

Improvement of Excretion Methods and Studying the Composition and Properties of Birch Bark Extracts

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Abstract

Results of the research into improved techniques to excrete the extracts of biologically active betulin, suberic and tanning agents from the wastes of birch bark are presented. A short-term activation of bark by superheated steam has been used to intensify the processes of excretion of extractives. Selection of optimum conditions for bark activation that ensure the maximum yield of betulin, suberic and tanning agents has been performed. It has been shown that the use of betulin extracts is viable to yield gastroprotective preparations, whereas the use of suberic materials is viable in production of film-forming materials.

INTRODUCTION

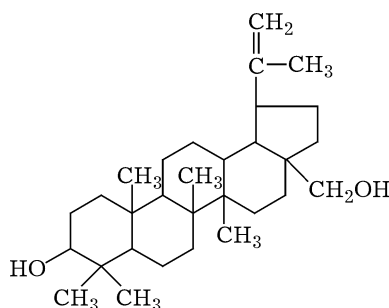
Birch is one of the most widespread kinds of trees in the forests of Siberia and European part of Russia [1]. The woodwork manufacturers and those of plywood and wood charcoal produce significant amount of a birch bark, which is practically out of use and, at the best, is burnt.

The birch bark of young trees and outgrowths is made up of the external layer (outer birch bark) and the inner layer (bast) that differ considerably from each other in their chemical composition [2, 3]. The birch bark is the champion among the other kinds of vegetative raw material as to the content of biologically active triterpenic compounds.

Betulin prevails within the composition of triterpens from the birch bark [4] (Scheme 1).

Betulin possesses antiseptic, anti-virus (herpes virus), anti-inflammatory, hepatoprotective, antioxidant, and other useful properties [5–7]. Betulinic acid and its

derivatives are capable to depress melanoma development [8] and HIV [9, 10]. Betulin itself and some of its ethers are applied as effective emulsifying agents of water-oil mixtures [11]. Betulin may also be applied to synthesise the new classes of biologically active compounds that contain the betulinic fragment [12]. The specified properties of betulin and its derivatives open wide opportunities for their



Scheme 1.

application in medicine, pharmaceutical, perfumery-cosmetic, food, and other industries.

The polymeric part of the birch bark is represented mostly by suberin that is a natural polyester, which includes fatty acids and hydroxyacids. Suberin from the bark of birch exhibits several potential application fields [13]. It can be used as a deflocculant to produce sooty and sizing compositions, paints, cement, boring solutions, and asphalt emulsions. Suberin is also applicable as a binder and a film-forming material [14].

The suberic part of the birch bark also provides a source of valuable chemicals [15]. Taking into account the etherous bonds available within the structure of suberin, the employment of the alkaline and acidic hydrolysis processes is possible to transform suberin into monomer products, specifically, mixtures of natural fatty acids [16, 17]. Suberin hydrolysates contain unique natural hydroxy fatty acids together with fatty acids. The former can be useful in production of lubricants and oils, coatings, insecticides and fungicides, polymers, polyesters, specialty chemicals, pharmaceuticals, and in the other fields [18, 19].

The known ways to excrete betulin are developed around the birch-bark extraction with various solvents and also around the alkaline hydrolysis of the birch bark followed by betulin extraction [20]. However, betulin is poorly soluble in the majority of solvents, which determines a low degree of its extraction. The maximum yield of betulin is attained only with a complete alkaline hydrolysis of the birch bark, which proceeds under reasonably severe conditions [21].

With the aim of increasing the yield of extractives and cutting-down the duration of extraction, various intensification methods are applied. Some of them enhance hydrodynamic regimes of extraction process [22, 23]; the others are based on the application of mechanical, chemical, and mechanochemical activation of the raw material [24–26]. In some cases, the positive effect is achieved with fractional extraction of wood bark with organic solvents of various polarities [2].

Significant supplies of the birch bark are represented by the bark of a bottom part of a birch trunk. There is no clear distinction line between the birch bark and bast in this part.

The processing techniques for such bark to yield betulin and other products are not described in the literature.

The present work presents the results of studying the new methods to produce betulin, suberic and tanning agents from the birch bark including that with no clearly defined boundary between the birch bark and the bast.

EXPERIMENTAL

The bark of drooping birch (*Betula pendula* Roth.) that was stocked in the vicinity of Krasnoyarsk served as the subject of the research. Specimens from an external part of the bark, that is the birch bark separated from the interior of the bark (bast), as well as the specimens of a bottom bark, wherein there was no clear distinction line between the birch bark and the bast, were used in the experiments. Some evidence on the composition of specimens is presented in Table 1.

Specimens of outer birch bark and combined bark were grinded in a disintegrator into the particles of the required size prior to their use.

Activation of the birch bark by steam under “explosive” autohydrolysis conditions was conducted using a set-up with a reactor capacity 0.85 l following a procedure described in [27]. After steaming for 15–240 s at a temperature of 240 °C, and under pressure of 3.4 MPa, the bark of a birch constituted a crumby pulp, which, upon desiccation, was subjected to a fractionation with sieves. A share of fractions with particle size less than 1 mm could amount from 60 to 90 % depending on the activation time.

In some cases, activation of outer birch bark was performed in the presence of alkali liquor of various concentrations. In this case, the hydrolysed birch bark looked like grey sour-cream-like pulp. Excretion of betulin from the hydrolysed pulp was performed by means of extraction with aliphatic alcohols (methyl, ethyl, and isopropyl alcohols). A flask under reflux was loaded with the hydrolysed material, and then it was filled with alcohol and boiled for 10–15 min. Hot reaction pulp was filtered, the filtrate was diluted with water, and alcohol was distilled off. The precipitated betulin was filtered off, washed out on the filter

TABLE 1
Composition of initial specimens of the birch bark and outer birch bark

Component	Content, % from the mass of a.d.w. ¹	
	Bark	Outer birch bark
Cellulose	23.0	3.4
Readily hydrolysable polysaccharides	18.0	–
Hardly hydrolysable polysaccharides	22.0	5.8
Lignin	35.0	13.4
Betulin	10.5	37
Suberin	–	38.7
Extractives	26.9	40.1
Including:		
betulin	39.0 ²	87.3 ²
ashes	1.8	2.1

¹ Absolutely dry wood.

² Recalculated to extractives.

with hot water, and dried up. The purified betulin was obtained by recrystallization from ethanol or isopropanol.

Excretion of suberic materials from the activated birch bark was performed by treatment with water solution of caustic soda followed by boiling in a flask under reflux for 20 min. The solution was cooled down to 70 °C; isopropanol was added in the reaction mixture and then it was boiled for additional 5 min. Hot solution was filtered; isopropanol was distilled off; the residue was diluted with water and betulin was separated by filtering. Water solution was acidified with sulphuric acid to pH 4–5. The suberin deposit was filtered off and washed out with cold water on the filter to achieve the neutral reaction of the washing water.

To extract the ground bottom bark of a birch, the following solvents were used: hexane, ethyl acetate, isopropanol, and water. Complete extraction with hexane was conducted in a Soxhlet apparatus for 30 h, and extraction with ethyl acetate, isopropanol, and water was conducted for 10–12 h. The amount of the recovered extractives was determined by the weight method upon removing a solvent. Each experiment in recovery of extractives was conducted for no less than three times and an average value of the extract weight was calculated.

Separation of neutral and acid matter in a hexane extract was performed with 20 % water solution of NaOH. The yield of acid matter was about 4.8 %. Excretion of betulin from ethyl acetate extract was conducted by treatment with mixture water – ethanol – NaOH at a boiling temperature for 4 h. Then ethanol was distilled off, reaction mass was cooled down, diluted with 10 ml of water, and filtered off. Betulin was washed out on the filter to a neutral reaction of washing water. Excretion of betulin from isopropanol extract was conducted in a similar way.

Betulin was identified by its melting point (257–259 °C) [28], from the IR spectra, which have been taken with Vector-22 Fourier IR spectrometer of Bruker Company, and from ¹H NMR spectra obtained in Bruker AM-400 spectrometer (200 MHz). Quantitative determination of polyphenols was made by the reaction with paranitroaniline [29] using KFK-3 photoelectric photometer. A content of tanning agents of bark specimens and of the extracts obtained was determined following the standard method [30].

Calculation of LD₁₆ (the minimal lethal dose) and LD₅₀ (the mid-lethal dose) was performed by the procedure [31] with the use of the programs, tables, and formulae supplied with the procedure. Studying the toxicological and gastroprotective properties of betulin and of hexane extract of the birch bark, which was composed predominantly of betulin, was performed by the following procedures.

Gastroprotective properties of betulin and those of the birch bark extract were explored with the model of indomethacin ulcer. Ulcer formation in mice was induced by the intragastric administration of Indomethacin. After 24 h, the animals were subjected to euthanizing under etherization and a quantity of cankers was calculated.

RESULTS AND DISCUSSION

Excretion of betulin and suberic materials from a birch bark

Betulin production methods described in the literature are based on the extraction of a birch

bark with various solvents or on the application of alkaline hydrolysis of a birch bark followed by extraction of betulin [20].

With the aim of intensifying the betulin excretion process, we suggested the prior activation of a birch bark by steam under “explosive” autohydrolysis conditions [32]. This work investigates the effect of temperature, of duration of the activation process, and of NaOH admixtures on betulin extraction degree from the birch bark. Activation temperature was varied from 180 to 240 °C, steam tension from 1.0 to 3.4 MPa, duration of treatment from 30 to 360 s. Based on the results of the completed research, the temperature 240 °C has been chosen as an optimum one, whereby water-vapour pressure is 3.4 MPa.

An effect of duration of activation of a birch bark in these conditions on the yield of betulin, which is recovered by aqueous-alkaline solutions of methanol, ethanol, and

isopropanol, is illustrated in Fig. 1, *a*. The highest yield of betulin is evidenced at duration of activation of a birch bark for 180–300 s, and the maximum quantity of betulin (37 % from the mass of absolutely dry birch bark) was recovered by ethanol. After prior activation of a birch bark by steam, the extraction degree of betulin increased by 25–40 % as compared to non-activated birch bark.

Further improvement of betulin production process involved an application of birch bark activating treatment by superheated steam in the presence of alkali [33], whereby the alkaline hydrolysis of the birch bark occurs coincidentally with its loosening. As a consequence of these concurrent processes of loosening and hydrolysis, the birch bark converts to grey sour-cream-like pulp, wherefrom betulin was extracted by ethanol.

Presented in Table 2 are the data as to an effect of the conditions of the birch bark

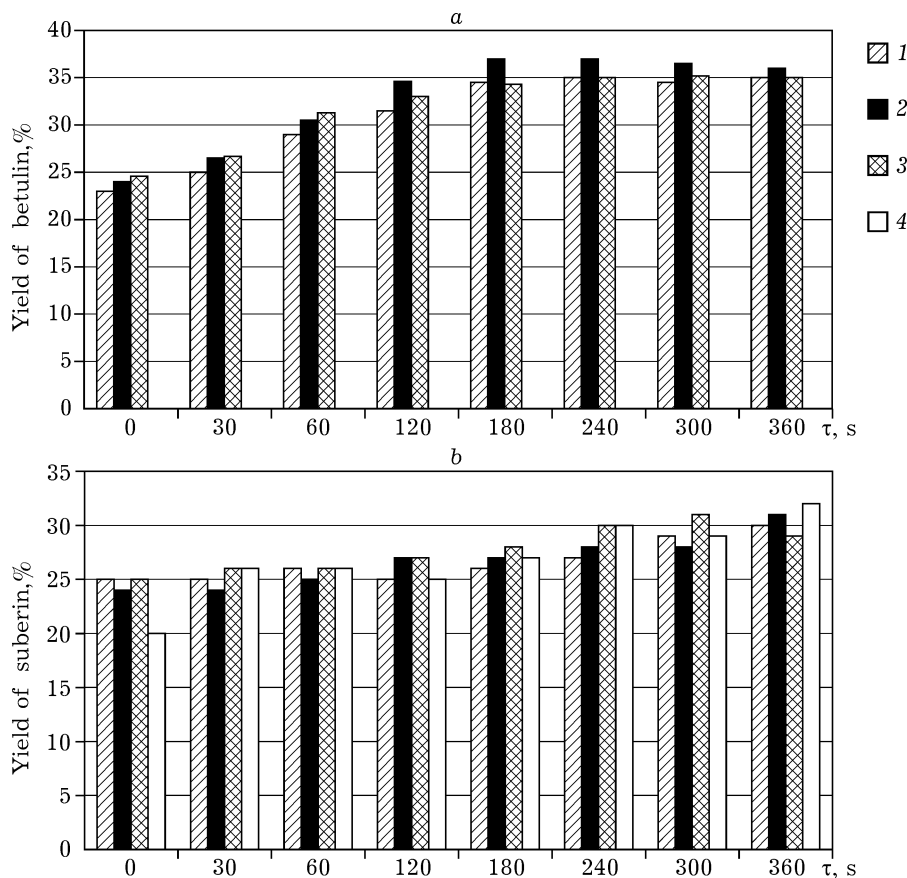


Fig. 1. Effect of duration of activation (t) of the birch bark by steam at 240 °C on the yield of betulin (*a*) and suberin (*b*) that are recovered by aqueous-alcohol-alkaline solution: 1 – methanol, 2 – ethanol, 3 – isopropanol, 4 – without alcohol.

TABLE 2

Effect of duration of birch bark activation by steam at 240 °C in the presence of alkali on the yield of betulin, which is extracted by ethanol, % from the mass of absolutely dry birch bark

NaOH concentration, %	Duration of activation, s						
	30	60	90	120	180	240	300
5	15 (43)	17 (49)	19 (54)	21 (60)	23 (66)	24 (67)	25 (71)
10	18 (51)	20 (57)	23 (66)	26 (74)	30 (86)	31 (89)	32 (91)
15	21 (60)	23 (66)	28 (80)	30 (86)	33 (94)	33.5(96)	33 (94)
20	24 (69)	26 (74)	30 (86)	31 (89)	33.5(96)	34 (97)	3.5 (96)

Note. Given in parentheses is betulin extraction degree.

activation in the presence of sodium hydroxide on the yield of betulin that is extracted by ethanol. As is suggested by the obtained results, the optimum duration of steam treatment of a birch bark in the presence of alkali that corresponds to the maximum extraction degree of betulin comprises no less than 180 s with alkali content being 15–20 % from the weight of absolutely dry birch bark. If concentration of alkali is less than 10 %, a high extraction degree of betulin can be achieved upon longer activation, which leads to partial decomposition of betulin and to a decrease in its yield. Decomposition of betulin in the course of birch bark activation is accompanied by an increase in the amount of evolving gaseous products as well as by tar formation.

The variation in the nature of water-soluble alcohols (methanol, ethanol, and isopropanol) has no considerable effect on the extraction degree of betulin when it is extracted from a birch bark activated in the presence of alkali.

Thus, activation that is carried out simultaneously with alkaline hydrolysis of a birch bark permits tangible intensification of betulin excretion process and raising its extraction degree relative to the birch bark activated with no alkali [32]. Under optimum activation conditions (temperature 240 °C, NaOH concentration of 20 %, duration 240 s), we succeeded in extracting 97 % betulin from the outer birch bark.

During hydrolysis of a birch bark in aqueous-alcohol-alkaline solution, suberin can be extracted together with betulin upon acidifying the hydrolysate. Among the known methods to produce suberin, the most effective one includes a stage of bark grinding in water solution of alkali; this requires higher energy consumption [34].

We suggested a new technique for suberin extraction on the basis of the application of activating treatment of a birch bark by superheated steam with the subsequent extraction of the activated birch bark with an alcohol-alkaline solution and precipitation of betulin [35]. Upon acidifying the filtrate, which remained after betulin excretion by sulphuric acid, up to pH 4–5, the yellow flocculent deposit of suberin is precipitated, which is separable by filtering.

An effect of duration of activation of a birch bark by steam at 240 °C on the yield of suberin is illustrated in Fig. 1, b. Its maximum yield (about 30 % from the mass of a. d. outer birch bark) was observed at duration of activation of a birch bark for 360 s. The extraction degree of suberin with methanol, ethanol, and isopropanol increased by 20–25 % as compared to non-activated birch bark, and in so doing, the nature of alcohol had no considerable effect on its yield. We succeeded in simultaneous extracting of betulin and suberin in the presence of aliphatic alcohols; however, only suberin can be recovered when the activated birch bark is extracted by aqueous-alkaline solution.

In the literature, there is evidence on the possibility to apply the products of thermal condensation of suberin as the constituents of paintwork compositions, whose properties are determined by suberin condensation degree [20]. We have examined the effect of conditions of thermal condensation of lignin, which is extracted from a birch bark as a by-product in betulin production, on some film-forming properties of resins around betulin. The main performance indexes of polycondensed resins from suberin are viscosity and solubility.

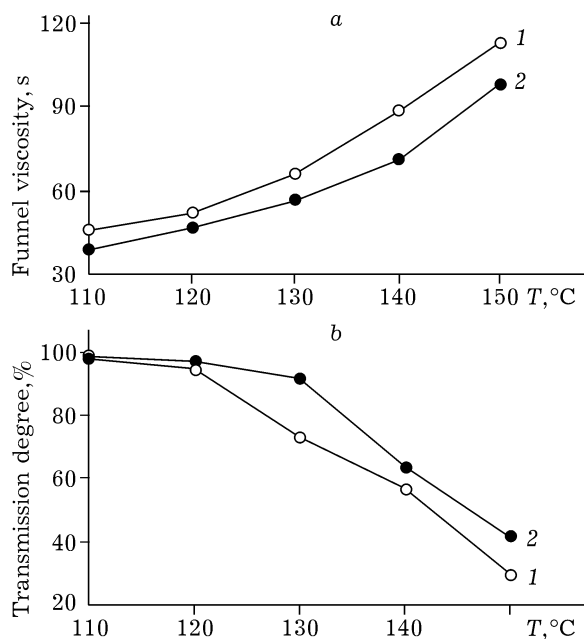


Fig. 2. Dependence of funnel viscosity (a) and of transmission degree (b) for polycondensed suberin solutions (content of 20.5 mass %) in styrene (1) and turpentine (2) on the temperature of suberin polycondensation.

Therefore, the quality of the produced specimens of film-forming varnishes was assessed from change in these parameters.

Adjusting the conditions of suberin polycondensation process that ensure the acceptable values of viscosity and transparency for its solutions in styrene and turpentine has been performed. As the process temperature is raised from 110 to 150 °C, the funnel viscosity of solutions of polycondensed suberin in turpentine and styrene increases from 45 and 38 up to 113 and 98 s, respectively (Fig. 2, a). The specimen of polycondensed suberin, which has been obtained through treatment at 160 °C, is insoluble in turpentine and styrene. As Fig. 2, b suggests, the transparency of solutions of suberin polycondensed at 110–130 °C decreases a little. With further increase of suberin treatment temperature, a sharp decrease in its solubility occurs, which gives rise to a colloidal solution. The transmission degree of solutions considerably decreases and comprises 30 % for styrene and 42 % for turpentine. Seemingly, as the temperature of heat treatment of suberin rises up to 130 °C, linear polymers are formed, and further temperature rise promotes formation of spatial elastomer that is insoluble in organic solvents.

Further research of the process to obtain the film-forming varnishes from suberin was performed by varying the duration of suberin treatment from 5 to 25 min. Optimum conditions for obtaining film-forming resins from suberin have been found: the temperature 125–135 °C, duration of exposition of 15–20 min, water content of suberin of no less than 10 %, and pH 6.0–7.0. When carrying out the process of suberin polycondensation under the specified optimum conditions and upon the subsequent dissolving of the polycondensed suberin in styrene and turpentine, film-forming materials with the performance characteristics, which are close to the characteristics of PF-060 commercial varnish (technical specifications TU 6-10-612-76), have been produced.

Excretion of extractives from a bottom bark of a birch

Methods that were applied for mechanical separation of combined bark of a birch to yield an outer birch bark and bast are complicated and power consuming. In addition, significant resources of birch bark are represented by a so-called bottom bark, wherein there is no clearly defined boundary between an outer birch bark and bast. We have investigated the potentials for extraction processing of the birch bark to yield betulin and tanning agents without prior separation of the bark into the outer birch bark and the bast.

Data concerning the yield and composition of extracts, which were excreted from the bottom bark of a birch with hexane, ethyl acetate, isopropanol, and water, are given in Fig. 3. It was found that the fraction of betulin is 90 % of hexane extract, and the yield of this extract comprises 10.5 % from the mass of dry bark. The extracts that were excreted with ethyl acetate and isopropanol with a yield of 15.5 and 20.6 % respectively contain three basic components: betulin, polyphenols, and sugars. Their content in ethyl acetate extract comprises 60.0, 27.1, 12.7 %, and in isopropanol extract, 46.0, 39.7, 14.3 %, respectively. Water recovers 9.7 % of extractives, which contain 71.7 % of polyphenols (tanning agents comprise 48.7 % from them) and 24.8 % of sugars.

Consecutive extraction of bark of a birch with hexane, ethyl acetate, isopropanol, and

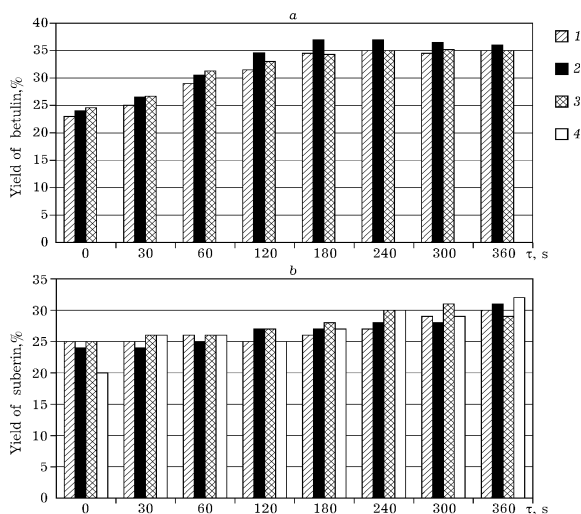


Fig. 3. Yield and composition of extracts that were excreted from the bottom bark of a birch with various solvents. * from the mass of extract.

then with water recovers totally 26.9 % extractives. In so doing, ethyl acetate extract, isopropanol extract, and aqueous extract are composed preferentially of polyphenols (78.3, 61.8, and 54.2 %, respectively) and their content of tanning agents is 67.5, 56.2, and 51.3 %, respectively. Quality of these extracts varies from 46.3 to 49.7 %. Tanning extracts contain from 5.3 (isopropanol extract) to 15.4 % (aqueous extract) of sugars that deteriorate their quality.

Established is the fact of significantly (by 1.5–2 times) increased yield of extractives that are recovered with hexane, ethyl acetate, isopropanol, and water from the bark of birch activated by superheated steam under conditions of “explosive” autohydrolysis (Table 3). Chemical composition of extractives that were excreted from the activated bark of birch by methods of individual and consecutive extraction has been investigated. It has been

found that betulin content of all hexane extracts is kept constant irrespective of duration of bark activation. The solvents that are more polar (ethyl acetate and isopropanol) recover polyphenols, tanning agents, and sugar, together with betulin, from the activated bark. An increase in solvent polarity in going from ethyl acetate to isopropanol and water promotes the growth in concentration of polyphenols and tanning agents in the produced extracts and exerts a little influence on the content of sugars.

Amount of polyphenolic and tanning agents in these extracts remains approximately constant after short-term (less than 30 s) activation of the bark; however, the content of sugars decreases 2.0–2.5 times as compared to non-activated bark (Fig. 4, a, b). As duration of bark activation increases to 240 s, the yield of ethyl acetate and isopropanol extracts grows to 29.0–30.2 %; however, their content of polyphenols, tanning agents, and sugars sharply decreases. The effects observed are related, obviously, to intensification of the processes of depolymerisation of polyphenols and the processes of extraction of the fixed low-molecular-mass phenols with an increase in duration of bark activation. Analogous regularities are observed also at consecutive extraction of the activated birch bark with solvents of increasing polarity: with hexane, then with ethyl acetate, isopropanol, and water (see Fig. 4, c, d).

Toxicological and gastroprotective properties of betulin and of hexane extract of the birch bark

TABLE 3

Effect of duration of activation of the bottom bark of birch by steam at 240 °C on the yield of extractives, which are recovered with various solvents, % from the mass of a. d. bark

Solvent	Duration of activation, s								
	0	15	30	45	60	90	120	180	
Hexane	10.5	12.4	13.5	14.6	15.4	14.7	13.5	12.7	12.5
Ethyl acetate	15.5	18.1	20.8	22.0	23.8	26.2	26.3	27.2	29.0
Isopropanol	20.6	23.8	26.2	27.7	29.2	30.1	31.6	31.2	30.2

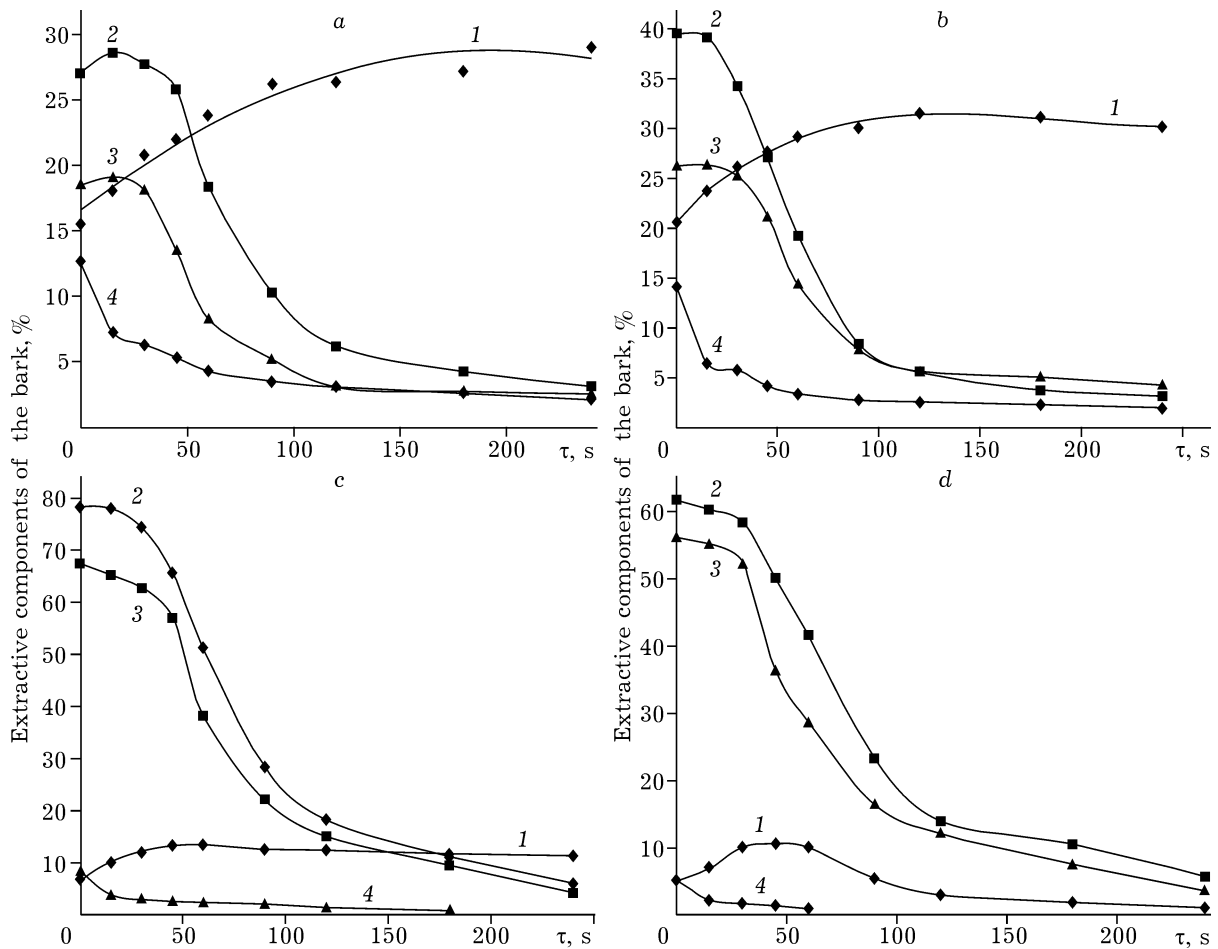


Fig. 4. Effect of duration of activation (τ) of the bottom bark of birch by steam at 240 °C on the yield and composition of the substances that are extracted by ethyl acetate (a) and isopropanol (b) and of ethyl acetate (c) and isopropanol (d) extracts, which have been produced upon consecutive extraction with hexane, ethyl acetate, and isopropanol: 1 – yield, 2–4 – content of polyphenols, tanning agents, and sugars, respectively.

Literature contains evidence that betulin is a low-toxicity material [36]; however, an exact value of its mid-lethal dose (LD_{50}) has not yet been determined.

We have studied toxicological properties of betulin and hexane extract of the birch bark that contains betulin. Determination of the minimal lethal dose (LD_{16}) and of the mid-lethal dose (LD_{50}) was conducted in white mice.

Upon the intragastric administration of betulin and birch bark extract (BBE) dozed from 3000 to 6000 mg/kg, animals did not die. General action of great doses of these biologically active compounds manifested itself as insignificant oppression of central nervous system. Death of part of animals was observed upon administration of 7000 mg/kg dozed BBE with and 7500 mg/kg dozed betulin. A picture of sharp poisoning with BBE and betulin was

dominated by symptoms of oppression of central nervous system, breath irregularity, and decrease in motion activity.

Based on the toxicological research performed, a conclusion is drawn that betulin and betulin-containing hexane extract of the birch bark are not poisonous and they comply with the 4th class of low-toxicity materials according to the international toxicological classification. For betulin, the minimal lethal dose (LD_{16}) comprises 6500 mg/kg; the mid-lethal dose (LD_{50}) does more than 9000 mg/kg. For the birch bark extract, LD_{16} comprises 5750 mg/kg and LD_{50} does 8500 mg/kg.

Study of gastroprotective properties of betulin and BBE was conducted with four groups of white mice, their mass being 10–12 g each (10 individuals in each group). Agents

were administered intragastrically (0.4 ml of 1.5 % starchy suspension) once per day, during 7 days, 600 mg/kg in dose. The test group was administered with 2 ml of water per 1 kg of mass. The last administration of the agent was made 1 h before the ulcer induction by the intragastric administration of Indomethacinum dosed 20 mg/kg. The quantity of cankers was counted up in all groups; the Pauls index was counted by the formula: $PI = AB/100$, where A was the mean quantity of ulcers per animal, B was the quantity of animals with ulcers. Antiulcerous activity was determined as the ratio of PI of the test group to PI of animals under experiment.

The obtained results testify that betulin and birch bark extract show high gastroprotective effect. Antiulcerous activity of betulin comprises 9.3 and that of betulin extract, 7.0.

CONCLUSIONS

Improved techniques to excrete the valuable extractives from the wastes of a birch bark have been offered and investigated. To intensify the processes of their extraction, a short-term activation of the bark by superheated steam followed by sharp pressure release has been used.

In our studies of betulin extraction from the external layer of a birch bark (referred to as an outer birch bark), it was found that the degree of its extraction with alcohol increases by 25–40 % upon an activation by steam as a result of loosening of a birch bark and its partial hydrolysis. Upon birch bark treatment with steam in the presence of alkali, we succeeded in combining the processes of its activation and alkaline hydrolysis; this has allowed us to reach 97 % extraction degree of betulin. Identification of betulin was performed by Fourier IR and H^1 NMR spectroscopy methods and chromato-mass-spectrometry.

The viability to apply betulin extracts for obtaining gastroprotective preparations has been revealed. As a consequence of the investigation of toxicological properties, it was found that betulin and its hexane extract do not exhibit toxic properties and they are classed with the 4th class of low-toxicity materials.

Selection of optimum conditions to excrete suberic materials that remain upon extraction of betulin from the activated birch bark has been performed, as well as selection of the conditions for thermal polycondensation of suberin that provide obtaining the film-forming materials (varnish) with the required technical characteristics.

Potentiality for extraction processing of the birch bark to yield betulin and tanning agents without prior separation of the bark into the outer birch bark and the bast has been demonstrated. Established is the fact of significant (by 1.5–2 times) increase in the yield of extractives, which are recovered with various solvents from the birch bark activated for several minutes by steam at 240 °C.

Studying chemical composition of bark extracts has demonstrated that the hexane extract predominantly contains betulin (content about 90 mass %). The solvents with more polarity (ethyl acetate, isopropanol, and water) recover predominantly polyphenols (including tanning matter) and sugars from the activated bark. The presence of sugars deteriorates the quality of tanning extract from the birch bark. Selection of conditions for the bark activation (240 °C, duration 15–30 s) that ensure an increase in quality of tanning extract due to the decreased content of sugars in this extract by a factor of 2.0–2.5 as compared to non-activated bark has been performed.

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