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Synthesis and Properties of Enterosorbent from the Birch Inner Bark with Supported Betulin

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Abstract

A method is developed for the production of enterosorbent from the birch inner bark with supported biologically active betulin with improved therapeutic and prophylactic properties.

Keywords: enterosorbent, birch inner bark, birch bark bast, polyphepan, betulin

INTRODUCTION

Broad application of antibiotics for the treatment and prophylactics of gastrointestinal diseases of animals and birds causes the development of resistance to these substances, the formation of toxic compounds and immunodeficient states, which affect the quality of the products of cattle breeding and poultry farming. An alternative method of the treatment and prophylactics of gastrointestinal diseases is the use of enterosorbents promoting a rapid removal of toxic and pathogenic substances from an organism, formation of healthy microflora and enhancement of stability against unfavourable factors. Enterosorption is a promising method to purify an organism from diverse exo- and endotoxins (xenobiotics, heavy metals, the products of metabolism of pathogenic micro- and mycoflora). Enterosorption allows using enterosorbents both for acute and for chronic diseases accompanied by intoxication, indigestion, metabolic disorders and a loss of immunity [1–4].

In the area of detoxication, industrial enterosorbent Polyphepan is successfully used. It is based on hydrolytic lignin and is characterized by the ability to sorb the toxins of different kinds and by the practical absence of any contraindications for application [5]. A decrease in the amount of hydrolytic lignin manufactured at present brings about the necessity to search for other kinds of plant raw materials for making enterosorbents. One of the possible alternative initial materials is the waste of birch bark and birch inner bark (birch bark bast) possessing good sorption properties. In our previous works, we demonstrated the methods to obtain sorbent from the inner bark and bark of birch with sorption properties meeting the requirements to enterosorbents [6–13].

The major extract of the outer layer of birch bark – betulin – exhibits a wide range of pharmacological activity. The high potential of the use of betulin in the pharmaceutical industry is defined by its antiseptic, antiinflammatory, antiviral, antioxidant and other characteristics [14–16]. Promising methods of obtaining betulin from birch bark were developed at the Institute of Chemistry and Chemical Technology SB RAS (Krasnoyarsk) [17, 18].

At present, much attention is paid to the studies of the possibility to apply enterosorbents as carriers for immobilization of prebiotics, medicinal or biologically active compounds [10, 19, 20]. A method for obtaining composite veterinary preparation based on porous carbon material in which betulin is dispersed from ethanol to ethanol-glycerol solution is presented in [20]. It is proposed to use this preparation for immunocorrection and detoxication in the case of poisoning as a medicinal agent with prolonged action against disorders of gastrointestinal tract (GT) and immune system, and for the enhancement of therapeutic effect of other medicinal agents for the treatment of the diseases of animals and birds. The gradual liberation of the carrier surface from the active component allows conserving its adsorption capacity with respect to toxic components along the entire GT. The joint action of these factors provides the therapeutic effect necessary for the treatment and prophylactics of a number of diseases, and for rehabilitation of an organism.

Due to the pharmacological properties, betulin may enhance the activity of some immunocompetent cells, rise the number of phagocytes, their functional ability to destroy viruses and bacterial cells, thus increasing the protective forces of an organism and promoting the facilitation of the course of a disease.

The goal of the present work was to develop a method for obtaining enterosorbent with improved medioprophylactic properties from the inner birch bark impregnated with biologically active betulin, and investigation of its properties.

EXPERIMENTAL

Materials

The initial raw material was ground inner bark of birch (Betula pendula Roth.) having the following composition (% of the mass of absolutely dry inner bark): polysaccharides (48.8–49.1); lignin (33.1–34.2); water-soluble substances (13.1–13.7); mineral substances (2.4–3.4). The birch inner bark was ground in a rotor knife mill RM 120 (Russia), and the fraction of 1–2 mm was separated by sieving.

Procedures for obtaining enterosorbents

Enterosorbent was prepared from the birch inner bark (sample 1 – EBB) through the extraction of the birch inner bark with a 0.5 % NaOH solution in a 20 % aqueous solution of ethanol. The mixture was boiled with a water bath for 1 h, filtered, neutralized, washed with water and dried to the air-dry state. Extraction of the inner bark with the alkaline alcohol mixture was carried out instead of multi-step extraction with solvents which was carried out previously to obtain enterosorbent from the birch inner bark [8]. A shortcoming of the method of enterosorbent preparation described in [8] was the use of a fine dust-like fraction of inner bark, 0.1–1.0 mm, with lower sorption properties, and preliminary multistep extraction of the sorbent with a number of solvents: hexane, ethylacetate, isopropanol and water, with subsequent treatment with an alkaline solution.

Sample 2 – EBB-B – was obtained by impregnating the EBB sorbent with a hot alcoholic solution of betulin. Its volume was determined relying on the capacity of the sorbent with respect to alcohol. Impregnation was carried out with small portions under thorough mixing. The impregnated sorbent was kept for 1 day in a closed vessel at room temperature and then dried at 100 °C for 3 h.

For comparison, we used a commercial enterosorbent Polyphepan (JSC Sayntek, Russia) manufactured from hydrolytic lignin (sample 3 – PPh). Образец 4 – PPH-B – was prepared similarly to sample 2 by impregnating PPH with the alcoholic solution of betulin. Betulin content in EBB-B and PPH-B samples was 10 mg/g of the sorbent.

Methods of investigation

The composition of the raw material and the obtained sorbents was determined using the procedures generally accepted in wood chemistry [21].

The sorption activity A of the samples was studied with respect to marker substances with different molecular masses: iodine (A_{J2}) [22], methylene blue (A_{MB}) [23] and gelatin (A_g) [24].

The morphology of the obtained samples was studied by means of scanning electron microscopy (SEM) using a TM 1000 scanning electron microscope (Hitachi, Japan).

Thermal analysis (TA) of the sorbents was carried out using an STA 449 F1 Jupiter thermo-analyzer (Netzsch, Germany) in the atmosphere of argon in crucibles made of corundum ceramics (Al_2O_3) with perforated caps. The samples were heated from 30 to 500 °C at a rate of 10 °C/min. The gas flow rate was 50 mL/min.

Elemental analysis was carried out with the help of a Flash EA 1112 element analyzer (Thermo Quest, Italy).

The efficiency of the therapeutic and prophylactic action of all samples was tested with respect to acute gastrointestinal infections (escherichiosis) in white line mice in the Krasnoyarsk State Agricultural University. The animals were infected through intraperitoneal introduction of an 18–24 h agar culture of *E. Coli* microorganisms of the O138 K99 serotype in the dose of 500 mln microbes per 1 mL.

Animals with the average body mass of 18 g were arranged in five groups, five animals in each. All mice received a standard feed. The animals of the experimental prophylactic groups received enterosorbent once a day, in the dose of 0.2 g per 1 kg of body mass, three days before infection with the culture of E. Coli microorganisms. The animals of experimental treatment groups received enterosorbent twice a day in the dose of 0.2 g per 1 kg of body mass, starting from the first signs of clinical symptoms till complete recovery.

The parameters evaluated during experiments were: the severity of disease; undamaged state of laboratory animals; morbidity percentage; death percentage; the time within which the clinical signs of the disease were manifested; the parameter of bacterial contamination of intestines with the test culture of *E. Coli*. The duration of observation of the animals in the experimental and reference groups was 3 to 5 days.

The quantitative parameter of bacterial contamination of intestines with the test culture of *E. Coli*, O138 K99 serotype, in 1 g of digestive chyme in the small intestines was studied using the procedure described in [25]. In the case of the death of animals, bacteriological analysis of the contents of the chyme of the small pathological material was carried out [26]. The material was taken for bacteriological analysis according to the methodic guide by the Federal Centre for Hygiene and Epidemiology of Rospotrebnadzor [27].

RESULTS AND DISCUSSION

Extraction of the inner bark with an alkali solution in the aqueous solution of ethanol allowed us to extract polyphenols and replaced multi-step extraction with organic solvents [8].

After the extraction of birch inner bark with a 0.5 % solution of alkali in a 20 % aqueous solution of ethanol, the sorbent was obtained with a yield of 60–62 % of the mass of initial inner bark. The content of the major components in the samples of sorbents after extraction with the ethanol-alkali mixture was (% of the mass of absolutely dry sorbent): polysaccharides (37.3–38.2); lignin (20.2–23.1); water-soluble substances (1.1–1.8); mineral substances (2.1–2.4).

A comparison of the elemental composition of enterosorbent from the birch inner bark with the composition of commercial enterosorbent Polyphepan was carried out in [9]. It was demonstrated that the samples under investigation are similar to each other in their elemental composition (EBB/PPh, mass %): C (64.0/68.9); H (6.8/6.1); Stotal (0.03/0.07). The EBB sorbent contains less sulphur than PPh, which is due to the nature of the raw material.

The morphology of the obtained sorbents was studied by means of SEM (Fig. 1). A developed porous structure and inclusions of white betulin crystals in sorbent pores are observed in enterosorbent EBB-B (see Fig. 1, *a*). The surface of the PPh-B sample is non-uniform (see Fig. 1, *b*), it contains a smaller number of micropores. Betulin crystals occur at a greater depth in sample pores. The presence of crystalline betulin on the surface of the sorbents is likely to be connected with the fact that a definite part of betulin is not sorbed, but instead, it is crystallized on the surface of the samples during their treatment with the ethanol solution of betulin.

The presence of the developed porous structure of the sorbents improves their sorption characteristics with respect to high- and medium-molecular compounds: various organic and inorganic substances, biogenic amines and bile acids, and toxins of protein origin.

Sorption properties were tested relying on the ability of the sorbents to absorb iodine, methylene blue and gelatin. Iodine molecules are rather small, so they are adsorbed on the surface of micropores of the sorbent. The molecules of methylene blue are larger and are absorbed on the surface of mesopores of the sorbent. Gelatin molecules model the class of high-molecular toxins and are adsorbed on the surface of macropores of the sorbent. The data on the sorption activity for enterosorbents made of birch inner bark in comparison with the commercial sorbent Polyphepan

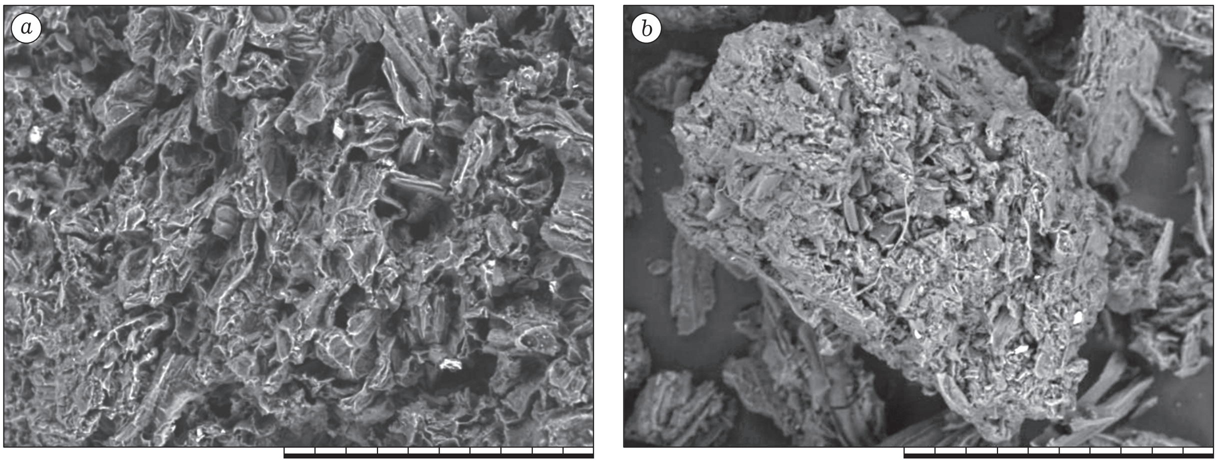

Fig. 1. SEM images of sorbent samples with supported betulin: EBB-B (*a*), PPh-B (*b*).

and the samples with supported betulin are presented in Table 1.

One can see that the sorption activity with respect to iodine is higher for PPh and PPh-B than for the samples of birch inner bark and remains practically unchanged after the deposition of betulin on the samples.

The sorption activity with respect to methylene blue for the EBB sample was 76.2 mg/g, after impregnation with betulin it increased to 89 mg/g for EBB-B sample and decreased to 26 mg/g for PPh-B sample.

The sorption activity with respect to gelatin was lower for the EBB sample than for the commercial PPh sample. However, after the deposition of betulin, the sorption activity of the EBB-B sample with respect to gelatin increased to 224 mg/g, and for the PPh-B sample, it decreased to 128 mg/g. It may be assumed that betulin crystals blocked the pore mouths or occupied the whole surface of meso- and macropores of PPh-B. It is possible that betulin is worse sorbed on the surface of Polyphepan sorbent than on the surface of the sorbent made of birch inner bark.

It is known that betulin (betulinol, lup-20(29)-ene-3β,28-diol) is a pentacyclic triterpenic alcohol of lupan series. Its molecule contains a primary and secondary hydroxyl groups and a double bond in the isopropenyl group at the five-membered ring [28]. These functional groups may form bonds with the functional groups of molecules in the sorbent structure. An increase in sorption activity with respect to methylene blue and gelatin for the EBB-B sorbent and its decrease for PPh-B enterosorbent may be explained by better bonding of the functional groups of enterosorbent from birch inner bark with betulin.

Thermogravimetric (TG), differential thermogravimetric (DTG) curves of mass loss and the curves of differential scanning calorimetry (DSC) for the EBB-B and PPh-B samples are shown in Fig. 2. One can see that, in addition to water removal at a temperature up to 100 °C, thermal transformations of the samples under investigation include several stages of mass loss characterized by the presence of peaks on DTG curves. In the case of EBB-B sample, the maximal mass loss (up to 52 %) occurs within the region 220–350 °C. For

TABLE 1

Sorption activity of enterosorbent samples

Sample No.	Sample	Impregnation with the ethanol solution of betulin	Sorption activity, mg/g of sorbent		
			A_{J_2}	A_{mb}	A_g
1	EBB	–	170.0±8.0	76.2±1.5	151.1±3.1
2	EBB-B	+	180.0±9.0	89.0±2.0	224.0±5.6
3	PPh	–	320.0±18.0	60.0±2.5	264.0±4.9
4	PPH-B	+	320.0±13.0	26.0±1.5	128.0±2.8

Note. Sorption activity (the amount of sorbed substance, in mg, per 1 g of the sorbent): with respect to iodine (A_{J2}), methylene blue (A_{mb}), and gelatin (A_g).

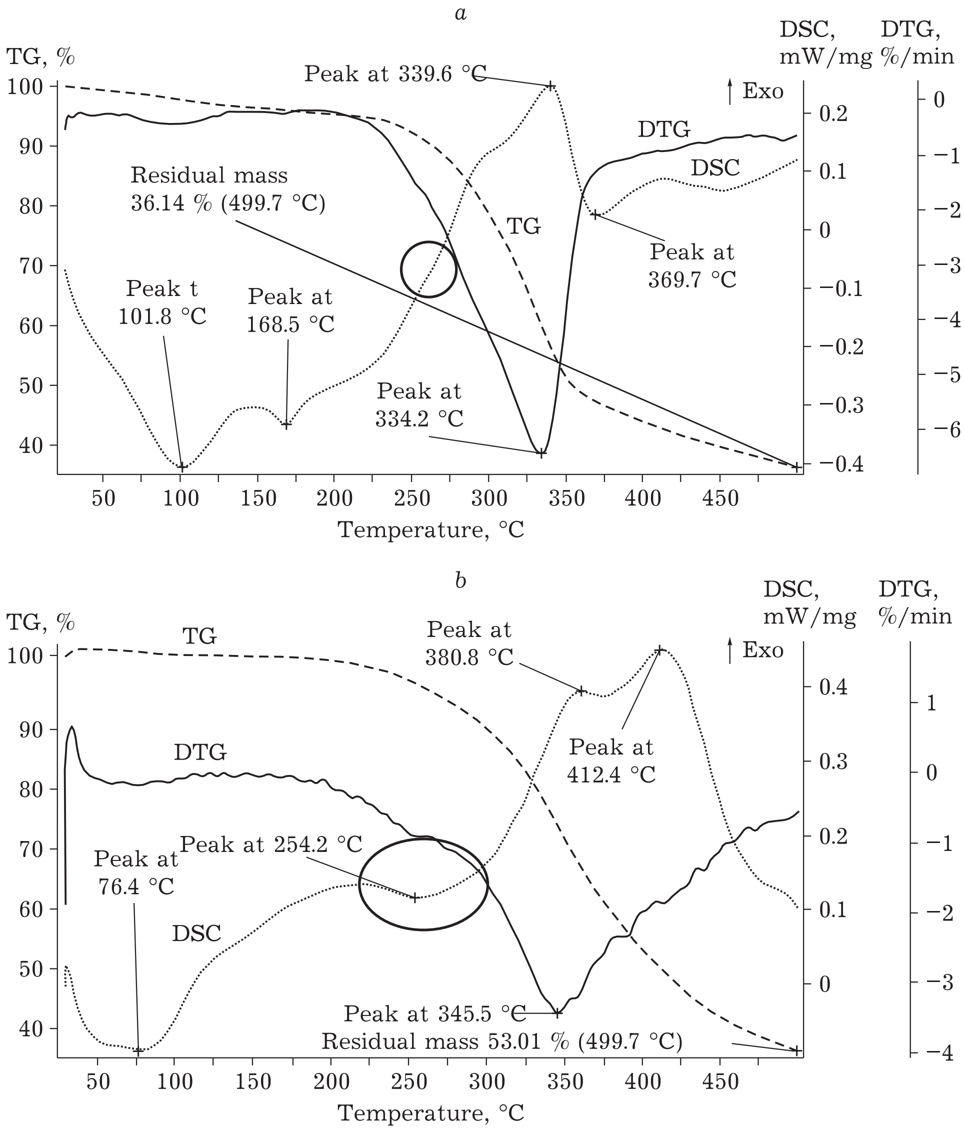

Fig. 2. Data of thermal analysis of the sorbents under investigation: EBB-B (*a*), PPh-B (*b*).

the PPh-B sample, mass loss starts at 220 °C, too. So, thermal stability of both kinds of sorbents is approximately the same.

An endothermic peak at 254 °C is present on the DSC curve of PPh-B sample. This point corresponds to the melting temperature of betulin recrystallized from ethanol (251–261 °C [27]). No endothermic heat effects are observed in the DSC curve of EBB-B sample within temperature range 250–270 °C. This fact confirms the assumption concerning the presence of a larger amount of crystalline betulin in PPh-B sample than in EBB-B.

Results of studying the possibility to use the obtained sorbents for prophylactics and treatment of escherichiosis in animals on the model of laboratory white mice are presented in Table 2.

Both samples of enterosorbents from the birch inner bark demonstrated good results in the prophylactics of escherichiosis. Better prophylactic properties were demonstrated by EBB-B sorbent. The disease of the animals of this group proceeded in the slight form, and the animals were healthy after 24 h. In the group of animals receiving EBB enterosorbent for the prophylactics of escherichiosis, manifestation of the disease were moderate, recovery occurred within 72 h, and the percentage of deaths was 40 %. After infection of the group of animals receiving PPh

TABLE 2

Results of the use of sorbents for the prophylactics and treatment of escherichiosis

Group No.	Entero-sorbent	Prophylactics			Treatment		
		Symptoms of the disease	Recovery time, h	Death percentage, %	Symptoms of the disease	Recovery time, h	Death percentage, %
1	EBB	moderate	72	40	moderate	48	20
2	EBB-B	slight	24	20	very slight	24	0
3	PPh	severe	120	80	severe	–	100
4	PPh-B	moderately severe	96	60	severe	–	100
5	Reference	severe	–	100	severe	–	100

enterosorbent for prophylactics, escherichiosis proceeded in the severe form, and the percentage of deaths was 80 %. The symptoms of the disease were also severe in the reference groups of animals.

In the case of the application of enterosorbents to treat a generalized form of escherichiosis in laboratory animals, good results were demonstrated also by both samples of enterosorbents from the birch inner bark. The group of animals receiving EBB exhibited moderate symptoms of the disease, and the animals recovered completely after 48 h. The percentage of deaths was 20 %. In the animals of the group receiving EBB-B enterosorbent, after infection with escherichiosis and treatment with this sorbent, rapid relief of clinical symptoms was observed, along with the recovery of appetite and activity, and the animals of this group were healthy after 24 h. In the experimental groups treated with PPh and PPh-B enterosorbents, the symptoms of the disease were severe, practically the same as those in the reference group, and death percentage was 100 %.

So, experiments demonstrated that the sorbents obtained on the basis of birch inner bark may be successfully used for prophylactics and treatment of acute and chronic gastrointestinal infections of animals. The obtained enterosorbent from birch inner bark with supported betulin possesses high sorption capacity, which is connected not only with the developed internal surface but also with the presence of different functional groups in its macromolecules. This allows enteral binding and removal of gases, alkaloids, bacterial cells and the products of their vital activity, exogenous and endogenous toxins entering the GT, cholesterol, bilirubin, as well as biologically active substances – serotonin, histamine, the products of mast cells. The enterosorbent recovered intestinal biocenosis, improves lipid exchange, normalizes the content of bilirubin, the activity of transaminases and amylase, causes a decrease in the concentrations of toxins in blood, plasma and ascitic fluid, other biochemical parameters than were affected by exo- and endotoxemia. The presence of immobilized betulin on sorbent surface enhances its antiinflammatory and immunomodulating properties.

Decreased activity of Polyphepan enterosorbent with supported betulin may be connected with the presence of a large amount of crystalline betulin on the sample surface, and crystalline betulin exhibits its antiinflammatory properties to a lower extent than betulin immobilized on the surface; in addition, it may block the pores of the sorbent.

CONCLUSION

Enterosorbent was prepared from the birch inner bark ground to the fraction of 1.0–2.0 mm, by means of extraction with a 0.5 % alkaline solution in a 20 % aqueous solution of ethanol and impregnation of the resulting product with the ethanol solution of betulin.

The enterosorbent from the birch inner bark with supported betulin exceeds the commercial sorbent Polyphepan with supported betulin in the ability to adsorb methylene blue and gelatin.

The tests of enterosorbents with the experimental groups of white mice for the prophylactics and treatment of gastrointestinal infections showed that the enterosorbent made of the birch inner bark with supported betulin possesses better prophylactic and therapeutic properties in comparison with other enterosorbent samples under investigation and provides the necessary enterosorption in the case of acute intestinal infections at different stages of the course of the disease.

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REFERENCES

1 Belyakov N. A., Enterosorption, St. Petersburg: Alternativnaya Meditsina, 1994. 462 p.

2 Verotchenko M. A., Gimadeeva L. S., Use of enterosorbents for normalization of metabolic processes and for obtaining ecologically safe products of cattle breeding, *Vopr. Normativno-Pravovogo Regulirovania v Veterinarii*, 2015, No. 2, P. 119–121.

3 Morozov A. S., Bessonov I. V., Nuzhdina A. V., Pisarev V. M., Sorbents for extracorporal removal of toxic substances and molecules with undesirable biological activity (a review), *Obshchaya Reanimatologiya*, 2016, Vol. 12, No. 6, P. 82–107.

4 Velikanov V. V., Belko A. A., Ignatenko A. S., Gaponenko S. S., Subbotina I. A., Enterosorbents and prebiotics in the prophylactics and treatment of gastrointestinal pathologies in animals, *Uchen. Zap. Uchrezhdeniya Obrazovaniya Viteb. Gos. Akademiya Veterinar. Meditsiny: Research and Practical Journal*, 2015, Vol. 51, Issue. 2, P. 19–22.

5 Babkin V. A., Levanova V. P., Isaeva L. V., Medical preparations from the wastes of hydrolysis works, *Chemistry for Sustainable Development* [in Russian], 1994, Vol. 2, No. 2–3, P. 559–581.

6 Kuznetsov B. N., Levdanskiy V. A., Kuznetsova S. A., Chemical Products from Tree Bark. Monograph, Krasnoyarsk: SFU, 2012. 259 p.

7 Kuznetsova S. A., Shchipko M. L., Kuznetsov B. N., Levdanskiy V. A., Veprikova E. V., Kovalchuk N. M., Preparation and properties of enterosorbents from the inner bark of birch, *Khimiya Rast. Syrya*, 2004, No. 2, P. 25–29.

8 RF Pat. No. 2311954, 2007.

9 Veprikova E. V., Kuznetsova S. A., Skvortsova G. P., Shchipko M. L., Properties and application of sorption materials from the birch inner bark, *Zhurn. Sibir. Feder. Un-ta. Khimiya*, 2008, Vol. 1, No. 3, P. 286–292.

10 Veprikova E. V., Kuznetsova S. A., Chesnokov N. V., Kuznetsov B. N., Properties of enterosorbents obtained by immobilization of furacilin and gelatin on the substrate made of the birch inner bark, *Zhurn. Sibir. Feder. Un-ta. Khimiya*, 2014, Vol. 7, No. 1, P. 100–111.

11 Veprikova E. V., Shchipko M. L., Kuznetsova S. A., Kovalchuk N. M., Kuznetsov B. N., Sorption of organic substances modeling various factors of intoxication, on enterosorbent made of birch inner bark, *Chemistry for Sustainable Development* [in Russian], 2010, Vol. 18, No. 3, P. 239–247.

12 RF Pat. No. 2389498, 2010.

13 RF Pat. No. 2611388, 2017.

14 Alakurtti S., Makela T., Koskimies S., Yli-Kauhaluoma J., Pharmacological properties of ubiquitous natural product botulin, *Pharm. Sci.*, 2006, Vol. 29, P. 1–13.

15 Soica C., Dehelean C., Peev C., Aluas M., Zupko I., Kasa P., Alexa E., Physico-chemical comparison of betulinic acid, betulin and birch bark extract and in vitro investigation of their cytotoxic effects towards skin epidermoid carcinoma (A431), breast carcinoma (MCF7) and cervix adenocarcinoma (HeLa) cell lines, *Nat. Prod. Res.*, 2012, Vol. 26, Iss. 10, P. 968–974.

16 Kuznetsova S. A., Skvortsova G. P., Malyar Yu. N., Skuridina E. S., Veselova O. F., Extraction of betulin from birch bark and study of its physicochemical and pharmacological properties, *Russ. J. Bioorg. Chem.*, 2014, Vol. 40, No. 7, P. 742–747.

17 RF Pat. No. 2264411, 2005.

18 RF Pat. No. 2458934, 2012.

19 Kovalenko G. A., Kuznetsova E. V., Adsorption of antiseptics (furacilin, chlorohexidin) and vitamin E on carbon-containing enterosorbents, *Khim.-Farm. Zhurn.*, 2000, Vol. 34, No. 6, P. 45–49.

20 RF Pat. No. 2477125, 2013.

21 Obolenskaya A. V., Elnitskaya E. P., Leonovich A. A., Laboratory Works on the Chemistry of Wood and Cellulose, Moscow: Ekologiya, 1991. 319 p.

22 GOST 6217–74. Active crushed wood charcoal, Moscow: IPK Izd-vo Standartov, 2003.

23 Reshetnikov V. I., Evaluation of the adsorption capacity of enterosorbents and their pharmaceutical forms, *Khim.-Farm. Zhurn.*, 2003, Vol. 37, No. 5, P. 28–32.

24 Markelov D. A., Nitsak O. V., Gerashenko T. T., *Pharm. Chem. J.*, 2008, Vol. 42, No. 7, P. 405–408.

25 Boytsov A. G., Kaftyreva L. A., Lastovka O. N., Chugunova Yu. A., Nilova L. Yu., Pustynnikova A. M., Emmanuel V. L., Recommendations Concerning the Pre-Analytical Stage of Microbiological Laboratory Investigation, Tver: Triada, 2008. 64 p.

26 Mari P. R., Shey I. R., Clinical Microbiology, A Brief Manual, Moscow: Mir, 2006. 425 p.

27 Technics of Collection and Transportation of Biomaterials in Microbiological Laboratory: Methodical Guides MU 4.2.2039-05, Federal Centre for Hygiene and Epidemiology, Rospotrebnadzor, 2006. 126 p.

28 Abyshev A. Z., Agaev E. M., Guseynov A. B., Investigation of the chemical composition of the extract from the bark of birch Cortex betula, Betulaceae family, *Khim.-Farm. Zhurn.*, 2007, Vol. 41, No. 8, P. 22–26.