Preparation, increasing production of nitrogen oxide by macrophages in vitro, based on humic acids of peat of tomsk region bogs and method for obtaining thereof

Abstract

FIELD: medicine.
SUBSTANCE: invention relates to medicine and veterinary. The method for obtaining humic acids, increasing production of nitrogen oxides by macrophages in vitro, from peat of Tomsk region bogs includes crushing initial raw material, treating with exractant with mechanical stirring for 8 hours, sedimentation from solution with inorganic acid, separation of liquid and solid phases and drying the latter, with preliminary drying of peat at room temperature to air-dry state, crushing sieving through sieve with hole diameter 3 mm, with further extraction with sodium pyrophosphate with concentration 2.0-4.0 wt % in mass ratio peat: extractant 1:50-1:100 with constant stirring at temperature 25-27°C; after that extract of humic acids is treated with hydrochloric acid to pH 1-2, sediment of obtained humic acids is separated by centrifugation, after that washed from acidic to pH 7 medium and dried at room temperature. Application of humic acids from peat of Tomsk region bogs to increase nitrogen oxide production by macrophages in vitro.
EFFECT: inventions make it possible to create effective technology of peat processing, which makes it possible to obtain water-soluble humic acids that possess capability to activate secretory properties in macrophages.
2 cl, 3 tbl, 4 ex

Classifications

A61K35/10 Peat; Amber; Turf; Humus

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Claims (2)

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translated from Russian

1. A method of producing humic acids that increase the production of nitric oxide by macrophages in vitro from peat of the marshes of the Tomsk region, including grinding the feedstock, processing with extractant under mechanical stirring for 8 hours, precipitation from a solution of inorganic acid, separation of liquid and solid phases and drying of the latter , characterized in that the peat is pre-dried at room temperature to an air-dry state, crushed, sieved through a sieve with a hole diameter of 3 mm, then extracted with pyrophos sodium ata concentration of 2.0-4.0 wt. % in the mass ratio of peat: extractant - 1: 50-1: 100 with constant stirring at a temperature of 25-27 ° C; then the humic acid extract is treated with hydrochloric acid to a pH of 1-2, the precipitate of humic acids released is separated by centrifugation, then the medium is washed from acidic to pH 7 and dried at room temperature. 2. The use of humic acids from peat bogs in the Tomsk region to increase the production of nitric oxide by macrophages in vitro.

Description

translated from Russian

The invention relates to means based on humic acids (HA) from peat, which increase the production of nitric oxide by macrophages for use in medicine and veterinary medicine and methods for their preparation.

Humic substances (GW) - the main organic component of soil, water, and also solid minerals - caustobiolites, formed during the decomposition of plant and animal residues under the influence of microorganisms and abiotic environmental factors. Humification is the second largest process of the conversion of organic substances after photosynthesis, the result of which is a stochastic, probabilistic mixture of molecules, where none of the compounds is identical to the other. Thus, humic substances are a complex mixture of natural compounds that does not exist in living organisms. Accordingly, it is difficult to apply the traditional method of numerically describing the structure of organic compounds to HS - to determine the number of atoms, the number and types of bonds between them. Therefore, chemists have proposed a classification method based on the solubility of HS in alkalis and acids [1]. Meanwhile, from the point of view of the chemistry of the future, the possibilities of HV are endless, and the areas of possible application, including in medicine, are extremely versatile.

In general, despite insufficient knowledge, HCs are quite widely used in various fields of human activity: in production (oil and gas production, manufacture of batteries, etc.), for remediation of ecosystems polluted by production, and also in agriculture as veterinary preparations and components of organic fertilizers [2]. The main problem of the development and use of humates in pharmacology and medicine is the difficulty of standardization and insufficient knowledge of the mechanisms that determine their therapeutic effectiveness [3]. At present, the physicochemical and biochemical properties of HA have been more or less well studied to obtain new highly

effective peloid preparations that contribute to the availability and effectiveness of peloid therapy [4]. Known sorbent "medical lignin" or polyphepan is widely used in clinical practice [5].

The experiment showed that the administration of HA to animals reduces the stress load, at the same time, they have a positive effect on the functioning of the liver [6]. In laboratory animals treated with HA feed, the concentration of cholesterol, lipids, glucose in the blood decreased, the content of globulins, hemoglobin, and the number of red blood cells increased [7]. Humic acids have immunostimulating properties, as well as antiviral activity and do not show toxic effects in a wide range of doses in experimental animals with oral or cutaneous use [8, 9]. The use of HA (in oxidized form) as an adjunctive therapy in HIV-infected patients has been shown [10].

Humic acids are used as part of agents that increase the body's resistance to the action of various adverse factors. For example, a natural immunomodulator is produced in Poland, including HA, which has an interferonogenic effect and is an inducer of tumor necrosis factor [11]. In the literature there is information about the positive effects of phenolic compounds of peat in the treatment of metabolic disorders of the digestive system, while there are no side effects and complete elimination of the drug from the body [12].

The described variety of biological effects of HA on living organisms cannot be reduced to a single mechanism. In general, the description and use of HA as medical or veterinary drugs, as subjects of stochastic origin, is a difficult task, since they do not have the usual stoichiometric composition and regular structure, they have heterogeneity of structural elements and polydispersity [13]. The difficulty in describing this class of drugs is aggravated by the fact that HAs differ in their properties depending not only on the feedstock, but also on the method of their extraction, the leaching reagent [14].

The prior art.

The methods of obtaining HA that are known today are mainly based on cavitation dispersion of caustobiolites in an aqueous solution of alkalis — aqueous solutions of sodium and potassium hydroxides until the HA comes out with the subsequent production of humates and is aimed at increasing the yield% [15].

A known method for the allocation of HA [16], in which natural raw materials (rich humus soils, chernozems with the addition of peat and sapropel) are treated with an alkaline solution in the presence of urea and complexon in the following ratio by weight of 1.0: 1.0-1.5: 0 , 1: 2.5. Ethylenediaminetetraacetic acid is used as complexone.

A known method for the separation of humic substances from peat [17], in which they carry out the cleaning and grinding of wet feedstock up to 70%. Peat is treated with extractant (NaOH pH 11-12) with stirring. The resulting peat-water mass is subjected to activation by at least one grinding, preferably on a screw and / or ultrasonic grinder, in which the physicochemical decomposition of peat occurs. In the humic product obtained after grinding, the content of humates in terms of dry matter is at least 50%.

A known method of producing organomineral humic fertilizer from a liquid humate-containing product [18] containing 98 wt. % liquid humic composition, humic and fulvic acids and assimilable phosphorus and potassium. As the humic composition, a product is used to treat wet sapropel with an aqueous solution of potassium pyrophosphate heated to 80 ° C, containing 15.0-33.4 g / l humic and 7.1-15.4 g / l fulvic acids.

Closest to the proposed, is a method for producing water-soluble humates [19], selected as a prototype. The method includes grinding the feedstock (peat or coal), treatment with an extractant (potassium or sodium alkali or ammonia water, organic bases) under mechanical stirring under normal conditions for 6-8 hours, separation of solid and liquid phases (solution in the form of salts of monovalent cations - humate of potassium, sodium or ammonium, respectively) and drying the latter. It was found that when using alkali NaOH in a concentration of from 0.03 M to 0.2 M, humus dissolves and a maximum and stable yield of the final product to the solution is ensured. The authors consider the concentration of 0.1 M to be the optimal concentration of alkali solutions for the separation of HA from natural raw materials, which is minimally necessary to replace the hydrogen molecule of all functional groups with alkali metal ions in the process of salt formation of soluble HA in an aqueous solution. A further increase in the concentration of alkali more than 0.2-0.3 M can lead to their chemical destruction.

The disadvantages of the methods are:

- instability of the resulting product and low reproducibility;

- the use of aggressive alkaline reagents (pH 11-12) and grinders to activate the physico-chemical decomposition of peat, which can contribute to artificial humification and, along with a high yield, provoke a chemical modification and prevent the production of native, close to natural HA;

- equipping with special equipment - screw or ultrasonic shredder;

- the use of high temperature (80 ° C), which contributes to the artificial humification of plant residues and the destruction of the native structure of peat HA with the formation of new substances, in particular polycyclic aromatic hydrocarbons related to carcinogens (3,4-benzofluorantene, 10,11-benzofluorantene, 3,4-benzpyrene, 1,12-benzanthracene, etc.), and prevents the development of medical and veterinary drugs based on them;

- most of the available methods for the extraction of HA and their salts from caustobiolites are aimed at increasing the yield of humic substances, increasing their solubility and maintaining the stability of solutions, as well as reducing production costs, possibly associated with the loss of some biological properties.

The objective of the invention is to provide a method that allows to obtain chemically pure HA while maintaining their native structure and having the ability to stimulate the production of nitric oxide by peritoneal macrophages of intact mice in vitro.

A new technical result of the invention is the creation of an effective technology for peat processing, which allows to obtain precisely such water-soluble humic acids that have the ability to activate the secretory properties of macrophages while simplifying the production process, reducing the complexity and material consumption of the process, and, accordingly, reducing the cost of the final product.

To achieve a new technical result in a method for producing humic acids that increase the production of nitric oxide by macrophages in vitro from peat of the marshes of the Tomsk Region, including grinding of raw materials, treatment with estragent with mechanical stirring for 8 hours, precipitation from a solution of inorganic acid, separation of liquid and solid phases and drying the latter, peat is preliminarily dried at room temperature to an air-dry state, ground, sieved through a sieve with a hole diameter of 3 mm, then extraction ruyut with sodium pyrophosphate concentration of 2.0-4.0 wt. % in the mass ratio of peat: extractant - 1: 50-1: 100 with constant stirring at a temperature of 25-27 ° C, then the humic acid extract is treated with hydrochloric acid to pH = 1-2 and the precipitate of humic acids separated is separated by centrifugation, then washed from acidic to neutral pH = 7 medium and dried at room temperature.

New in the invention is the creation of a method for producing humic acids using pre-dried to air-dry state and sifted peat, the use of low concentrations of extractants, moderate mechanical stress and room temperature at the stage of activation, as well as the elimination of the release of HA in the form of humate salts having biological activity, in particular, the ability to stimulate the production of nitric oxide, which allows us to consider the use of this method of obtaining material in for development based on these medications that have specific immunomodulatory properties.

The method is as follows: a sample of peat is dried at room temperature to an air-dry state, crushed in a rotary knife mill and sifted through a sieve with a hole diameter of 3 mm, which ensures the most optimal conditions for the physicochemical process of the interaction of material particles and solution - effective mass transfer, and, accordingly, the completeness and acceleration of extraction. Then peat is treated with a solution of sodium pyrophosphate ($Na_4P_2O_7$) in a concentration of 2.0-4.0 wt. % in a mass ratio of peat: extractant - 1: 50-1: 100 for 8 hours with constant stirring in a reactor R-100 at a temperature of 25-27 ° C. Carrying out the extraction of peat from their state of the art allows preserving the natural composition of HAs without changes, since this extractant at the indicated concentration excludes the destruction of HAs and ensure the extraction of HAs with the highest possible yield and minimum acceptable losses. Next, the liquid phase (HA extract) is separated from the solid phase (peat residue) using a vacuum filtration system (suction filter), which helps to accelerate the process of obtaining HA. The extract obtained is treated with

hydrochloric acid (HCl) to a pH of 1-2 to precipitate HA from the liquid phase, after which HA precipitated in the field of centrifugal forces is separated by centrifugation, the HA precipitate is washed with purified water, pH 7, to release the obtained HA from the acid residue and dried at room temperature.

The method of obtaining HA does not follow explicitly from the prior art for the specialist and the proposed combination of features is not found by the authors in the patent and medical literature.

To illustrate the invention, the following examples.

Example 1. Eight batches of peat from various marshes (places of sampling / sampling) of the Tomsk region (table 1 of the appendix) were dried at room temperature to an air-dry state, crushed in a rotary knife mill and sieved through a sieve with a hole diameter of 3 mm, processed a solution of sodium pyrophosphate (concentration 2.0-4.0 wt.%) in a mass ratio of peat: extractant - 1: 50-1: 100 for 8 hours with constant stirring in a reactor R-100 at a temperature of 25-27 ° C, the liquid phase (HA extract) was separated from the precipitate (peat residue) by filtration Using a vacuum filtration system (suction filter), to precipitate HA from the extract, the liquid phase was treated with hydrochloric acid to pH 1-2, the released HA was separated in a centrifugal field (centrifugation), washed with purified water to pH 7 and dried at room temperature . The HA content in peat was determined gravimetrically.

The proposed method for the production of native chemically pure HA from peat in the marshes of the Tomsk Region, which are capable of increasing the production of nitric oxide by peritoneal macrophages of intact mice in vitro, allows the extraction of HA with a yield of at least 3.1% and no more than 26.0% of the organic mass of peat.

Example 2. Next, the same eight weights of peat were taken, dried at room temperature to an air-dry state, ground in a rotary knife mill and sieved through a sieve with a hole diameter of 3 mm, treated with a solution of sodium hydroxide in a concentration of 0.4 wt. % in the mass ratio of peat: extractant - 1: 50-1: 100 for 8 hours with constant stirring in the R-100 reactor at a temperature of 25-27 ° C, the liquid phase (HA extract) was separated from the precipitate (peat residue) by filtration at using a vacuum filtration system (suction filter), to precipitate HA from the extract, the liquid phase was treated with hydrochloric acid to pH 1-2, the released HA was separated in a centrifugal field (centrifugation), washed with purified water to pH 7 and dried at room temperature. The HA content in peat was determined gravimetrically.

This method, which differs from the method proposed in the invention in Example 1 only by extractant sodium hydroxide, allows extracting at least 6.5% and not more than 38.6 masses from the dry mass of the starting material. % HA, which significantly exceeds the yield of humic acids during extraction with sodium pyrophosate: in the sample M-20-70 2 times, BP-10-50 - 2.4 times, G-20-70 - 3.4 times, KV-10-50 - 1.5 times, NR-200-250 - 3.2 times, T-50-100 - 2.2 times, in samples OT-230-270 and G-100-120 - 4 times (table 1 of the appendix).

A new property of HA obtained by the proposed method to stimulate the production of nitric oxide by macrophages of intact mice in vitro was discovered as a result of experimental studies

Experiments to confirm the biological activity of isolated HA were performed on linear C57BL / 6 mice aged 8-10 weeks. Animals are obtained from the Department of Experimental Biological Models NIIIFiRM them. E.D. Goldberg. All procedures (keeping, killing) were carried out in accordance with the principles of the European Convention for the Protection of Vertebrate Animals (dated March 18, 1986; Strasbourg; ETS No. 123). Macrophages (MF) were obtained from a suspension of peritoneal cells, for which the animals were sacrificed by dislocation of the cervical spine, the abdominal cavity was washed with ice-cold isotonic sodium chloride (RF) solution, the cells were besieged, resuspended in culture medium and their viability was evaluated in a test with 0.1% trypan in blue. Then the cells were placed at 1.5-2.0 × 10^6 / ml in plastic Petri dishes, cultivated for 2 hours at 37 ° C (in an atmosphere of 5% CO_2 and absolute humidity) in an environment of the following composition: RPMI 1640 ("Sigma") with the addition of 10% ETS (Nus1ope), 20 mM HEPES (Sigma), 0.05 mM 2-mercaptoethanol (Sigma), 50 μg / ml gentamicin (Sigma), 2 mM L-glutamine ("Sigma"), after which cells adhering to the plastic were collected. MFs obtained after adhesion (2.5-3.0 × 10^6 cells / ml) were placed in flat-bottomed 96-well plates and cultured under the above conditions for 48 hours in the presence of selected concentrations of HA (the most effective concentrations of active substances were identified in preliminary in vitro experiments) or 1 μg / ml LPS (serotype O111: B4, Sigma). After 48 hours from the start of cultivation, the production of nitric oxide (NO) in the supernatants was assessed by nitrite content using Grace reagent [20], which was mixed with an equivalent volume of supernatant, and absorption was measured using a Titertek Multiskan® MCC multichannel spectrophotometer (Labsystems, Finland) at a wavelength of 540 nm. The nitrite concentration was determined by a calibration curve constructed using standard solutions of sodium nitrite.

To determine the endotoxin impurity, the studied HA samples were incubated under the same conditions with 50 μg / ml polymyxin B (InvivoGen, USA), bacterial toxic substances, which are structural components of bacteria, for example, structural components of membranes of gram-negative bacteria, are understood as endotoxins. . lipopolysaccharides (LPS) By itself, endotoxin is not a toxic substance, but its presence in drugs, especially for injection, is a huge problem, since it can lead to activation of the immune system, namely, increased synthesis of a number of anti-inflammatory mediators by monocytes and macrophages. The development of this cascading anti-inflammatory reaction, accompanied by fever and fever (endotoxic shock), which can lead to death, is usually denoted by the term pyrogenicity. Endotoxin is quite stable and persists at high temperatures and in a wide pH range. Impurities of endotoxin are often found in samples of plant origin of both higher and lower plants. The absence of such an impurity in a natural substance, when identifying directed pharmacological effects, provides significant advantages for the further development of various drugs on its basis.

Example 3. The biological activity of HAs isolated from eight different types of peat of the marshes of the Tomsk Region as proposed in Example 1 was evaluated by their NO-activating effect on peritoneal macrophages of intact C57BL / 6 mice in vitro (table 2). Incubation of macrophages in the presence of HA samples led to an increase in nitric oxide production: sample M-20-70 (concentration 10 μg / ml) 26 times, sample G-100-120 (10 μg / ml) 32 times, sample KV-10 -50 (10 μg / ml) 39 times, sample HP-200-250 (10 μg / ml) 30 times, sample BP-10-50 (100 μg / ml) 6.5 times, sample G-20 -70 (10 μg / ml) 32 times, sample OT-230-250 (50 μg / ml) 20 times, sample T-50-100 (50 μg / ml) 19 times. Moreover, the use of a standard activator of NO-producing activity of LPS macrophages as a control increased the value of the indicator by 35 or 14 times in different series of experiments.

The cultivation of LPS-stimulated cells in the presence of polymyxin B (control 1) led to a decrease in the concentration of nitrites in the supernatant by 1.7 and 2.8 times, respectively, in the series of the experiment.

The addition of an inhibitor to three samples of M-20-70, G-100-120 and BP-10-50 HCs in order to exclude the possibility of NO-activating action by the presence of endotoxin revealed the absence of an endotoxin impurity, moreover, the concentration of nitric oxide was 1, 8 times more compared to the same indicator when incubating MF with LPS in the presence of polymyxin B.

During the processing of HA samples KV-10-50, HP-200-250, Г-20-70, OT-230-250 and T-50-100 with polymyxin B, the concentration of produced nitric oxide decreased slightly, however, it was significantly 1.5, 1 , 3, 4, 2.5 and 1.7 times, respectively, exceeded the control value 1 with polymyxin B.

Thus, HAs isolated using sodium pyrophosphate (Example 1) have a significant advantage in the activation of macrophages, causing a specific increase in their production of nitric oxide, due to their exceptionally special properties.

Example 4. Incubation of macrophages in the presence of HA isolated using the method of Example 2 from eight different types of peat of the marshes of the Tomsk Region revealed an increase in nitric oxide production: 26 times in sample M-20-70 (concentration 50 μg / ml), G- 100-120 (100 μg / ml) 9.1 times, KV-10-50 (50 μg / ml) 27 times, HP-200-250 (100 μg / ml) 27.5 times, BP-10 -50 (50 μg / ml) 3.5 times, G-20-70 (50 μg / ml) 4 times, OT-230-270 (10 μg / ml) 19 times, T-50-100 (50 μg / ml) 3.3 times (table 3).

The addition of polymyxin B to the cell culture in order to exclude the possibility of the NO-activating action of the samples by the presence of endotoxin completely reversed the revealed stimulating effect of the HA isolated by the method of Example 3, the concentration of nitric oxide in the cell supernatants decreased in sample M-

20-70 in 3 , 9 times, G-100-120 by 2.3 times, KV-10-50 by 5 times, HP-200-250 by 3.8 times, BP-10-50 by 1.6 times, G-20- 70 2 times, OT-230-270 2.3 times, T-50-100 1.7 times, which was significantly on average 3-5 times lower than the incubation rate of MF with LPS in the presence of polymyxin B and, thus indicate for the presence of endotoxin in the impurity HA samples isolated at alkali extraction.

Thus, it was experimentally established that the proposed method for producing HA using sodium pyrophosphate (Example 1) from peat allows obtaining HAs that retain their native chemical structure, are free from impurities and have a significant advantage in stimulating the NO-producing properties of macrophages in vitro - to induce specific their enhanced nitric oxide production. HA obtained by the method in example 3 using sodium hydroxide do not possess such properties, their stimulating activity is due to the presence of endotoxin, although at the output of this method (example 2) allows you to get more material.

The revealed properties of HA stimulate the production of nitric oxide, indicating their ability to classically activate antigen-presenting cells and, accordingly, stimulate the development of a Th1-dependent type of immune response, can serve as the basis for the development of low toxic and highly effective herbal remedies for the correction of the immune system in chronic, sluggish, infectious inflammatory processes in medicine and veterinary medicine.

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application

Table 1 General characteristics of various types of peat of the Tomsk region with various methods of material extraction

Table 2 The effect of humic acids (extraction using sodium pyrophosphate) on the production of nitric oxide by peritoneal macrophages of intact C57BL / 6 mice (X ± m)

● - различия показателя по сравнению с инкубацией с ЛПС (контроль 1) без полимиксина В достоверны, p<0,05; ♦ - различия показателя по сравнению с инкубацией МФ с действующими веществами без полимиксина В (контроль 2) достоверны, p<0,05; ■ -различия показателя по сравнению с инкубацией с ЛПС в присутствии полимиксина В достоверны, p<0,05. Концентрация полимиксина В - 50 мкг/мл, n=6.Note: * - differences in the indicator with the environment are significant, p <0.05; ● - differences in the indicator compared with incubation with LPS (control 1) without polymyxin B are significant, p <0.05; ♦ - differences in the indicator compared with incubation of MF with active substances without polymyxin B (control 2) are significant, p <0.05; ■ -differences of the indicator compared with incubation with LPS in the presence of polymyxin B are significant, p <0.05. The concentration of polymyxin B is 50 μg / ml, n = 6.

Table 3. The effect of humic acids (extraction with sodium hydroxide) on the production of nitric oxide by peritoneal macrophages of intact C57BL / 6 mice (X ± m)

● - различия показателя по сравнению с инкубацией с ЛПС (контроль 1) без полимиксина В достоверны, p<0,05; ♦ - различия показателя по сравнению с инкубацией МФ с действующими веществами без полимиксина В (контроль 2) достоверны, p<0,05; ■ -различия показателя по сравнению с инкубацией с ЛПС в присутствии полимиксина В достоверны, p<0,05. Концентрация полимиксина В - 50 мкг/мл, n=6.Note: * - differences in the indicator with the environment are significant, p <0.05; ● - differences in the indicator compared with incubation with LPS (control 1) without polymyxin B are significant, p <0.05; ♦ - differences in the indicator compared with incubation of MF with active substances without polymyxin B (control 2) are significant, p <0.05; ■ -differences of the indicator compared with incubation with LPS in the presence of polymyxin B are significant, p <0.05. The concentration of polymyxin B is 50 μg / ml, n = 6.

Таблица 1

Вид торфа, глубина отбора, шифр	Место отбора проб торфа	Степень разложения, %	Зольность, %	Выход ГК на органическую массу при экстракции, %	
				$Na_4P_2O_7$	NaOH
Верховой сфагново-мочажинный, 20-70 см **М-20-70**	Мочажина грядово-мочажинного комплекса олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	5-10	2,8	3,1	6,5
Верховой сосново-сфагново-пушицевый, 10-50 см **ВР-10-50**	Высокий рям олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	30-35	7,2	13,2	31,4
Верховой магелланикум, 100-120 см **Г-100-120**	Гряда грядово-мочажинного комплекса олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	10-15	2,7	4,2	16,9
Верховой фускум торф, 50-70 см **Г-20-70**		5-10	2,6	3,9	13,3
Низинный древесный, 10-50 см **КВ-10-50**	Эвтрофное болото Клюквенное	25-30	8,9	26,0	38,2
Низинный травяно-моховый, 200-250 см **НР-200-250**	Низкий рям олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	35-40	4,5	6,8	21,5
Низинный травяной, 230-270 см **ОТ-230-270**	Осоково-сфагновая топь олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	40-45	16,3	4,2	16,9
Низинный древесный торф, 50-100 см **Т-50-100**	Эвтрофное болото Таган	30-35	6,4	17,9	38,6

Таблица 1

Вид торфа, глубина отбора, шифр	Место отбора проб торфа	Степень разложения, %	Зольность, %	Выход ГК на органическую массу при экстракции, %	
				$Na_4P_2O_7$	NaOH
Верховой сфагново-мочажинный, 20-70 см **М-20-70**	Мочажина грядово-мочажинного комплекса олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	5-10	2,8	3,1	6,5
Верховой сосново-сфагново-пушицевый, 10-50 см **ВР-10-50**	Высокий рям олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	30-35	7,2	13,2	31,4
Верховой магелланикум, 100-120 см **Г-100-120**	Гряда грядово-мочажинного комплекса олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	10-15	2,7	4,2	16,9
Верховой фускум торф, 50-70 см **Г-20-70**		5-10	2,6	3,9	13,3
Низинный древесный, 10-50 см **КВ-10-50**	Эвтрофное болото Клюквенное	25-30	8,9	26,0	38,2
Низинный травяно-моховый, 200-250 см **НР-200-250**	Низкий рям олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	35-40	4,5	6,8	21,5
Низинный травяной, 230-270 см **ОТ-230-270**	Осоково-сфагновая топь олиготрофного болота Бакчарского болотного массива юго-восточных отрогов Васюганского болота в междуречье Икса и Бакчар	40-45	16,3	4,2	16,9
Низинный древесный торф, 50-100 см **Т-50-100**	Эвтрофное болото Таган	30-35	6,4	17,9	38,6

Таблица 2

Исследуемое вещество		Концентрация нитритов, мкМ	
Шифр пробы	Концентрация, мкг/мл	инкубация без полимиксина В (контроль 2)	инкубация с полимиксином В
среда	–	1,08±0,04	1,10±0,08
ЛПС (контроль 1)	1	38,40±0,62*	21,91±0,41*♦
М-20-70	10	**33,75±0,95***•	**38,90±1,32***♦■
Г-100-120	10	**34,68±0,50***•	**38,60±0,13***♦■
КВ-10-50	10	42,05±0,57*•	33,23±0,27*♦■
НР-200-250	10	33,16±0,81*•	29,27±0,49*♦■
среда	–	1,56±0,02	1,28±0,10
ЛПС (контроль 1)	1	22,08±2,77*	7,74±0,28*♦
ВР-10-50	100	**10,00±0,95***•	**13,77±0,20***♦■
Г-20-70	10	39,10±0,70*•	32,67±0,16*♦■
ОТ-230-250	50	31,87±0,53*•	19,26±0,37*♦■
Т-50-100	50	29,81±1,12*•	13,20±0,30*♦■

Таблица 2

Исследуемое вещество		Концентрация нитритов, мкМ	
Шифр пробы	Концентрация, мкг/мл	инкубация без полимиксина В (контроль 2)	инкубация с полимиксином В
среда	–	1,08±0,04	1,10±0,08
ЛПС (контроль 1)	1	38,40±0,62*	21,91±0,41*♦
М-20-70	10	**33,75±0,95***•	**38,90±1,32***♦■
Г-100-120	10	**34,68±0,50***•	**38,60±0,13***♦■
КВ-10-50	10	42,05±0,57*•	33,23±0,27*♦■
НР-200-250	10	33,16±0,81*•	29,27±0,49*♦■
среда	–	1,56±0,02	1,28±0,10
ЛПС (контроль 1)	1	22,08±2,77*	7,74±0,28*♦
ВР-10-50	100	**10,00±0,95***•	**13,77±0,20***♦■
Г-20-70	10	39,10±0,70*•	32,67±0,16*♦■
ОТ-230-250	50	31,87±0,53*•	19,26±0,37*♦■
Т-50-100	50	29,81±1,12*•	13,20±0,30*♦■

Таблица 3.

Исследуемое вещество		Концентрация нитритов, мкМ	
Шифр пробы	Концентрация, мкг/мл	инкубация без полимиксина В (контроль 2)	инкубация с полимиксином В
среда	–	1,56±0,02	1,28±0,10
ЛПС (контроль 1)	1	22,08±2,77*	7,74±0,28*♦
1/ВР-10-50	50	5,51±0,74*•	3,37±0,12*♦■
4/ОТ-230-270	10	29,97±0,50*•	12,82±0,33*♦■
5/Г-20-70	50	6,20±0,59*•	3,07±0,30*♦■
9/Т-50-100	50	5,18±0,61*•	3,02±0,10*♦■
среда	–	1,08±0,04	1,10±0,08
ЛПС (контроль 1)	1	38,40±0,62*	21,91±0,41*♦
7/М-20-70	50	28,33±0,77*•	7,27±0,16*♦■
8/КВ-10-50	50	28,99±0,66*•	5,82±0,20*♦■
6/Г-100-120	100	9,87±0,76*•	4,33±0,65*♦■
3/НР-200-250	100	29,76±0,39*•	7,77±0,10*♦■

Таблица 3.

Исследуемое вещество		Концентрация нитритов, мкМ	
Шифр пробы	Концентрация, мкг/мл	инкубация без полимиксина В (контроль 2)	инкубация с полимиксином В
среда	–	1,56±0,02	1,28±0,10
ЛПС (контроль 1)	1	22,08±2,77*	7,74±0,28*♦
1/ВР-10-50	50	5,51±0,74*•	3,37±0,12*♦■
4/ОТ-230-270	10	29,97±0,50*•	12,82±0,33*♦■
5/Г-20-70	50	6,20±0,59*•	3,07±0,30*♦■
9/Т-50-100	50	5,18±0,61*•	3,02±0,10*♦■
среда	–	1,08±0,04	1,10±0,08
ЛПС (контроль 1)	1	38,40±0,62*	21,91±0,41*♦
7/М-20-70	50	28,33±0,77*•	7,27±0,16*♦■
8/КВ-10-50	50	28,99±0,66*•	5,82±0,20*♦■
6/Г-100-120	100	9,87±0,76*•	4,33±0,65*♦■
3/НР-200-250	100	29,76±0,39*•	7,77±0,10*♦■

Patent Citations (3)

Publication number	Priority date	Publication date	Assignee	Title
CN1232007A *	1999-04-26	1999-10-20	文希良	Organic humic acid fertilizer and its preparing process
RU2212391C2 *	2001-12-11	2003-09-20	Шульгин Александр Иванович	Method of composting organic and organomineral materials and wastes (options)
RU2370478C2 *	2007-09-17	2009-10-20	Государственное образовательное учреждение высшего профессионального образования "Югорский государственный университет"	Method of producing oxyhumates from peat
Family To Family Citations				

* Cited by examiner, † Cited by third party

Cited By (1)

Publication number	Priority date	Publication date	Assignee	Title
RU2727692C1 *	2019-12-02	2020-07-22	Федеральное государственное бюджетное учреждение "Сибирский федеральный научно-клинический центр Федерального медико-биологического агентства" (ФГБУ СибФНКЦ ФМБА России)	Humic substance agent for increasing physical performance and endurance

Family To Family Citations

* Cited by examiner, † Cited by third party, ‡ Family to family citation

Similar Documents

Publication	Publication Date	Title
JP4709203B2	2011-06-22	Argin oligosaccharide and its derivatives, and their preparation and use
Hu et al.	2020	Construction and structure-activity mechanism of polysaccharide nano-selenium carrier
JP2018538328A	2018-12-27	Composition comprising ginseng saponin as an active ingredient
CN102070727B	2012-07-04	Extraction method of sodium heparin
US20110081319A1	2011-04-07	Composition and use of phyto-percolate for treatment of disease
CA2631773C	2022-04-26	Composition and use of phyto-percolate for treatment of disease
WO2016041258A1	2016-03-24	Method for preparing bamboo fungus polysaccharide-zinc chelate and use thereof
RU2662094C1	2018-07-23	Agent of humic nature of immunomodulating activity
RU2610446C2	2017-02-13	Preparation, increasing production of nitrogen oxide by macrophages in vitro, based on humic acids of peat of tomsk region bogs and method for obtaining thereof
CN101724089B	2011-08-24	Acidic aloe polysaccharide as well as preparation and purification method and application thereof
EP1928247A2	2008-06-11	Composition and use of phyto-percolate for treatment of disease
CN110790848A	2020-02-14	Preparation method and application of total polysaccharides of sea buckthorn
CN108047343B	2020-11-10	Preparation method and application of fritillaria pallidiflora total polysaccharide
Hafez et al.	2020	Humic substances as an environmental-friendly organic wastes potentially help as natural anti-virus to inhibit COVID-19
RU2716504C1	2020-03-12	Humic substance having immunomodulatory activity
CN107857826B	2020-12-11	Separation and purification method of blood sugar-reducing banana flower polysaccharide
RU2657782C1	2018-06-15	Solid-phase composition having antibacterial and detoxification effect
RU2652347C1	2018-04-25	Liquid phase composition with antibacterial and detoxicative properties
RU2618398C1	2017-05-03	Method of producing melanins from chaga
RU2597160C1	2016-09-10	Method of producing melanin and dry extract of biologically active substances of shelf fungus
RU2425686C1	2011-08-10	Method of producing precipitated preparation of shelf fungus
WO2001028575A1	2001-04-26	Proteoglycan, a bioactive substance from plants
Klavina et al.	2020	FRESHWATER SAPROPEL: BIOLOGICALLY ACTIVE COMPONENTS AND METHODS OF EXTRACTION
KR100348870B1	2002-08-17	Peptido-glyco Compounds from Chinese elm and Process for Preparing the Same
Jasim et al.	2018	Synthesis of herb silver nanoparticle and study the effect against some bacterial infection

Priority And Related Applications

Priority Applications (1)

Application	Priority date	Filing date	Title
RU2015131867A	2015-07-30	2015-07-30	Preparation, increasing production of nitrogen oxide by macrophages in vitro, based on humic acids of peat of tomsk region bogs and method for obtaining thereof

Applications Claiming Priority (1)

Application	Filing date	Title
RU2015131867A	2015-07-30	Preparation, increasing production of nitrogen oxide by macrophages in vitro, based on humic acids of peat of tomsk region bogs and method for obtaining thereof

Concepts

machine-extracted

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Name	Image	Sections	Count	Query match

humic acid	title,claims,abstract,description	76	0.000
nitric oxide 	title,claims,abstract,description	52	0.000
peat	title,claims,abstract,description	48	0.000
manufacturing process	title,claims,abstract,description	32	0.000
Macrophages	title,claims,abstract,description	17	0.000
in vitro	title,claims,abstract,description	12	0.000
bog	title,claims,abstract	4	0.000
nitrogen oxide	title,abstract	3	0.000
preparation method	title,description	7	0.000
HCl 	claims,abstract,description	10	0.000
liquid phase	claims,abstract,description	10	0.000
stirring	claims,abstract,description	7	0.000
centrifugation	claims,abstract,description	6	0.000
separation method	claims,abstract,description	6	0.000
drying	claims,abstract,description	5	0.000
solid phase	claims,abstract,description	5	0.000
mechanical stirring	claims,abstract,description	4	0.000
acidificating	claims,abstract,description	3	0.000
mineralic acids	claims,abstract,description	3	0.000
grinding	claims,description	6	0.000
precipitate	claims,description	5	0.000
sodium	claims,description	5	0.000
sodium 	claims,description	4	0.000
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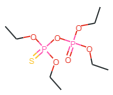
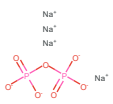
precipitation	claims,description	2	0.000
diethoxyphosphinothioyl diethyl phosphate 	claims	1	0.000
substance	abstract,description	18	0.000
drug	abstract,description	13	0.000
extraction	abstract,description	13	0.000
effects	abstract,description	12	0.000
Tetrasodium pyrophosphate 	abstract,description	7	0.000
sodium pyrophosphate	abstract,description	7	0.000
tetrasodium diphosphate	abstract,description	7	0.000

tetrasodium phosphonato phosphate	abstract,description	7	0.000
raw material	abstract,description	4	0.000
engineering process	abstract,description	2	0.000
secreting	abstract,description	2	0.000
sedimentation	abstract	1	0.000
sieving	abstract	1	0.000

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