

## Obtaining Tanning Agents, Dyes, and Enterosorbents from Bast of a Birch Bark

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### Abstract

Optimization of conditions for obtaining of tanning agents, anthocyanidin dyes, and enterosorbents from bast of a birch bark has been performed. By choosing the appropriate conditions for alkali treatment of a porous fixed residue from extraction processing of bast, enterosorbent was produced, which has exhibited high efficiency when treating acute intestinal infections in animals and dysbacterioses that were caused by application of antibiotics. The comprehensive processing of bast of a birch bark makes it possible to produce about 3 % of resinous matter, 35–40 % of polyphenolic products, 13–15 % of anthocyanidin dye, and about 40 % of enterosorbent.

### INTRODUCTION

At present, the manufacturers, which perform a woodwork, production of plywood and wood charcoal, produce the significant amount of waste of a birch bark. The birch bark, typically, is made up of the external layer (outer birch bark) and the inner layer (bast), which show various chemical composition [1, 2].

The outer birch bark is applied to produce the subjects of folk trade, birch oil, and for medical purposes. A promising direction for applying a birch bark is excretion of biologically active triterpens from it (betulin, lupeol, *etc.*) [3].

The main part of a birch bark (about 80 mass %) constitutes the bast, which contains up to 10 % tanning matter, specifically, the water-soluble polyphenols, which are represented by catechols, leucoanthocyanidins, flavonols, phenolcarboxylic acids, and other compounds [4].

It is common knowledge that acidic hydrolysis of leucoanthocyanidins and condensed catechols yields low-molecular-weight compounds of catechol group, which are capable of oxidizing to form coloured anthocyanidins [5]. Anthocyanidin compounds are applied as food colours; they are constituents of preparations with anti-inflammatory, healing action and of preparations that promote protective behaviour of organism. They are currently excreted for the most part from berries, vegetables, and fruits [6], although there are known methods to produce anthocyanidin dyes from the bark of fir and larch [7, 8].

During recovery of extractives of a bark, an opening of pore structure of raw material occurs. After extraction with polar solvents (alcohol, ethyl acetate, *etc.*), fixed residue of bark is virtually free from individual phenolic compounds. This enables its

application as enterosorbents (of the polyphepane type) [9, 10].

The literature contains no information on procedures to produce high-quality tanning extracts, anthocyanidin dyes, and enterosorbents from bast of a birch bark.

The purpose of the present work is to optimize the conditions for production of tanning agents, anthocyanidin dyes, and enterosorbents from the birch bark bast and to develop the process for its wasteless recycling on this basis.

## EXPERIMENTAL

The bast of the bark of drooping birch (*Betula pendula* Roth.) that was stored in the vicinity of Krasnoyarsk was used in this work. Before being used, the air-dried bast was grinded in a disintegrator to obtain particles less than 1.5 mm in size. Hydraulic beating of bast with cold water was performed in some cases.

The composition of bast was analysed according to the schematic diagram, which is presented in Fig. 1.

Initial bast contains 26.3 % of readily hydrolysable polysaccharides, 22.9 % of hardly hydrolysable polysaccharides, 34.8 % of lignin matter, 13.7 % of substances extractable by hot water, 3.4 % of mineral substances.

Investigation of chemical composition of bast before and after extraction was carried out in compliance with standard procedures in chemistry of wood [11]. The basic components, specifically, lignin materials, polysaccharides, and extractives, were determined in the samples under investigation. Quantitative determination of tanning agents was performed in compliance with BEM standard procedure by means of hide powder [12].

Sodium hydroxide of various concentrations (from 0.5 to 1.5 %) in water solution of ethanol was used as an extractant that provides the most complete recovery of substances of phenolic nature. Alcohol concentration was varied from 10 to 20 %. Extraction of tanning agents was conducted with the liquid ratio (extractant : bark ratio) being equal to 10 and a temperature of 70 °C in a laboratory temperature-controlled unit. Duration of extraction process was varied from 0.5 to 1.5 h.

Anthocyanidin dye was isolated through treatment of previously deresinified bast with boiling ethanol in the presence of muriatic acid. To recover a resinous matter, a batch of air-dried bast (50 g) was extracted with hexane, petroleum ether, or with BR-1 grade petrol in a Soxhlet apparatus of capacity 250 ml during 8 h. The yield of resinous matter comprised 3.0–3.2 % from the weight of absolutely dry bast 10 g of absolutely dry resin-free bast was

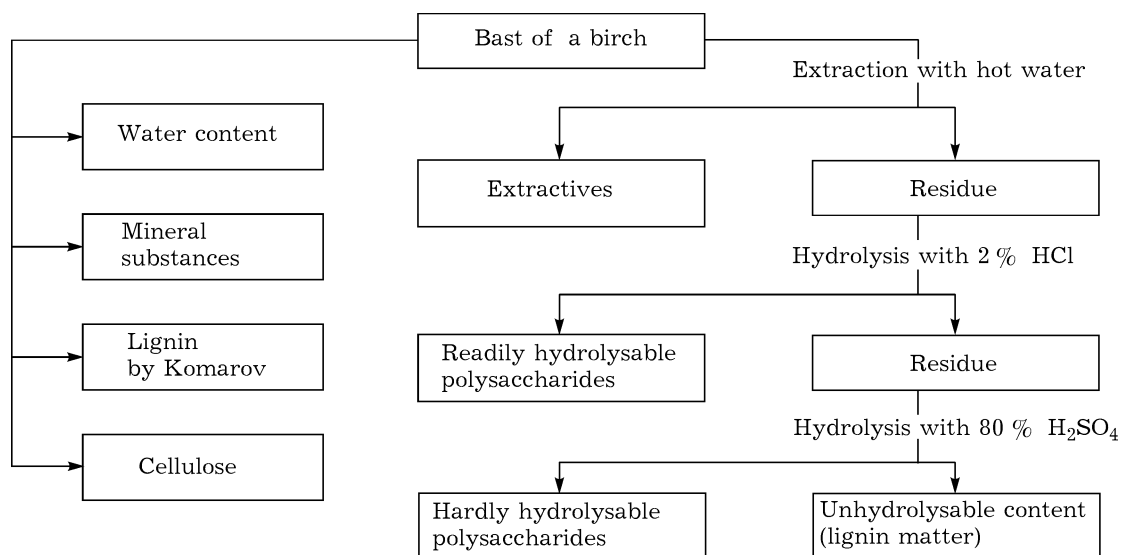


Fig. 1. Schematic diagram for determination of composition of a birch bark bast.

loaded in a round-bottomed flask of capacity 0.5 l that was equipped with a reflux condenser; then the required amount of ethanol and muriatic acid (the concentration of 33–37 %) was added. A liquor ratio was varied from 8 to 14; the concentration HCl was varied from 0.5 to 4.0 %. After boiling for 4 h, the solution in the flask turned dark-cherry. Reaction mass was filtered off from bast on the filter with no cooling. The resulting dye solution was concentrated so that the volume was equal to 20–25 ml; then it was diluted with 150–160 ml of water; the deposit was separated by filtering and dried in a shaded place at the room temperature.

Paper chromatography (Whatman drawing paper No. 1) was applied for separation and identification of the obtained anthocyanidin compounds. Cyanidin chloride, which was obtained through counter synthesis from quercetin by the procedure [13], was used as tracking substance. The best results were produced with chromatography in acetic acid–hydrochloric acid–water system that were taken in a ratio 30 : 3 : 10, respectively.

Column chromatography with a polyamide sorbent was applied for quantitative separation of the mixture of anthocyanidins of a birch bark. Before being used, the sorbent was reprecipitated from acetic acid in compliance with the procedure described in the work [9]. Elution was conducted with 30 % methanol, which contained 0.01 % of hydrochloric acid. Two clearly defined zones of colouring were observed along the eluent movement in a column. Upon cutting fractions and their concentrating (methanol stripping), the substances **I** (0.69 g) and **II** (0.43 g) have been obtained. The yield of these materials amounts to 46 and 29 % respectively, which corresponds in the aggregate to 75.1 % from the weight of the resulting product. Recovered compounds constitute dark-claret coloured crystals; they melt with decomposition.

UV spectra of anthocyanidins were taken in methanol and ethanol, which contained 1 % of hydrochloric acid, in sample pans 1 cm in thickness with UV-30 Shimadzu unit. To perform measurement in the ultraviolet region of a spectrum, the anthocyanidin concentration comprised  $0.2 \cdot 10^{-4}$  mol/l, and

it comprised  $1.0 \cdot 10^{-4}$  mol/l for the visible spectrum range.

IR-spectra registration was performed in KBr on the Specord-75 IR spectrophotometer in the region 400–4000  $\text{cm}^{-1}$ .

Preparation of enterosorbents was performed through treatment of resin-free bast with a dilute solution of sodium hydroxide by analogy with obtaining polyphosphate from the hydrolysing lignin [10]. Concentration of alkali varied from 1 to 2 %; the treatment temperature varied from 60 to 100 °C; the duration varied from 1 to 2 h. Given the specified processing conditions, the yield of sorbent comprised 41–42 % from the mass of initial bast. The yield of sorbent from the fixed residue of bast, which has been subjected to consecutive extraction with hexane, ethyl acetate, isopropanol, and water, was, upon processing with alkali liquor, as great as 67–71 % from the mass of bast previously subjected to extraction.

Sorption characteristics of porous materials that were obtained from bast have been investigated with iodine adsorption and methylene blue adsorption. These reagents are traditionally applied for testing the quality of carbon sorbents [14].

Efficiency of their medical action in acute gastroenteric infections has been studied on the basis of Achinsk zonal veterinary bacteriological laboratory. A material for investigations was received from pigs of an early postnatal period (less than 10 days) with symptoms of acute gastroenteric infections.

Investigation of pathological material, isolation of pure growth, and studying the properties were conducted according to methodical instructions now in use [15]. Availability of adherent antigens from the isolated strains was determined in the reaction of agglutination on glass with agglutinating sera to adherent antigens of *Escherichias* K99, K88.A20, 987P, and F41. The quantity of the colonies grown in Petri dishes was counted by means of the device for calculation of colonies. Strain *E. coli*, which possesses hemolytic properties to adherent antigens K99, has been used in the study of saturation capacity of enterosorbent in the *in vivo* experiment in white mice.

TABLE 1

Characteristics of aqueous and water–alcohol–alkaline extracts from bast of a birch bark (70 °C, 15 % of ethanol, liquor ratio 10, duration 1 h)

Characteristics	Aqueous extract*NaOH concentration, mass %		
	0.5	1	1.5
Yield of substances (% from the mass of a. d. bast):			
soluble	9.0	18.4	29.0
tannic (tanning matter)	4.0	–	10.6
other	5.0	–	18.4
Quality (%)	44.6	–	36.5

\*Obtained by hydraulic beating of bast with cold water.

## RESULTS AND DISCUSSION

### Recovery of tanning agents

Effect of conditions for carrying out the water–alcohol–alkaline extraction of bast of a birch bark on the yield and composition of extracts has been investigated. Table 1 presents data regarding an effect of concentration of sodium hydroxide on the yield of substances recovered from bast and on the quality of tanning extracts.

Some gain in yield of tanning agents with an increase in NaOH concentration from 1.0 to 1.5 % is likely to be related to intensification of depolymerisation processes for the high-molecular-mass tanning matter. The possible reason for a low quality of tanning extracts from the birch bark bast may be their high content of the low-molecular-mass phenols that do not exhibit tanning properties.

Effect of ethanol concentration on the yield of substances and quality of tanning extracts

was studied at NaOH concentration being equal to 1.5 % (Table 2). An increase in ethanol concentration from 10 to 15 % leads to only insignificant increase in the yield of extractives. Further growth of concentration from 20 to 30 % is followed by a decrease in yield of both total extractives and tanning matter with a small increase in the quality of the latter.

Optimisation of the process for obtaining extractives from the birch bark bast was performed using a mathematical method of design of experiments with Box–Wilson design [16]. The following parameters were chosen as independent variables: concentration of sodium hydroxide (%), ethanol concentration (%), duration of extraction (h). The yield of extractives (%) was chosen as optimisation parameter.

The graphic representation of the yield of extractives *versus* technological parameters is presented in Fig. 2. Concentration of NaOH varied from 0.5 to 1.5 %; that of ethanol varied from 10 to 20 %; duration of extraction varied from 0.5 to 2 h.

As suggested by the data acquired, the concentration of NaOH exerts the greatest effect on the yield of extractives. This dependence shows a linear character. An increase in the concentration of alkali from 0.5 to 1.5 % causes the yield of extractives to increase from 21 to 40 % from the mass of abs. dry raw material. Ethanol concentration in the selected interval has less tangible influence on the yield of extractives.

It was found that in the case of liquor ratio being equal to 10, the maximum yield of

TABLE 2

Effect of ethanol concentration on the characteristics of extracts from the birch bark bast (70 °C, liquor ratio 10, duration of 1 h)

Characteristics	Ethanol concentration, mass %			
	10	15	20	30
Yield of substances (% from the mass of a. d. bast):				
soluble	33.0	33.2	30.8	22.7
tannic (tanning matter)	–	–	11.6	9.3
other	–	–	19.2	13.4
Quality of tanning matter (%)	–	–	37.8	40.9

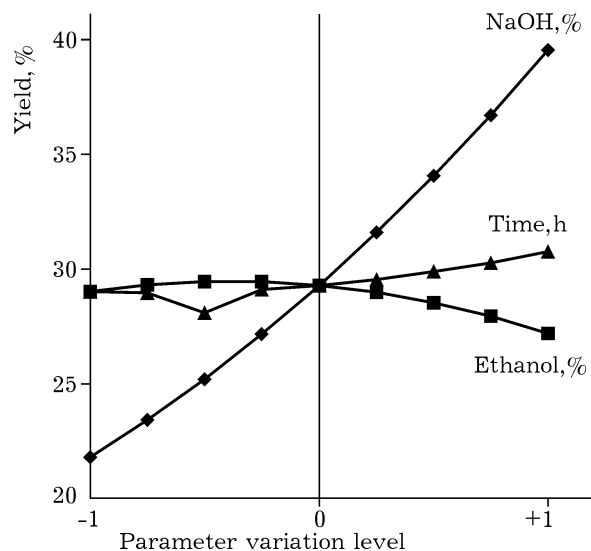


Fig. 2. Dependence of the yield of extractives on technology factors. Parameter variation level: 0 – basic level, +1 – top level, and -1 – bottom level.

tanning agents is attained under the following conditions: the temperature of 70 °C, NaOH concentration in the reaction mass of 1.5 %, ethanol concentration of 15 %, and duration of extraction of 1 h. In these conditions, tanning extract was obtained with a yield of 38.6 % from the mass of absolute dry of raw material. By virtue of the fact that sugars and the lignin nature substances, which exhibit no tannic properties, pass into solution upon alcohol-alkaline extraction of bast, the produced extract shows rather low quality, 43 %. To enhance the performance of alcohol-alkaline extract of the birch bast, the method of membrane ultrafiltration was applied, which has allowed increasing its quality up to 82 %.

#### Recovery of anthocyanidin dyes

Obtaining anthocyanidin dye was made through treatment of previously deresinified bast with 96 % ethanol in the presence of HCl. Effect of liquor ratio magnitude on the extraction of anthocyanidins at fixed temperature, HCl concentration, and duration of treatment is shown in Fig. 3. The maximum extraction of anthocyanidin dye is attained with the liquor ratio being 12. Further increase in ethanol content of the reaction mixture has no effect on the yield of anthocyanidins.

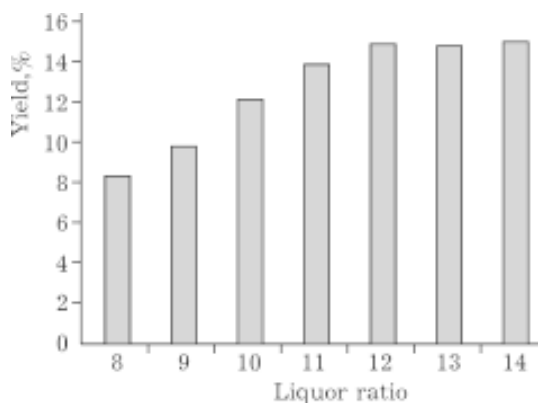


Fig. 3. Influence of liquor ratio (ethanol : bast ratio) on the yield of anthocyanidin dye. Concentration of HCl is 3.5 %; the temperature is 78 °C; duration of treatment is 3 h.

The influence of HCl concentration in ethanol on the yield of the anthocyanidins, which are recovered for 3.5 h from deresinified bast of birch bark at a temperature of 78 °C and at liquor ratio 12, has been studied.

As suggested by data that are presented in Fig. 4, the maximum yield of anthocyanidins from the birch bark bast (about 15 mass %) is attained under the specified conditions at HCl concentration of 3.0–3.5 mass %. Further increase in the HCl concentration decreases their yield.

Paper chromatography was applied for qualitative identification of anthocyanidins. Quantitative separation of anthocyanidin mixture was conducted in a column with a polyamide sorbent, and elution was performed with 80 % methanol that contained 1 % HCl. Two clearly defined zones of colouring were observed along the movement of this eluent in

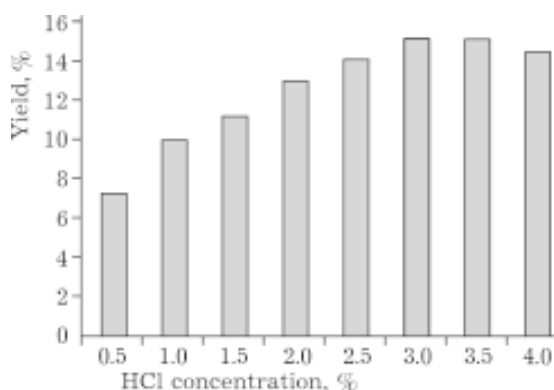


Fig. 4. Dependence of the yield of anthocyanidin dye from the birch bark bast on the ethanol concentration of hydrochloric acid. The temperature is 78 °C; the liquor ratio is 12; duration of treatment is 210 min.

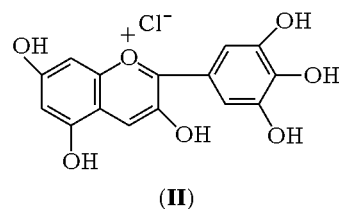
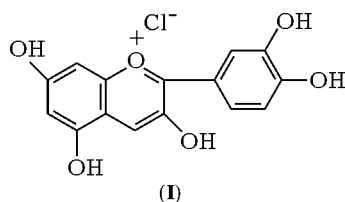
TABLE 3  
Spectral characteristics of anthocyanidins from the birch bark bast

Substance	Colouring	$\lambda_{\max}$ of the visible range of electronic spectrum, nm					
		Ethanol	Ethanol + AlCl <sub>3</sub>	$\Delta\lambda$	Methanol	Methanol + AlCl <sub>3</sub>	$\Delta\lambda$
Cyanidin chloride* (Standard)	Crimson	547	570	23	537	553	18
Cyanidin chloride	«	547	570	23	537	553	18
Delphinidin chloride	Mauve	553	581	28	547	569	23

\*Obtained through counter synthesis from quercetin [13].

the column. Compounds **I** and **II** have been obtained upon cutting these two fractions and their evaporation to dryness. UV and IR spectroscopy methods were used for their identification.

It is common knowledge that the position of absorption maximum in an electronic spectrum is determined by the anthocyanidin nature [6]. The nature of solvent also has an influence on the position of maximum in the visible spectrum range. Cyanidin that is dissolved in methanol with 1 % HCl shows a maximum at 537 nm, whereas in the ethanol with the same quantity of HCl, the peak position corresponds to 546 nm. Still more essential bathochromic shift with a colour deepening is observed when anthocyanidins that contain hydroxyl groups in *ortho*-positions of phenolic ring form stable complexes with Fe<sup>3+</sup>, Al<sup>3+</sup>, and Sn<sup>4+</sup> [17, 18]. It was found that cyanidin chloride and delphinidin chloride, which have been obtained from the birch bark bast in the presence of AlCl<sub>3</sub>, change a solution colour to dark blue with essential displacement of absorption maximum in the visible spectral range (Table 3).



The spectra of compounds **I** and **II** in methanol contain absorption maxima in the region about 537 nm (compound **I**) and 547 nm (compound **II**). The mentioned maxima of absorption are characteristic of cyanidin chloride (537 nm) and delphinidin chloride (547 nm) [6].

Thus, it is believed that the composition of anthocyanidin dye, which has been obtained from the birch bark bast, includes cyanidin chloride (I) and delphinidin chloride (II). Their total content in the isolated dye comprises 70–75 %.

#### Obtaining and properties of enterosorbents

The possibility to obtain enterosorbents of the polyphepane type from the birch bark bast and fixed residue from its extraction processing has been studied. As suggested by the data presented in Table 4, the composition of bast of a bark of drooping birch is dominated by

TABLE 4  
Chemical composition of the birch bark bast before and after extraction (70 °C, liquor ratio is 10, concentrations of NaOH 1.5 %, of ethanol 15 %, duration 1 h), % from the mass of the feedstock

Substances	Content in the bast	
	Initial	After extraction
Water extractable	13.71	1.46
Readily hydrolysable		
polysaccharides	26.29	17.89
Hardly hydrolysable		
polysaccharides	22.86	19.97
Lignin matter	34.82	20.20
Total of polysaccharides	49.09	37.86
Mineral substances	3.42	2.36

TABLE 5

Sorptive capacity of sorbents, which were produced by alkali treatment of previously extracted bast of a birch bark

Sample	Method of producing	Sorptive capacity	
		for methylene blue, mg/g	for iodine, %
1	Treatment of bast with 1 % NaOH at 60 °C, 1 h	47.5	25.4
2	Treatment of bast with 2 % NaOH at 60 °C, 1 h	42.5	27.9
3	Treatment of bast with 2 % NaOH at 100 °C, 2 h	44.5	29.8
Polyphepane (commercial sample)	From the hydrolysing lignin of "Santec" JSC	10.0	43.2

Note. The bast was previously extracted by hexane and water-ethanol-NaOH mixture.

polysaccharides, and the amount of readily hydrolysable polysaccharides is more than that of hardly hydrolysable polysaccharides. The bast is also rich in substances of the lignin nature. Along with the basic components, the composition of bast includes the water-soluble and mineral substances. Water-soluble matter is almost half (42.5 %) represented by tanning matter, *i.e.* complex high-molecular compounds of polyphenolic nature that possess tanning properties.

Content of water-soluble matter in the fixed residue from extraction of the birch bark bast by aqueous-alcoholic solution of alkali under conditions, which provide the maximum extraction of tanning agents, sharply drops (from 13.71 to 1.46 mass %). A decrease in content of polysaccharides (from 49.09 to 37.86 mass %), of lignin matter (from 34.82 to 20.20 mass %), and mineral substances (from 3.42 to 2.36 mass %) is observed too.

As is known, an opening of pore structure of vegetative raw materials occurs during extraction recovery of substances. Therefore, one might expect that removing significant amount of extractives, readily hydrolysable polysaccharides, and low-molecular-mass substances of lignin nature from bast of a birch bark will contribute to the development of the pore structure of fixed residue.

Sorbents were produced by treatment of initial or previously extracted bast of a birch bark with 1–2 % NaOH solution at a temperature from 60 to 100 °C for 1–2 h. Table 5 gives the results of comparison of sorptive capacity of sorbents from the birch bark bast and commercial enterosorbent, namely, polyphepane.

From the data acquired it follows that the characteristics comparable to polyphepane are shown by sorbents, which have been produced by treatment of previously extracted bast with 1–2 % NaOH solution at 60 °C for 1–2 h.

The sorbent samples, which have been produced from bast of a birch bark, more than 4 times exceed the commercial enterosorbent (polyphepane) in their capability to adsorb methylene blue, but rank 1.5 times below it in sorptive capacity for iodine. The difference in ability of samples from bast and polyphepane to adsorb iodine and methylene blue may be consequence of a varied ratio between micro- and mesopores in these sorbents. More extensive molecule of methylene blue cannot penetrate into the narrow micropores that are accessible to a small molecule of iodine. With allowance made for the circumstance, it can be inferred that the sorbents from bast of a birch bark exhibit more developed mesopore structure than that of polyphepane. For this reason, enterosorbents from bast must possess higher capability to capture bacteria and large organic molecules as compared to polyphepane.

Distinctions in the properties of sorbents from bast of a birch bark and polyphepane are likely to be controlled by the difference in their permolecular structure and chemical composition. In particular, the lignin content of sorbents from bast can amount from 20 to 28 %, and that of polyphepane can be no less than 77 %.

Enterosorbent from bast of a birch bark (EBB) has been tested as a preparation for treatment of acute intestinal infections. It features the following characteristics: water content of 7.4 %, ash percentage of 4.9 %, and

lignin content of 28.0 %, sorptive capacity for iodine of 27.9 %, sorptive capacity for methylene blue of 42.5 mg/g, and pH of aqueous extract of 7.0. When tested, the preparation was administered to test white mice perorally (both with primary and with repeat infection) in a dose of 240 mg/kg (the single dose could amount from 3.5 to 6.0 mg depending on the mass) until clinical signs of disease disappeared. The enterosorbent was applied in the acute phase of escherichiosis in mice, when the first signs of diarrhea appeared, but no later than the second day of the disease and also at a stage of moderately severe clinical manifestation of colibacilliosis (2–3 days later).

Clinical tests of EBB have demonstrated that the preparation provides the necessary enterosorption in practice of acute intestinal infections. EBB exerted fast cupping of colibacilliosis signs at various stages of disease in all tested mice groups; with more mild disease, a quicker convalescence was observed than in the check group of mice, which received antibiotics.

Before the repeat infection, the enterosorbent in a dose of 200 mg/kg was administered to all test groups of mice within 10 days, and then infection was conducted. Food and water intake, their motor performance, and dynamics of the body weight did not differ from the healthy animals, which were not

previously exposed to the infection. A group of check animals, which were on antibiotics instead of the enterosorbent, exhibited the indices that were much worse.

The enterosorbent from the birch bark bast had an equally positive effect also upon the repeat infection of test animals. When it was applied, an increase of immunity to infection was evidenced in all groups of test mice. An acute course of colibacilliosis in the group of check animals happened in 12–24 h upon administration of the first dose of pathological culture; meanwhile, no signs of infection were observed in the animals that were previously treated with enterosorbent.

## CONCLUSIONS

As a result of the research completed, the optimised methods are suggested to produce tanning agents, anthocyanidin dyes, and enterosorbents from the birch bark bast. Necessity of recycling of the last-mentioned will appear, predictably, in the commercial development of manufacturing the biologically active betulin and its derivatives from external layer of a birch bark (the outer birch bark). Considered in the previous sections are the methods to produce valuable products from bast. By integration of these methods, it is possible to attain the non-waste

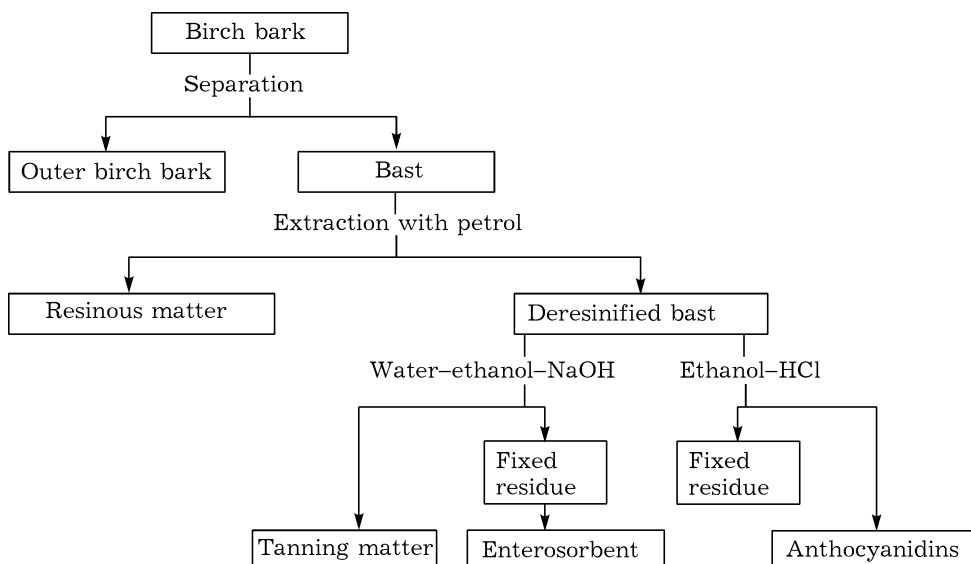


Fig. 5. Schematic diagram for comprehensive processing of the birch bark bast.



recycling of bast within the limits of the flow chart, which is presented in Fig. 5.

Bast of a birch bark contains resinous matter to the extent of 3.0–3.2 % from the weight of absolutely dry bast. Prior removal of resinous matter enhances the quality of tanning agents and anthocyanidin dyes obtained from bast. The research performs selection of optimum conditions to deresinify bast of a birch bark with the following non-polar solvents: petroleum ether, hexane, and BR-1 grade petrol. The optimum duration of bast treatment with the specified solvents in the Soxhlet apparatus is as great as 6–8 h. The extraction degree for resinous matter ranges up to 97–99 %.

Resin-free bast can be further processed in two ways. Treatment with water–ethanol–NaOH mixture is applied to isolate tanning matter, which can be used in the subsequent process to produce natural tanning agents, antioxidants, preservatives, and other important phenolic products. The alternative version of processing involves recovery of the coloured anthocyanidin substances from bast. Their maximum yield can be attained when the bast is processed with ethanol containing 3.0–3.5 % of muriatic acid.

The recovered anthocyanidins that consist predominantly of cyanidin chloride and delphinidin chloride can be applied as food dyes, dietary supplements, dyes for wood, etc. They are close compositionally to the anthocyanidins, which were recovered from the bark of Siberian larch under resembling conditions [19].

Extraction processing of bast of a birch bark yields a porous fixed residue, which was used to recover a significant part of polyphenols, resinous and water-soluble matter. Adsorptive capacity of the fixed residue increases with the enhanced extraction degree of extractives from bast of a birch bark. Selection the appropriate processing conditions for previously extracted bast produces enterosorbents, the properties of which are in many respects similar to the properties of commercial enterosorbent that is produced from the hydrolysing lignin.

Results of enterosorbent testing with animals have shown their high efficiency in treatment of acute intestinal infections and dysbacterioses, which have been caused by application of antibiotics. Thus, the new enterosorbents from a birch bark can find application in medicine and veterinary practice.

In general, optimum regimes of processing of bast of a birch bark at all technological stages makes it possible to obtain about 3.0 % of resinous matter, 35–40 % of polyphenolic and phenolic products, 15 % of anthocyanidin dye, and about 41–42 % of enterosorbent. The mentioned materials can be applied in the perfumery-cosmetic industry, food industry, tanning industry, in medicine, and veterinary practice.

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