Research Article

Michal Jablonský, Jozef Šima, Petra Strižincová, Katarína Hroboňová, Veronika Majová*, and Aleš Ház

Valorization of birch bark using a low transition temperature mixture composed of choline chloride and lactic acid

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Abstract: This article presents the results obtained in the extraction of birch bark with a green solvent. A low transition temperature mixture (LTTM) consisting of choline chloride (ChCl) and lactic acid in a molar ratio of 1:1 was used as the solvent. Extraction was performed at 60°C and 80°C. At both temperatures, the effect of extraction time on the yield of the extracted substances was monitored. The yields reached were compared with those obtained in studies using common solvents such as ethanol, methanol, D-limonene, ethyl acetate, and others. The extract was quantitatively analyzed by HPLC-UV to determine betulin and betulinic acid (0.491–1.788 mg/g dry bark and 0.106–0.316 mg/g dry bark, respectively). Total phenolic content was determined spectrometrically using Folin–Ciocalteu reagent and expressed as Gallic acid equivalents (GAE; 18.6–56.8 mg GAE/g dry bark). This study has shown that extraction with a green solvent composed of ChCl and lactic acid can be an effective method for extracting polyphenolic compounds from birch bark. The process for extracting triterpenes (betulin and betulinic acid) is less suitable compared to conventional methods using the mentioned organic extractants.

Keywords: low transition temperature mixtures, birch bark, triterpenes, extraction

1 Introduction

Plant biomass is a source of a large number of value-added substances [1]. An important part of biomass is the bark of trees. It is now generally accepted that the incineration of bark of any origin to obtain thermal energy is the least efficient way of its valorization. Many publications have highlighted the potential of different species of tree bark in terms of their valorization to obtain value-added substances. Illustrative examples include the extraction of spruce bark using deep eutectic solvents (DES) [2], several woody plants [3], or *Acacia nilotica* [4]. Considerable attention has also been paid to the processing of bark from various species of the Betulaceae family consisting of six plant genera, including *Betula* (birches), which is the largest genus in this family [5]. Birch is a medium-sized deciduous tree with a typical height of about 20 m. Of the genus *Betula*, silver birch (*Betula pendula*), and paper birch (*Betula pubescens*) predominate on the European continent. In addition to cellulose, hemicellulose, and lignin, birch bark also contains other types of biologically active substances – lupane-type triterpenes (betulin, betulinic acid, betulin aldehyde, and lupeol), suberin (a complex lipophilic polyester composed of long-chain fatty acids and glycerol), phenolic compounds [6] as well as lower levels of other substances such as hydrocarbons and their epoxides, steroids, tannins, and flavonoids [7–9]. Established extraction techniques are focused on the isolation of mainly betulin and its derivatives, lupeol, and polyphenols.

Researchers have focused on obtaining value-added substances, examining their properties (especially biological ones) and implementing the knowledge gained in

* **Corresponding author: Veronika Majová,** Institute of Natural and Synthetic Polymers, Department of Wood, Pulp and Paper, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, SK-812 37 Bratislava, Slovakia, e-mail: veronika.majova@stuba.sk
Michal Jablonský, Petra Strižincová, Aleš Ház: Institute of Natural and Synthetic Polymers, Department of Wood, Pulp and Paper, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, SK-812 37 Bratislava, Slovakia
Jozef Šima: Institute of Inorganic Chemistry, Technology and Materials, Department of Inorganic Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, SK-812 37 Bratislava, Slovakia
Katarína Hroboňová: Institute of Analytical Chemistry, Faculty of Chemical and Food Technology, Slovak University of Technology in Bratislava, Radlinského 9, SK-812 37 Bratislava, Slovakia

several industries [6,7,10–17]. To extract value-added substances from birch bark, organic solvents and their mixtures with water are used in most cases. However, such extractants do not meet the requirements for the use of green solvents and green chemistry practice [18]. In recent years, several methods have been developed to obtain value-added compounds from birch bark. The most common way to get betulin is solid–liquid extraction with organic solvents–ethanol, methanol, *n*-hexane, ethyl acetate, dichloromethane, etc. An effective way to improve the extraction process is to activate the bark with steam in the presence of sodium hydroxide and subsequently treat the bark with an organic solvent. By using an ethyl acetate/ethanol/water mixture in the presence of sodium or potassium hydroxide, it was possible to obtain an extract with betulin content of 74–75% and 85–89%, respectively [19]. More advanced methods include extraction of betulin by microwave extraction with limonene. Such a method made it possible to obtain betulin in high purity [20].

This article presents the first results of the employment of a low transition temperature mixture (LTTM), namely choline chloride (ChCl) and lactic acid in a molar ratio of 1:1 in the extraction of value-added compounds from birch bark, total phenolic content (TPC), the content of triterpene, such as betulinic acid and betulin, and comparison of our achievements with those obtained by other mentioned techniques. The work is aimed at determining the possibilities of extraction of the mentioned substances under different extraction conditions such as extraction time (45, 75, 105, and 150 min) and temperature (60°C and 80°C).

Using subcritical water extraction, it is possible to extract betulinic acid from birch bark in high purity and yield reaching 28 mg from 10 g bark in half an hour [21]. Recent research shows the use of wet ethyl acetate in the presence of microwaves. Treatment of such an extract with different types of stable monoterpenes, limonene, pinane, etc., made it possible to obtain betulin with a purity of 95–97% with high yields. Such a method is also green and economical, as it is possible to recover up to 85% of the solvents used [16]. To obtain pure betulin, Grazhdannikov's team used a sequence consisting of energy-saving extraction of the outer birch bark with ethyl acetate, saponification of the extract, and purification of betulin in limonene or hydrogenated monoterpenes. The refining of plant metabolites in monoterpenes can be considered as a promising method that successfully competes with known multi-step processes and methods using toxic solvents [16]. It is surprising (at least to our knowledge) that no one has tried to isolate value-added compounds from birch bark using LTTMs, which are true green solvents and whose extraction efficiency has been confirmed by the extraction of other bark species, such as spruce bark [2].

In research and technological practice, many LTTMs are used as extraction agents to recover value-added compounds from plant waste materials, including tree bark. In many cases, the yield of these substances is higher than when using conventional organic extractants. The advantages of LTTMs include their low toxicity, recyclability, and biodegradability, and LTTMs are considered environmentally friendly extractants. Our previous work on the extraction of value-added compounds from spruce bark as well as works of other authors has documented the benefits of using LTTM for extraction [22]. In this work, we only tried to find out whether the mentioned LTTM are also suitable for the extraction of value-added compounds from birch bark. We consider it important to publish the obtained negative result, so that in the next period no attention will be paid to the use of LTTM for birch bark extraction. As the primary results on the extraction yield indicated the inappropriateness of such a procedure, we did not address other otherwise important aspects, such as the recycling of the extractant, etc.

This article presents the first results of the use of LTTMs, specifically ChCl and lactic acid in a molar ratio of 1:1 in the extraction of value-added compounds from birch bark, TPC, the content of triterpenes such as betulinic acid and betulin and comparing our achievements with other mentioned techniques. The work was focused on verifying the possibilities of extraction of the mentioned substances under various extraction conditions, such as extraction time (45, 75, 105, and 150 min) and temperature (60°C and 80°C).

2 Materials and methods

2.1 Materials and chemicals

The birch (outer and inner) bark (*Betula pendula*) from F-Dental Hodonin, Czech Republic was used as raw material. The moisture content of the bark (5.9 ± 1.1%) was determined by drying approximately 2 g of birch bark at 105°C for 24 h until complete water was removed. All reagents were of analytical grade. ChCl (≥98.0%), and the Folin–Ciocalteu reagent were purchased from Sigma-Aldrich (Germany). A 90.0% lactic acid solution was obtained from VWR International (Bratislava, Slovakia).

ChCl was dried in vacuum. Standards of betulinic acid (97.0%) and betulin (98.0%) were purchased from Nature Science Technologies (Latvia). Acetonitrile for mobile phase preparation (HPLC gradient grade) was obtained from Centralchem (Slovak Republic) and deionized water was obtained from an AquaMax ultra water purification system (370 series). In this work, birch bark with a thickness of 2 mm after grinding was used.

2.2 Preparation of LTTM

The solvent (LTTM) consisted of ChCl and lactic acid in a molar ratio of 1:1 and water content of 5.4% by weight. The preparation of the solvent was carried out with constant stirring at a temperature of about 60°C until a homogeneous liquid mixture was formed [23].

2.3 Extraction of bark with LTTM

Birch bark and LTTM in a weight ratio of 1:10 (3 g of bark and 30 g of solvent) were used for extraction. The tube with these two components was shaken to mix them properly. The mixtures thus prepared in tubes were placed in an oven at a constant temperature of 60°C or 80°C and extracted for 45, 75, 105, or 150 min. A tube rotator was used to ensure constant stirring of the samples. Subsequently, an extract from each sample was obtained by filtration under reduced pressure through a fritted glass funnel with a porosity of S2. The obtained extract was stored in a closed tube in a dark and cool place until further use. After filtration, the rest of the bark was washed with about 0.5 L of pure water to remove the residual extract. The yield of extractants Y (%) was determined after each experiment by drying the samples at 105°C to constant weight. The results were expressed on the basis of dry matter before and after extraction (Eq. 1):

$$Y(\%) = 100 \times \frac{(m_i - m_j)}{m_i} \quad (1)$$

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.

2.4 HPLC instrumentation and conditions

The analysis was performed using an Agilent Technologies HPLC System (1100 series) consisting of a degasser, a binary solvent dosing pump, an autosampler, a column thermostat, and a diode array detector (DAD). Chromatographic separation was performed on a LiChrospher® 100 RP-18 column (250 mm × 4.6 mm i.d.) with 5 µm particle size (Merck, Darmstadt, Germany). The mobile phase was composed of acetonitrile and water (85:15, v/v). The analysis was performed at a flow rate of 1 mL·min^{-1}. The column temperature was maintained at 25°C and the injection volume was 20 µL. The DAD operated in the wavelength range of 190–400 nm and the detection wavelength was set at 210 nm. The run time was set at 20 min. The retention times of betulinic acid and betulin were 11.3 and 17.9 min, respectively. Quantitative analysis was performed by the calibration curve method. The values of the retention time, resolution, peak symmetry factor, high equivalent of theoretical plate, linearity, limit of detection (LOD), and limit of quantification (LOQ) are summarized in Table 1.

2.5 Determination of TPC

The TPC in the extracts was determined by the Folin–Ciocalteu test based on the redox reactions of the phenolic compounds. A volume of 0.5 mL of Folin–Ciocalteu reagent and 0.5 mL of extract or LTTM (as a blank) was pipetted into the test tube. After 3 min, 1.5 mL of 20% sodium carbonate solution and distilled water were added to the tube. After stirring, the mixture was incubated in a sealed dark-colored flask at room

Table 1: HPLC system suitability parameters for the separation of betulinic acid and betulin in the mixed standard solution and validation parameters for their determination

	Betulinic acid	Betulin
HPLC system suitability parameters[a]		
Retention time (t_R [min])	11.3	17.9
Repeatability RSD (%) t_R	0.55	0.39
Repeatability RSD (%) A	1.79	1.13
Peak symmetry	0.955	0.953
HEPT (µm)	41.7	32.5
R_S	9.42	
Validation parameters		
Calibration curve	$A = -19.181 + 8135.183 \times c$	$A = -2.336 + 11655.415 \times c$
R^2	0.9994	0.9981
Linear range (µg·mL^{-1})	10–500	10–500
LOD (µg·mL^{-1})	1.39	2.16
LOQ (µg·mL^{-1})	4.22	7.10

[a] The concentration of betulinic acid and betulin was 75 and 50 µg·mL^{-1}, respectively, t_R – retention time, A – peak area, HEPT – height equivalent of a theoretical plate, c – concentration of analyte, RSD – relative standard deviation ($n = 6$).

temperature for 120 min and then the absorbance of the solution at 765 nm was recorded. The TPC in the extracts was determined using a calibration curve based on the absorbance at 765 nm and expressed in Gallic acid equivalent (GAE) as mg GAE/g dry bark. All measurements were performed in triplicate for each sample. The data in Table 2 represent average values; the differences in measurements did not exceed 3.8%.

3 Results and discussion

Extraction of birch bark with LTTM consisting of ChCl and lactic acid in a molar ratio of 1:1 and 5.4% water by weight was performed at two temperatures (60°C and 80°C) and four extraction times (45, 75, 105, or 150 min). The contents of extracted betulin, betulinic acid, and TPC for the individual extraction conditions are listed in Table 2.

Based on the results shown in Table 2, it is clear that the yield of extractives at extraction temperatures of 60°C and 80°C is in the range of 10.2–19.2% and 12.9–24.1%, respectively. At both temperatures, a slight increase in the content of extractants in the extract can be observed with the extraction time. At 80°C and an extraction time of 150 min, a yield of up to 24.1% is achieved, but this may also be influenced by the fact that the extractant can also extract other biomass components such as lignin and hemicellulose. This hypothesis is supported by the fact that ChCl and lactic acid are often used as delignifying agents to remove lignin from wood and plants [23].

When our LTTM was applied at 60°C, most betulin was obtained at the extraction time of 75 min, at 80°C it was at 105 min. It has been shown that an increase in temperature leads to a higher content of betulinic acid and betulin. This is because the increase in temperature allows better penetration of LTTM into the substrate due to a decrease in the viscosity of LTTM.

In addition to the content of betulinic acid and betulin in the extracts, Table 1 also documents the content of these substances in relation to dry bark. The betulinic acid content at 60°C was in the range of 0.106–0.224 mg/g dry bark and for betulin it was 0.491–1.217 mg/g dry bark. At 80°C, the content of the extracted substances increased. The betulinic acid content was 0.263–0.317 mg/g dry bark and for betulin 1.192–1.329 mg/g dry bark. As can be seen from the data in Table 2, the proportion of betulinic acid to betulin in the extracts ranges from 12.0% to 23.8%, i.e., the extracts contain higher amounts of betulin.

The polyphenol content in the extracts was expressed as GAE and was determined in relation to both dry bark and extract. As can be seen from Table 2, depending on

Table 2: Determination of the yield of extractives (%) and contents of the betulinic acid (BA) and betulin (B) in birch bark extract (μg·mL^{-1}) and dry birch bark (mg/g dry bark), and total phenolic content (TPC; mg GAE/g dry bark; mg GAE/100 g extract)

Temp. (°C)	Time (min)	Yield (%)	BA in extract (μg·mL^{-1})	B in extract (μg·mL^{-1})	BA content (mg/g dry bark)	B content (mg/g dry bark)	BA/B × 100 (%)	TPC (mg GAE/g dry bark)	TPC (mg GAE/100 g extract)
60	45	10.2 ± 0.1	11.1	51.3	0.106	0.491	21.6	18.6 ± 0.7	174.2
60	75	12.4 ± 0.4	23.6	128.0	0.224	1.217	18.4	41.7 ± 1.2	394.4
60	105	13.8 ± 0.5	12.8	106.6	0.122	1.015	12.0	47.0 ± 0.8	441.4
60	150	19.2 ± 0.3	15.2	94.9	0.141	0.878	16.0	31.0 ± 0.5	298.4
80	45	12.9 ± 0.7	31.2	131.5	0.283	1.192	23.7	33.4 ± 1.1	306.3
80	75	14.0 ± 0.4	34.7	146.0	0.317	1.332	23.8	50.4 ± 0.7	465.6
80	105	14.2 ± 0.9	30.1	187.8	0.287	1.788	16.0	56.5 ± 0.6	513.0
80	150	24.1 ± 0.8	27.2	137.6	0.263	1.329	19.8	56.8 ± 1.4	519.9

the temperature and time of extraction, the TPC ranged from 18.6 to 56.8 mg GAE/g dry bark and from 174.2 to 519.9 mg GAE/100 g extract.

The effects of ultrasound, microwave radiation, negative pressure cavitation, and other supporting techniques are known [1,24–31]. On the other hand, it is known that some systems of these solvents are able to effectively dissolve lignin and just the application of ultrasound and microwaves cause significantly better extraction of this component (lignin). Since there are a significantly higher content of lignin than the content of extractives in the substrate, the effect of these methods would also increase the proportion of the extracted lignin, which would increase the extraction efficiency and this would be reflected in an increase in TPC. In the case of ChCl and lactic acid, extraction of hemicelluloses and lignin may also occur, as documented by other studies where this combination is used to remove lignin from wood or annual plants [32–36]. Lactic acid is a weak organic acid ($K_a = 1.34 \times 10^{-4}$). The acidity of the medium can affect protolytic reactions, but should not increase the yield of value-added compounds. The results of several recent studies suggest that DES/LTTMs may have a unique ability to dissolve phenolic substances as well as lignin from plant materials [37–41]. Based on the principles of the interaction between hydrogen bond donors and acceptors, DES/LTTMs can provide a mild acid-base catalytic mechanism that will initiate the controlled cleavage of labile ether bonds between phenylpropane units. This results in the depolymerization of lignin and its separation from biomass [37]. The authors have found that between ChCl and phenols, ChCl can bind to phenolic groups. The high acidity of hydrogen bonds leads to disruption of the structure of lignin-saccharide complexes [37]. Furthermore, it was found that at the same molar ratio of lactic acid and ChCl (1:2), increasing the temperature can maximize ionic properties and increase the molecular polarity of DES/LTTMs, which promotes breakdown of the intramolecular hydrogen bond network and increases lignin and hemicellulose solubility [37]. Recent studies have shown that DES/LTTMs can effectively cause the cleavage of the primary bond between xylan and lignin, thus selectively separating the lignin fraction from the lignocellulosic biomass [32–38]. Value of pH has essential impact on the chemical reactions. Kumar et al. [39] divided green solvents into three groups: acidic (pH values up to 3.0), slightly acidic (pH range 4.0–4.5), and neutral (pH values from 6.0 to 7.0). The results of this report indicated that acidity may affect the extraction of various structures of lignin compounds; consequently, the pH value has a significant effect on the extracted lignin composition and properties, which should be considered in biomass treatment using DESs. De Dios [40] used ChCl and lactic acid systems in a ratio of 1:2 and 1:9, and the lignin removal efficiency from pine sawdust at 60°C and 14 h was 4.9% and 9.7%, respectively. In contrast, Liu et al. [41], using a system containing ChCl and oxalic acid (molar ratio 1:1), extracted lignin from Poplar wood flour and reached up to 81.8% lignin removal at 3 min using microwaves (800 W, 80°C). These results are also in agreement with other authors who have applied various systems and extraction techniques to the extraction of lignin from biomass [23].

As the extraction time and temperature increased, especially the temperature, the polyphenol content increased, which may be due to the extraction of lignin from the substrate. This was shown at 80°C where the TPC content was highest. However, in our work, we did not focus on the analysis of lignin extraction, but our intention was to extract triterpenoids.

The results of the analysis showed that the LTTM used can extract betulin and betulinic acid, but not in the same amount as other types of both polar and non-polar solvents [14,20,42–46]. If we compare the yields of extractives obtained by various extraction methods, these range from 4.1% to 44.7% [14,20,42–46]. Rizhikovs et al. [45] achieved low yields using Soxhlet apparatus for non-polar solvents such as cyclohexane (8.3%), *n*-hexane (4.5%), petroleum ether (7.8%), and *n*-heptane (4.1%), which is up to 6 times lower than when using polar solvents. This can be explained by the fact that betulin is sparingly soluble in non-polar solvents (cyclohexane at 15.2°C, betulin solubility 0.1 g·L^{-1}) [47]. Rizhikovs et al. [45] also noted that when non-polar solvents are used, the extract is usually white, but the application of polar solvents leads to color change to brown due to the extraction of phenolic compounds.

Ostapiuk et al. [48] refluxed black and silver birch bark (outer and inner layers) for 4 h by ethanol. Chemical profiling revealed the similar composition of birch bark and an equally high content of triterpenes in black birch as in white birch. Analysis revealed a higher content of betulin and lupeol in the inner bark extract of *B. obscura* than in *B. pendula*, while the opposite was in the outer bark extract. The lupeol content in the outer bark extract was similar. Outer bark extract: for *B. pendula*, betulinic acid was 97.42 ± 3.91 mg/g dry extract and betulin was 295.93 ± 3.94 mg/g dry extract, and for *B. Obscura*, betulinic acid was 49.89 ± 0.74 mg/g dry extract and betulin was 154.86 ± 1.01 mg/g dry extract. Inner bark extract: for *B. pendula*, betulinic acid was 56.89 ± 2.43 mg/g dry extract and betulin was 417.49 ± 2.02 mg/g dry extract, and for *B. obscura*, betulinic acid was 48.77 ± 3.50 mg/g

dry extract, and betulin was 424.45 ± 5.87 mg/g dry extract [48]. The content of chemical substances is distinct in the case of the inner and outer barks [48–51]. Ajao et al. [49] extracted fresh-cut yellow birch (*Betula alleghaniensis*) and the content of total polyphenols in yellow birch bark was 333.375 ± 59.975 mg of GAE/g oven-dry extract. Athanasiadou et al. [52] extracted condensed tannins in the inner and outer layers of birch bark (*Betula pubescens*, not separated) and found that the content of condensed tannins is not connected with the age of the tree.

Betulinic acid was also identified and determined in the extraction of *Eucalyptus globulus* by Silva et al. [53], who used hydrophobic DESs as extractants. In this case, the authors focused on the analysis of the content of ursolic acid, oleanolic acid, and betulinic acid. As a result, the use of hydrophobic solvents such as menthol:thymol (1:2) led to higher yields of ursolic, oleanolic, and betulinic acids at room temperature, 60°C, and 90°C than those using the Soxhlet extraction with dichloromethane. At 60°C, the extract yield was 2% by weight for ursolic acid, 1% for oleanolic acid, and 0.38% for betulinic acid. They have shown that the ratios of biomass to solvent at 90°C affect the yield. Holonec et al. [54] determined the concentration of betulinic acid and betulin in birch bark from different areas and areas in the range of 7.3–15.4 mg/g bark and 57.4–165.6 mg/g bark. Using extractants such as dichloromethane, acetic acid ester, acetone, chloroform, methanol, and 95% ethanol, the betulin/betulinic acid (mg/g bark) content in the white bark was as follows: 122.0/10.8, 160.0/17.5, 130.2/15.1, 170.0/13.3, 121.6/10.7, and 202.2/18.6 mg/g bark [55]. The higher content of betulin compared to betulinic acid is consistent with our observations. Since the content of triterpenes was up to a hundred times lower in terms of the use of the chosen LTTM than in the case of the use of other extractants, it can be concluded that the choice of this LTTM was not the most suitable. On the other hand, the extracts have been colored brown and must therefore contain phenolic compounds.

In our previous work devoted to spruce bark, it has been documented that the use of DESs makes it possible to obtain a relatively high yield of polyphenols [2,22]. The content of polyphenols in DESs extracts ranged from 41 to 463 mg of GAE/100 g extract. ChCl with lactic acid, glycolic acid, malonic acid, tartaric acid, oxalic acid, citric acid, glycerol, maleic acid, or malic acid [22] was used as DESs. When spruce bark was treated with the DESs composed of ChCl with lactic acid, 1,3-propanediol, 1,5-pentanediol, 1,4-butanediol, 1,3-butanediol, and water, the yields ranging from 10.9 to 16 mg GAE/100 g extract were achieved [2].

Nakurte et al. [56] extracted polyphenols with two solvents – ethanol and 2-propanol – by treatment of various samples of birch bark. The yield of total extracts was 22.6–32.8%, but the extraction was performed subsequently 2–3 times (in the first extraction step, the yield reached from 13.3% to 22.1%). TPCs were also determined and ranged from 2.56 to 7.42 mg GAE/100 g dry extract (results for one extraction step).

Kähkönen et al. [57] summarized in their review the TPC content for different types of biomass. The content of TPC in the silver birch phloem was 85.5 mg GAE/g dry matter and in the bark 2 mg GAE/g dry matter.

A detailed analysis of the composition of the extract was published by Liimatainen et al. [58], who treated samples of the inner bark of 21 separate silver birches and identified 30 substances. The study showed that birch inner bark is a rich source of phenolic substances such as flavonoids, arylbutanoids, diarylheptanoids, simple phenolic compounds, phenolic acids, lignans, and procyanidines. Comparing our results with those obtained from other authors, it can be stated that the LTTM used leads to a comparable level of TPC extraction.

Methods for purifying extracts with LTTM are described in detail in the cited works of Cvjetko Bubalo et al. [59], Cravotto et al. [60], Panić et al. [61], Tang and Row [62], and Makris [63]. Cvjetko Bubalo et al. [59] applied 80% water to grape-pomace extract containing ChCl and citric acid prior to adsorption chromatography, and anthocyanin yield reached ~99.46%, and solvent recycling 96.8%. ChCl and citric acid recycling and anthocyanin regeneration were also followed on a pilot scale in an automatic two-column system [60]. The recycling yield of the extractant in this system was 77.91% and the yield of anthocyanin was about 90%. Zhuang et al. [64] and Wang et al. [65] reported that polyphenols reached 77.4–98.2% and 75.3–85.5% by a macroporous resin recovery, respectively, from a NADES extract of plants. When applying the ChCl and lactic acid system to extract lignin from biomass, it has been shown that recycling of this system is possible using water and even more advantageous if ethanol is used to recover lignin and to recycle the solvent system [66]. The mentioned results suggested that if the extracted substance obtained is of sufficient value for a given purity, a system for recycling and recovering value-added substances may be economically advantageous.

Hitherto, there has been a tendency to use in practice value-added compounds isolated from extracts, e.g., by chromatographic methods [67]. However, we consider the direct application of entire extracts to be a more advantageous mode. The condition is, of course, the safety of the LTTMs used and the sufficient content of

the desired value-added compounds in the extracts. The mixture of ChCl and lactic acid used by us can be considered from ecological and health aspects as a suitable extractant (meeting the characteristics of NADES), but the content of value-added compounds in the extract was too low to be able to advantageously use the entire extract in practice.

4 Conclusion

In this work, an LTTM composed of ChCl and lactic acid in a molar ratio of 1:1 and water content of 5.4 wt% was developed for the extraction of TPC and triterpenoids from birch bark. The total yield of extractives ranged from 10.2% to 24.1%. The concentration of betulin and betulinic acid in the extract were determined by HPLC-UV. The content of betulin ranged from 0.491 to 1.788 mg/g dry bark and betulinic acid ranged from 0.106 to 0.317 mg/g dry bark. This study showed that the used LTTM do not belong to the most suitable reagents for the extraction of triterpenoids. On the other hand, this agent was shown to extract polyphenolic compounds and the TPC content ranged from 18.6 to 56.8 mg GAE/g dry birch bark. The results showed that increasing the temperature improves the extraction process of triterpenoids and especially polyphenols.

It can reasonably be expected that it is only a matter of time before the ecological criteria for extraction and processing will be as important as the economic ones.

Comparing the results of the extraction of value-added substances from biomass faces one key problem. The analysis of inorganic minerals, ores, and products is performed using standardized methods for independent determination of the total content (e.g., metal element) in these materials. That is, the isolation yield of a component of such a material can be expressed on an absolute scale. Regarding the yields of individual value-added substances extracted from phytomass, there is no standardized independent analytical method that would determine the total actual content of these substances in phytomass. Any results regarding the efficiency or yield of the extraction do not provide absolute information.

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Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on request.

References

[1] Jablonsky M, Nosalova J, Sladkova A, Haz A, Kreps F, Valka J, et al. Valorisation of softwood bark through extraction of utilizable chemicals: A review. Biotechnol Adv. 2017;35(6):726–50. doi: 10.1016/j.biotechadv.2017.07.007.

[2] Jablonsky M, Majova V, Strizincova P, Sima J, Jablonsky J. Investigation of total phenolic content and antioxidant activities of spruce bark extracts isolated by deep eutectic solvents. Crystals. 2020;10(5):402. doi: 10.3390/cryst10050402.

[3] Tanase C, Coșarcă S, Muntean DL. A critical review of phenolic compounds extracted from the bark of woody vascular plants and their potential biological activity. Molecules. 2019;24(6):1182. doi: 10.3390/molecules24061182.

[4] Banso A. Phytochemical and antibacterial investigation of bark extracts of Acacia nilotica. J Med Plants Res. 2009;3(2):082–5. doi: 10.5897/JMPR.9000985.

[5] Petruzzello M. List of plants in the family Betulaceae. Encyclopedia Britannica, 7 Apr. 2021, https://www.britannica.com/topic/list-of-plants-in-the-family-Betulaceae-2075381. Accessed 31 May 2021.

[6] Godiņa D, Paze A, Rizhikovs J, Stankus K, Virsis I, Nakurte I. Stability studies of bioactive compounds from birch outer bark ethanolic extracts. Key Eng Mat. 2018;762:152–7. doi: 10.4028/www.scientific.net/KEM.762.152.

[7] Abyshev AZ, Agaev EM, Guseinov AB. Studies of the chemical composition of birch bark extracts (Cortex betula) from the Betulaceae family. Pharm Chem J. 2007;41(8):419–23. doi: 10.1007/s11094-007-0091-5.

[8] Blondeau D, St-Pierre A, Bourdeau N, Bley J, Lajeunesse A, Desgagné-Penix I. Antimicrobial activity and chemical composition of white birch (Betula papyrifera Marshall) bark extracts. MicrobiologyOpen. 2019;9(1):e00944. doi: 10.1002/mbo3.944.
[9] Miranda I, Gominho J, Mirra I, Pereira H. Fractioning and chemical characterization of barks of Betula pendula and Eucalyptus globulus. Ind Crop Prod. 2013;41:299–305. doi: 10.1016/j.indcrop.2012.04.024.
[10] Rastogi S, Pandey MM, Rawat AK. Medicinal plants of the genus Betula – traditional uses and a phytochemical–pharmacological review. J Ethnopharmacol. 2015;159:62–83. doi: 10.1016/j.jep.2014.11.010.
[11] Vinod M, Singh M, Pradhan M, Iyer SK, Tripathi DK. Phytochemical constituents and pharmacological activities of Betula alba Linn: A review. Int J Pharmtech Res. 2012;4(2):643–7. ISSN: 0974-4304.
[12] Drag M, Surowiak P, Drag-Zalesinska M, Dietel M, Lage H, Oleksyszyn J. Comparision of the cytotoxic effects of birch bark extract, betulin and betulinic acid towards human gastric carcinoma and pancreatic carcinoma drug-sensitive and drug-resistant cell lines. Molecules. 2009;14(4):1639–51. doi: 10.3390/molecules14041639.
[13] Wani MS, Gupta RC, Munshi AH, Pradhan SK. Phytochemical screening, total phenolics, flavonoid content and antioxidant potential of different parts of Betula utilis D. Don from Kashmir Himalaya. Int J Pharm Sci Res. 2018;9(6):2411–7. doi: 10.13040/IJPSR.0975-8232.9(6).2411-17.
[14] Popov SA, Sheremet OP, Kornaukhova LM, Grazhdannikov AE, Shults EE. An approach to effective green extraction of triterpenoids from outer birch bark using ethyl acetate with extractant recycle. Ind Crop Prod. 2017;102:122–32. doi: 10.1016/j.indcrop.2017.03.020.
[15] Koptelova EN, Kutakova NA, Ivanovich S, Tretjakov AV, Razumov E, Barcík Š. Extraction of betulin from the birch bark balance at pulp and paper production. Wood Res-Slovakia. 2020;65(5):833–41. doi: 10.37763/wr.1336-4561/65.5.833842.
[16] Grazhdannikov AE, Kornaukhova LM, Rodionov VI, Pankrushina NA, Shults EE, Fabiano-Tixier AS, et al. Selecting a green strategy on extraction of birch bark and isolation of pure betulin using monoterpenes. ACS Sustain Chem Eng. 2018;6(5):6281–8. doi: 10.1021/acssuschemeng.8b00086.
[17] Krasutsky PA. Birch bark research and development. Nat Prod Rep. 2006;23(6):919–42. doi: 10.1039/B606816B.
[18] Anastas P, Eghbali N. Green chemistry: principles and practice. Chem Soc Rev. 2010;39(1):301–12. doi: 10.1039/B918763B.
[19] Lugemwa FN. Extraction of betulin, trimyristin, eugenol and carnosic acid using water-organic solvent mixtures. Molecules. 2012;17(8):9274–82. doi: 10.3390/molecules17089274.
[20] Ferreira R, Garcia H, Sousa AF, Freire CSR, Silvestre AJD, Kunz W, et al. Microwave assisted extraction of betulin from birch outer bark. RSC Adv. 2013;3(44):21285–8. doi: 10.1039/C3RA43868F.
[21] Liu J, Chen P, Yao W, Wang J, Wang L, Deng L, et al. Subcritical water extraction of betulinic acid from birch bark. Ind Crop Prod. 2015;74:557–65. doi: 10.1016/j.indcrop.2015.05.064.
[22] Škulcova A, Haščičová Z, Hrdlička L, Šima J, Jablonský M. Green solvents based on choline chloride for the extraction of spruce bark (Picea abies). Cell Chem Technol. 2017;52(3–4):171–9.
[23] Jablonský M, Šima J. Deep eutectic solvents in biomass valorization. Bratislava: Spektrum STU; 2019.
[24] Ekezie FG, Sun DW, Cheng JH. Acceleration of microwave-assisted extraction processes of food components by integrating technologies and applying emerging solvents: A review of latest developments. Trends Food Sci Tech. 2017;67:160–72. doi: 10.1016/j.tifs.2017.06.006.
[25] Bajkacz S, Adamek J. Evaluation of new natural deep eutectic solvents for the extraction of isoflavones from soy products. Talanta. 2017;168:329–35. doi: 10.1016/j.talanta.2017.02.065.
[26] Bosiljkov T, Dujmić F, Cvjetko Bubalo M, Hribar J, Vidrih R, Brnčić M, et al. Natural deep eutectic solvents and ultrasound-assisted extraction: green approaches for extraction of wine lees anthocyanins. Food Bioprod Process. 2017;102:195–203. doi: 10.1016/j.fbp.2016.12.005.
[27] Cao J, Yang M, Cao F, Wang J, Su E. Well-designed hydrophobic deep eutectic solvents as green and efficient media for the extraction of artemisinin from Artemisia annua leaves. ACS Sustain Chem Eng. 2017;5(4):3270–8. doi: 10.1021/acssuschemeng.6b03092.
[28] Jeong KM, Ko J, Zhao J, Jin Y, Han SY, Lee J. Multi-functioning deep eutectic solvents as extraction and storage media for bioactive natural products that are readily applicable to cosmetic products. J Clean Prod. 2017;151:87–95. doi: 10.1016/j.jclepro.2017.03.038.
[29] Huang Y, Feng F, Jiang J, Qiao Y, Wu T, Voglmeir J, et al. Green and efficient extraction of rutin from tartary buckwheat hull by using natural deep eutectic solvents. Food Chem. 2017;221:1400–5. doi: 10.1016/j.foodchem.2016.11.013.
[30] Wang T, Xu WJ, Wang SX, Kou P, Wang P, Wang XQ, et al. Integrated and sustainable separation of chlorogenic acid from blueberry leaves by deep eutectic solvents coupled with aqueous two-phase system. Food Bioprod Proces. 2017;105:205–14. doi: 10.1016/j.fbp.2017.07.010.
[31] Chen Z, Wan C. Ultrafast fractionation of lignocellulosic biomass by microwave-assisted deep eutectic solvent pretreatment. Bioresource Technol. 2018;250:532–7. doi: 10.1016/j.biortech.2017.11.066.
[32] Alvarez-Vasco C, Ma R, Quintero M, Guo M, Geleynse S, Ramasamy KK, et al. Unique low-molecular-weight lignin with high purity extracted from wood by deep eutectic solvents (DES): a source of lignin for valorization. Green Chem. 2016;18(19):5133–41. doi: 10.1039/C6GC01007E.
[33] Jablonsky M, Haz A, Majova V. Assessing the opportunities for applying deep eutectic solvents for fractionation of beech wood and wheat straw. Cellulose. 2019;26(13):7675–84. doi: 10.1007/s10570-019-02629-0.
[34] Jablonský M, Škulcová A, Kamenská L, Vrška M, Šima J. Deep eutectic solvents: fractionation of wheat straw. BioResources. 2015;10(4):8039–47. doi: 10.15376/biores.10.4.8039-8047.
[35] Zhang CW, Xia SQ, Ma PS. Facile pretreatment of lignocellulosic biomass using deep eutectic solvents. Bioresource Technol. 2016;219:1–5. doi: 10.1016/j.biortech.2016.07.026.
[36] Li T, Lyu G, Liu Y, Lou R, Lucia LA, Yang G, et al. Deep eutectic solvents (DESs) for the isolation of willow lignin (Salix

matsudana cv. Zhuliu). Int J Mol Sci. 2017;18(11):2266. doi: 10.3390/ijms18112266.
[37] Francisco M, van den Bruinhorst A, Kroon MC. New natural and renewable low transition temperature mixtures (LTTMs): screening as solvents for lignocellulosic biomass processing. Green Chem. 2012;14(8):2153–7. doi: 10.1039/C2GC35660K.
[38] Chen Y, Zhang L, Yu J, Lu Y, Jiang B, Fan Y, et al. High-purity lignin isolated from poplar wood meal through dissolving treatment with deep eutectic solvents. Royal Soc Open Sci. 2019;6(1):181757. doi: 10.1098/rsos.181757.
[39] Kumar AK, Parikh BS, Shah E, Liu LZ, Cotta MA. Cellulosic ethanol production from green solvent-pretreated rice straw. Biocatal Agric Biotechnol. 2016;7:14–23. doi: 10.1016/j.bcab.2016.04.008.
[40] de Dios SLG. Phase equilibria for extraction processes with designer solvents [dissertation]. Santiago de Compostela: University of Santiago de Compostela; 2013.
[41] Liu Y, Chen W, Xia Q, Guo B, Wang Q, Liu S, et al. Efficient cleavage of lignin–carbohydrate complexes and ultrafast extraction of lignin oligomers from wood biomass by microwave-assisted treatment with deep eutectic solvent. ChemSusChem. 2017;10(8):1692–700. doi: 10.1002/cssc.201601795.
[42] Pakdel H, Népo Murwanashyaka J, Roy C. Extraction of betulin by vacuum pyrolysis of birch bark. J Wood Chem Technol. 2002;22(2–3):147–55. doi: 10.1081/WCT-120013359.
[43] Guidoin MF, Yang J, Pichette A, Roy C. Betulin isolation from birch bark by vacuum and atmospheric sublimation: A thermogravimetric study. Thermochim Acta. 2003;398(1–2):153–66. doi: 10.1016/S0040-6031(02)00358-1.
[44] Paze A, Zandersons J, Rizikovs J, Dobele G, Jurkjane V, Spince B, et al. Apparatus and selective solvents for extraction of triterpenes from silver birch (Betula pendula Roth.) Outer bark. Baltic For. 2014;20(1):88–97. ISSN 2025-9230.
[45] Rizhikovs J, Zandersons J, Dobele G, Paze A. Isolation of triterpene-rich extracts from outer birch bark by hot water and alkaline pre-treatment or the appropriate choice of solvents. Ind Crop Prod. 2015;76:209–14. doi: 10.1016/j.indcrop.2015.06.053.
[46] Diouf PN, Stevanovic T, Boutin Y. The effect of extraction process on polyphenol content, triterpene composition and bioactivity of yellow birch (Betula alleghaniensis Britton) extracts. Ind Crop Prod. 2009;30(2):297–303. doi: 10.1016/j.indcrop.2009.05.008.
[47] Cao D, Zhao G, Yan W. Solubilities of betulin in fourteen organic solvents at different temperatures. J Chem Eng Data. 2007;52(4):1366–68. doi: 10.1021/je700069g.
[48] Ostapiuk A, Kurach Ł, Strzemski M, Kurzepa J, Hordyjewska A. Evaluation of antioxidative mechanisms *in vitro* and triterpenes composition of extracts from silver birch (betula pendula roth) and black birch (betula obscura kotula) barks by FT-IR and HPLC-PDA. Molecules. 2021;26(15):4633. doi: 10.3390/molecules26154633.
[49] Ajao O, Benali M, Faye A, Li H, Maillard D, Ton-That MT. Multi-product biorefinery system for wood-barks valorization into tannins extracts, lignin-based polyurethane foam and cellulose-based composites: techno-economic evaluation. Ind Crop Prod. 2021;167:113435. doi: 10.1016/j.indcrop.2021.113435.
[50] Bachořík J, Urban M. Biocatalysis in the chemistry of lupane triterpenoids. Molecules. 2021;26(8):2271. doi: 10.3390/molecules26082271.
[51] Raitanen JE, Järvenpää E, Korpinen R, Mäkinen S, Hellström J, Kilpeläinen P, et al. Tannins of conifer bark as nordic piquancy – sustainable preservative and aroma? Molecules. 2020;25(3):567. doi: 10.3390/molecules25030567.
[52] Athanasiadou S, Almvik M, Hellström J, Madland E, Simic N, Steinshamn H. Chemical analysis and anthelmintic activity against teladorsagia circumcincta of nordic bark extracts *in vitro*. Front Vet Sci. 2021;8:666924. doi: 10.3389/fvets.2021.666924.
[53] Silva NH, Morais ES, Freire CS, Freire MG, Silvestre AJ. Extraction of high value triterpenic acids from Eucalyptus globulus biomass using hydrophobic deep eutectic solvents. Molecules. 2020;25(1):210. doi: 10.3390/molecules25010210.
[54] Holonec L, Ranga F, Crainic D, Truta A, Socaciu C. Evaluation of betulin and betulinic acid content in birch bark from different forestry areas of Western Carpathians. Not Bot Horti Agro. 2012;40(2):99–105. doi: 10.15835/nbha4027967.
[55] Zhao G, Yan W, Cao D. Simultaneous determination of betulin and betulinic acid in white birch bark using RP-HPLC. J Pharmaceut Biomed. 2007;43(3):959–62. doi: org/10.1016/j.jpba.2006.09.026.
[56] Nakurte I, Stankus K, Virsis I, Paze A, Rizhikovs J. Characterization of antioxidant activity and total phenolic compound content of birch outer bark extracts using micro plate assay. Environment. Technologies. Resources. Proceedings of the 11th International Scientific and Practical Conference; 2017 Jun 15; Rezekne, Latvia. Rezekne: Rezekne Academy of Technologies; 2017. doi: 10.17770/etr2017vol1.2554.
[57] Kähkönen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, et al. Antioxidant activity of plant extracts containing phenolic compounds. J Agr Food Chem. 1999;47(10):3954–62. doi: 10.1021/jf990146l.
[58] Liimatainen J, Karonen M, Sinkkonen J, Helander M, Salminen JP. Characterization of phenolic compounds from inner bark of Betula pendula. Holzforschung. 2012;66:171–81. doi: 10.1515/HF.2011.146.
[59] Cvjetko Bubalo M, Vidović S, Radojčić Redovniković I, Jokić S. Green solvents for green technologies. J Chem Technol Biot. 2015;90(9):1631–9. doi: 10.1002/jctb.4668.
[60] Cravotto G, Mariatti F, Gunjevic V, Secondo M, Villa M, Parolin J, et al. Pilot scale cavitational reactors and other enabling technologies to design the industrial recovery of polyphenols from agro-food by-products, a technical and economical overview. Foods. 2018;7(9):130–44. doi: 10.3390/foods7090130.
[61] Panić M, Gunjević V, Cravotto G, Redovniković IR. Enabling technologies for the extraction of grape-pomace anthocyanins using natural deep eutectic solvents in up-to-half-litre batches extraction of grape-pomace anthocyanins using NADES. Food Chem. 2019;300:125185. doi: 10.1016/j.foodchem.2019.125185.
[62] Tang W, Row KH. Design and evaluation of polarity controlled and recyclable deep eutectic solvent based biphasic system for the polarity driven extraction and separation of compounds. J Clean Prod. 2020;268:122306. doi: 10.1016/j.jclepro.2020.122306.

[63] Makris DP. Green extraction processes for the efficient recovery of bioactive polyphenols from wine industry solid wastes – recent progress. Curr Opin Green Sustain Chem. 2018;13:50–5. doi: 10.1016/j.cogsc.2018.03.013.
[64] Zhuang B, Dou LL, Li P, Liu EH. Deep eutectic solvents as green media for extraction of flavonoid glycosides and aglycones from Platycladi Cacumen. J Pharmaceut Biomed. 2017;134:214–9. doi: 10.1016/j.jpba.2016.11.049.
[65] Wang T, Jiao J, Gai QY, Wang P, Guo N, Niu LL, et al. Enhanced and green extraction polyphenols and furanocoumarins from Fig (Ficus carica L.) leaves using deep eutectic solvents. J Pharmaceut Biomed. 2017;145:339–45. doi: 10.1016/j.jpba.2017.07.002.
[66] Smink D, Kersten SR, Schuur B. Process development for biomass delignification using deep eutectic solvents. Conceptual design supported by experiments. Chem Eng Res Des. 2020;164:86–101. doi: 10.1016/j.cherd.2020.09.018.
[67] Isci A, Kaltschmitt M. Recovery and recycling of deep eutectic solvents in biomass conversions: a review. Biomass Conv Bioref. 2021;1–30. doi: 10.1007/s13399-021-01860-9.