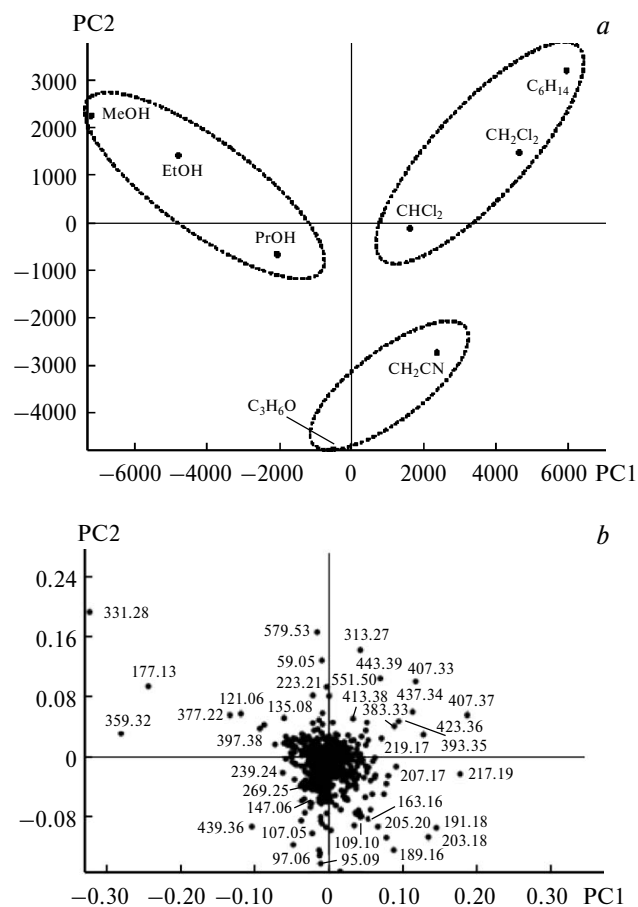
Fig. 4. Comparison of mass spectra of birch bark extracts obtained using different solvents using principal component analysis: plots of scores (*a*) and loadings (*b*).

References

1. S. Alakurtti, T. Makela, S. Koskimies, J. Yli-Kauhaluoma, *Eur. J. Pharm. Sci.*, 2006, **29**, 1.
2. A. N. Kislytsyn, *Khimiya drevesiny* [*Chemistry of Wood*], 1994, **3**, 3 (in Russian).
3. G. A. Tolstikov, O. B. Flekhter, E. E. Shultz, L. A. Baltina, A. G. Tolstikov, *Chem. Sustain. Develop.*, 2005, **13**, 1.
4. P. Krasutsky, *Nat. Prod. Rep.*, 2006, **23**, 919.
5. H. Pakdel, J. N. Murwanashyaka, C. J. Roy, *Wood Chem. Technol.*, 2003, **22**, 147.
6. L. I. Selyanina, *Lesnoy zhurnal* [*Forestry Journal*], 2004, **4**, 93 (in Russian).
7. V. A. Levdanskii, B. N. Kuznetsov, I. V. Korobkova, N. M. Ivanchenko, A. V. Levdanskii, *Zhurn. Sib. Fed. Univ.* [*Journal of Siberian Federal University*], 2012, **5**, 302 (in Russian).
8. V. A. Levdanskii, B. N. Kuznetsov, N. I. Polezhaeva, N. M. Ivanchenko, A. V. Levdanskii, *Khimija rastitel´nogo syr´ya* [*Chemistry of Plant Raw Material*], 2005, **2**, 15 (in Russian).

9. RF Pat. 2131882, *Byul. Izobret. [Invention Bull.]*, 1999, 17 (in Russian).

10. RF Pat. 2264411, *Byul. Izobret. [Invention Bull.]*, 2005, 32 (in Russian)

11. RF Pat. 2458934, *Byul. Izobret. [Invention Bull.]*, 2012, 23 (in Russian).

12. Y. Zhang, T. Yu, Y. Wang, *J. For. Res.*, 2003, **14**, 202.

13. A. Felfoldi-Gavaa, S. Szarkaa, B. Simandi, B. Blazicsa, B. Simonc, A. Kerya, *J. Supercrit. Fluids*, 2012, **61**, 55.

14. N. V. Ul´yanovskii, D. S. Kosyakov, K. G. Bogolytsin, A. D. Ivahnov, A. S Amosova, *Khimija rastitel´nogo syr´ya* [*Chemistry of Plant Raw Material*], 2012, **4**, 147 (in Russian).

15. D. L. Luthria, *J. Funct. Foods*, 2012, **4**, 842.

16. X. J. Chen, B. L. Guo, S. P. Li, Q. W. Zhang, P. F. Tu, Y. T. Wang, *J. Chromatogr. A*, 2007, **1163**, 96.

17. D. S. Kosyakov, N. V. Ul´yanovskii, D. I. Falev, *J. Anal. Chem.* (*Int. Ed.*)*, 2014, **69** 1264 [*Mass-spektrometriya*, 2013, **10**, 237].

18. M. Co, P. Koskela, P. Eklund-Akergren, K. Srinivas, J. W. King, J. R. Sjoberg, C. Turner, *Green Chem.*, 2009, **11**, 668.

19. C. M. Hansen, *Hansen Solubility Parameters: A Userrs Handbook*, CRC Press, Boca Raton, 2007, 544 pp.

20. S. Kim, R. W. Kramer, P. G. Hatcher, *Anal. Chem.*, 2003, **75**, 5336.

21. R. G. Brereton, *Chemometrics: Data Analysis for the Laboratory and Chemical Plant*, John Wiley & Sons, Chichester, UK, 2003, 489 pp.