

Process for the manufacture of a preparation having immunomodulating activity and stimulating cytokine formation by extracting plants and plant residues

Abstract

A non-toxic preparation having immunomodulating activity and stimulating cytokine (interferons, tumor necrosis factor and others) formation is manufactured by extracting plants and plant residues and is suitable for therapeutic and preventive use in humans and animals. From the extract, preferably from peat extract, humic substances are eliminated by means of acidification with hydrochloric acid to pH 1,5 - 3,0 and separation. The extract is then concentrated by evaporation under reduced pressure and/or by means of nano-filtration with simultaneous elimination of inorganic salts. Next, the pH of the concentrated solution is brought to 6,0 - 7,1. The resulting mixture is then concentrated to a thick syrup and heated up to 70-90 °C until completion of Amadori rearrangement reaction of the products resulting from the reaction of simple sugars, oligo- and polysaccharides with amino acids and/or peptides extracted from peat. The reaction is then stopped by cooling and freed from hydrophobic substances - which would reduce the immunotropic activity of the product - by selective adsorption on a specific resin. The product thus obtained is converted into a sterile solid form by spray-drying.

Classifications

■ **A61K35/10** Peat; Amber; Turf; Humus

WO1994012197A1

WIPO (PCT)

[Download PDF](#)

[Find Prior Art](#)

[Similar](#)

Other languages: [French](#)

Inventor: [Jan Zbigniew Mioduszewski](#), [Krystyna Witkiewicz](#), [Magdalena Kowalska](#), [Joanna Goral](#), [Magdalena Klimecka](#)

Worldwide applications

1992 [PL](#) 1993 [PE](#) [ZA](#) [CO](#) [AU](#) [WO](#) [EP](#) [TW](#) 1994 [EE](#)

Application PCT/EP1993/003362 events

Priority claimed from PLP.296811

1993-12-01 Application filed by Torf Establishment

1993-12-01 Priority to AU56295/94A

1994-06-09 Publication of WO1994012197A1

Info: [Patent citations \(3\)](#), [Non-patent citations \(2\)](#), [Cited by \(1\)](#), [Legal events](#), [Similar documents](#), [Priority and Related Applications](#)

External links: [Espacenet](#), [Global Dossier](#), [PatentScope](#), [Discuss](#)

Claims

[Hide Dependent](#) ^

C L A I M S

1. Process for the manufacture of a preparation having immunomodulating activity and stimulating cytokine formation in living organisms and suitable for therapeutic and preventive use in humans and animals, in which raw plants and plant residues are extracted and from the obtained extract humic substances are separated by means of precipitation in an acidic environment, characterized in that

- humic substances are eliminated from the peat extract by means of acidification with hydrochloric acid to pH 1,5 - 3,0 and separation of the precipitate, whereafter the extract is concentrated by evaporation under reduced pressure and/or by means of nano-filtration with simultaneous elimination of inorganic salts and the pH of the concentrated solution is brought to 6,0 - 7,1;

- the resulting mixture is concentrated to a thick syrup and heated up to 70-90°C, preferably 80°C until completion of Amadori rearrangement of the amino acid and/or peptide products substituted at the terminal free NH₂- group (not involved in any peptide bond) with suitable 1-desoxy-2-ketose C1-radical, resulting from the reaction of simple sugars, oligo- and polysaccharides with amino acids and/or peptides extracted from peat;

- the reaction is stopped by cooling down the reaction mixture and eliminating hydrophobic substances (that would reduce the immunotropic activity of the product) by means of selective adsorption on a specific adsorptive resin of the AMBERLITE XAD type, preferably of XAD-2 or XAD-4 type or other resin having similar properties.

2. Process according to claim 1, characterized in that the starting extract is a mixture of alkaline and water extracts obtained by means of extracting natural raw peat, preferably a low peat of humification degree H6 - H10 - optionally first washed with an acidic solution - with an alkaline aqueous solution and then with water.

3. Process according to claim 1 or 2, characterized in that the Amadori rearrangement reaction is carried out in a concentrated aqueous solution or in a concentrated water-alcohol solution.

4. Process according to any one of the preceding claims, characterized in that the substances to be eliminated from the final product are adsorbed from their acidic or neutral solution in columns which afterwards are eluted with distilled water and diluted alkali solutions.

5. Process according to any one of the preceding claims, characterized in that the final solution - optionally after sterilization - is spray-dried.

Description

Process for the manufacture of a preparation having immunomodulating activity and stimulating cytokine formation by extracting plants and plant residues

The present invention relates to a process for obtaining out of plants and plant residues a non-toxic preparations having immunomodulating activity and stimulating the formation of cytokines (interferons, tumor necrosis factor, etc.) when administered to humans and animals.

Many disclosures have been made relating to the manufacture of different extracts, especially of peat, having immunotropic or antitumor activity:

Hyoshita Takuya et al (Chem. Abstr. 1974, 00, 47021j) teach a process in which peat is extracted several times with an 8% sodium hydroxide solution; the obtained extract is acidified, centrifuged to separate the solid particles, then dialyzed and purified on chromatographic columns. The fraction is mainly (95 %) composed of

polysaccharides and has antineoplastic activity.

St. Tolpa et al (in Polish patent no. 124110) describe the same peat extract but differ insofar as they treat the extract further. After the acidification step, it is alkalized, then acidified again, concentrated by means of evaporation, neutralized, concentrated again, extracted with an alcohol-water mixture, then partially concentrated again and extracted with ether or other water immiscible organic solvent. Finally, the aqueous layer is separated and purified by means of chromatographic columns on a filtration gel. It is, however, not indicated which fractions may be considered to constitute the preferred final product. Contrary to the alkaline extracting media known from the two above mentioned documents, the Polish patent application 279.475 (Tarchomińskie Zakłady, Farmaceutyczne POLFA, published in BUF 24/90) covers a process of peat extraction with an acidified aqueous extracting medium. The acidic extract is neutralized and concentrated by means of ultrafiltration or reverse osmosis employing membranes having a retention limit - cut off - from 500 to 10.000 daltons. Then the product is separated by means of column gel filtration or on anion exchange resins. Fractions differing in salt concentrations, and in the ratio of polysaccharides to glycoproteides, may also be obtained. It is, however, indicated that all mixtures obtained are non-toxic and show immunotropic activity.

The above described processes have a number of common features; it has been known for long to isolate active substances from natural raw materials while preserving the active substances and thus avoiding secondary reactions which may be the cause of decomposition or inactivation of the active substances. Unfortunately none of the prior art teaches the exact structure or chemical character of the compounds responsible for the biological activity of the product (being a mixture of different substances) manufactured by the respective processes. Very little information is given on polysaccharides and the use of separation methods in which low molecular weight fractions are discarded (dialysis and ultrafiltration); this indicates that in the products obtained the biological activity is meant to relate to the high molecular weight fractions.

In the present studies on peat extracts it was found that the extracts obtained even under preserving conditions either have a relatively low immunotropic activity or contain hardly separable ballast substances - mainly inorganic salts - the presence of which limits the use of the product in desired pharmaceutical preparations; they also increase labor and energy consumption in further processing steps and for a number of therapeutical applications even exclude the use of the product.

It is a main aim of the present invention to provide a new method for extracting plants and plant residues and processing such extracts, allowing to obtain - with a higher yield

- preparations having immunotropic properties able to stimulate cytokine formation, free from the drawbacks of the known processes.

Unexpectedly it was found that the immunotropic activity of each production lot is maintained within the preferred range and the yield of organic substances in the final product is significantly increased when the extraction conditions are changed in one or more of the following features:

- some secondary reactions between the extracted compounds may intentionally occur in a controlled direction and to a controlled extent so that new compounds are formed that in known - e.g. peat - extracts would either be absent, or be present in rather low concentrations only;

- the processing of extracts thus obtained is modified so that valuable and beneficial components of the extract are not eliminated when the ballast substances - both extracted from natural peat and/or introduced with the extracting medium - are separated.

The organic substances related to the biological and therapeutic activity of peat derived preparations - including TTP (TOEPA TORF PREPARATION, Trademark protected in the name of TORF CORPORATION, Poland) - are Amadori rearrangement compounds and products obtained in the course of such rearrangement of aldoses and aminoacid reaction products, i.e. the compounds of a general formula $R_1-CO-CH_2-NH-R_2$, wherein R_1 is a residue of 1-desoxy-2-keto-sugar, oligo- or polysaccharide in which such 1-desoxy-2-keto group is an end group of a carbon atom chain, and wherein R_2 is an aminoacid residue or peptide residue in which the end group is a free NH_2 -group not involved in the peptide bond. The process for manufacturing such compounds as well as their pharmaceutical applications are disclosed in PCT/EP93/00327.

It has now been found that it is possible to obtain extracts of plants and plant residues which are rich in both substrates required for a synthesis of such active compounds, i.e. sugars, oligo- and polysaccharides on one hand, as well as aminoacids and peptides on the other hand, and eventually including organic acids and/or compounds with at least one active methylene group as catalysts for the above described reaction.

The instant process allows to manufacture non-toxic preparations, e.g. peat derived ones, having immunomodulating activity and stimulating cytokine (interferons, tumor necrosis factor and others) formation in the living organisms, suitable for therapeutic and preventive use in humans and animals. Raw peat is extracted; humic substances are separated from the obtained extract by means of precipitation in acidic environment; acidification is carried out with hydrochloric acid to pH 1,5 - 3,0; the precipitate is separated and the extract concentrated by evaporation under reduced pressure and/or by means of nano-filtration, simultaneously eliminating inorganic salts; the pH of the concentrated solution is brought to 6,0 - 7,1; the resulting mixture is concentrated to a thick syrup and heated up to 70- 90°C, preferably to 80°C until completion of the Amadori rearrangement of the aminoacid and/or peptide products substituted at the terminal free NH_2 -group (not involved in any peptide bond) with a suitable 1-desoxy-2-ketose C1- radical, resulting from the reaction of simple sugars, oligo- and polysaccharides with aminoacids and/or peptides extracted from peat; the reaction is then stopped by cooling down the reaction mixture; hydrophobic substances - reducing the immunotropic activity of the product - and are eliminated from the obtained post-reaction mixture by means of selective adsorption on a specific adsorptive resin such as the AMBERLITE XAD type, preferably XAD-2 or XAD-4 or any other resin having the similar properties.

Preferably the peat extract to start with is a mixture of alkaline and water extracts obtained by means of extracting natural peat - optionally first washed with an acidic solution - first with an alkaline aqueous solution and then with water. Preferred raw peat is low in humification degree H6 to H10.

The Amadori rearrangement reaction is preferably carried out in a concentrated aqueous solution or concentrated water-alcohol solution.

Preferably, the adsorption of substances to be eliminated from the final product is carried out from the neutral or slightly acidic solution; the columns are eluted with distilled water and diluted alkaline aqueous solution. From the final solution, biologically and therapeutically active substances are isolated in a solid form by spray-drying.

The Amadori rearrangement reaction can be monitored by means of a test reaction, i.e. reduction of potassium ferricyanide with the rearrangement products in alkaline solution. At room temperature, the potassium ferricyanide is reduced within the first minute by reducers such as ascorbic acid, within the first five minutes by the desired rearrangement products and within 15-30 minutes by reducing sugars. The reduction product potassium ferrocyanide with ferrous sulfate forms prussian blue. Such reaction can be used for photometric quantitative monitoring of the rearrangement reaction. Samples of the reaction mixture are taken in predetermined time intervals and subjected to the test reaction. The rearrangement reaction is stopped when the intensity of the prussian blue in a reduced sample is not increasing any longer.

It was unexpectedly found out that from a mixture of processing substrates such as peat extract, the formation of desired Amadori rearrangement products proceeds easier than in the laboratory pre-mixed composition of the same sugars and aminoacids.

It was further determined that some substances contained in the peat extract increase the biological effectiveness of the final preparations obtained in the process according to the present invention in comparison with the analogous synthetic products, whereas other substances decrease such effectiveness.

The initial peat extract used in the process according to the invention, being rich in sugars and amino-groups, is obtained in a way described in literature. Preferred starting material is a low peat showing a high degree of humification (for example H6 to H10 in a scale known from Moor- und Torf- Kunde, ed. Karl-Hans Gottlich, Stuttgart, 3rd edition, 1990) and a carbon to nitrogen ratio of from 15 to 35.

Aqueous extracts may be obtained by any - preferably by a combination - of the above described methods, i.e. with acid-, neutral and/or alkaline aqueous extracting media. Preferably, the raw peat is first shortly (1 to 2 hours) extracted with an acidic aqueous extraction medium in order to leak out inorganic substances, such as carbonates, metal oxides and the like; then for a prolonged time - of several to more than twenty hours - with aqueous alkaline extraction medium (for example a 0,2% to 0,6% NaOH-solution) at 20-50°C in order to dissolve humic and fulvic acids; and finally with water, preferably distilled water at 30-60°C for another 12 or more hours; the acidic extract is then discarded; the alkaline and neutral extract are combined and further processed according to the present invention.

It is known that post-extraction peat is very difficult to be separated from the extract and that an expensive equipment - such as centrifuge separators - is needed to obtain clear extracts with a good yield. Such difficulties can be eliminated when special extractors are used in which the peat hard is in a stationary bed and the extracting medium is circulating throughout the bed. Details of the extraction process will be given in further parts of the specification.

According to the present invention, the alkaline aqueous extract is acidified to pH 1,5-3,0 - preferably 2,3-2,5 - in order to precipitate humic acids. 6N hydrochloric acid is a suitable acidification medium. The precipitates sediment within several to more than 20 hours, and the clear extract can be decanted. Centrifugation or filtration may be used to collect the remaining portion of the extract.

The clear solution is then concentrated in a rotary evaporator down to 1/10 of the initial volume. Alternatively, the concentration and elimination of inorganic salts may be achieved by means of nano-filtration while adapting the pH-value of the solution. The term "nano-filtration" is used to define a modified reverse osmosis process in which membranes permeable only for small size molecules, such as simple inorganic salts, are used. Such membranes are not characterized by a retention time but more frequently - by permeability for NaCl. It was found that when using membranes of a HC50PP type, having NaCl permeability of 40-60%, or a CA865PP type, having NaCl permeability of 66-74 % (products of Dow Denmark), satisfactory results are obtained. The process carried out while using such membranes or membranes of a similar type, allows concentration of the solution and simultaneous elimination of electrolytes, practically without any loss of low molecular weight organic substances, which are considered to be the most important in the process according to the present invention (while in the known methods higher molecular weight components of the extracts were rather considered as the valuable ones) and contributing to the final activity of the desired final product obtained.

The two just described ways of concentration may also be employed in sequence in the same process. Preferably, first the solvent is evaporated in a rotary vacuum evaporator; then, the solution is neutralized and subjected to nano-filtration until a concentration of 8-12 g dry solids in 100 ml of solution is reached. If for some particular application, a high degree of desalination, i.e. low content of mineral salts, is desired, the solution may be diluted in the course of nano-filtration with distilled water (dia-filtration) either periodically or in a continuous manner. Cations of a bigger size, such as Ca, Fe, Al, which migrate through the membranes much slower, may be substituted with sodium cation before the nano-filtration process is started by means of passing the solution to be concentrated through a column with a weak ion exchanger (for example AMBERLITE E IRC50) in sodium form.

At the end of the nano-filtration, the pH of the remaining solution is controlled and if necessary adjusted to 6,0-7,2, preferably to 6,5-6,8. The solution is then placed in a rotary vacuum evaporator and is concentrated at temperature of 35-40°C down to approximately half of its initial volume (until a syrup-like, thick solution is obtained). Then, under atmospheric pressure, while continuously stirring, the temperature of the solution is raised to 70-90°C, preferably to 80°C, and heating is continued. Test samples are taken at the beginning every 30 min, after 1,5 hours every 15 min. Monitoring of the reaction progress is accomplished by measuring the intensity of colour in the test reaction described above. If the intensity of the colour in the test reaction performed for a given sample is the same or lower than for the preceding sample, the reaction is stopped by rapidly cooling down the heating bath.

Alternatively, the reaction may be carried out in the following manner: The reaction mixture is subjected to the same concentration process as previously described. Then it is diluted with a 50% aqueous solution of ethanol in an amount sufficient for dissolving the product; then, the diluted solution is heated under reflux, and the progress of the reaction is monitored and controlled as described above, except that the solvent is evaporated from each sample prior to subjecting it to the test reaction. When the reaction is complete, alcohol is distilled off under reduced pressure.

After determination of the dry solids content in the post-reaction mixture it is diluted with distilled water so that a 10-12% solution is obtained which is then passed slowly through a column filled with a non-ionic adsorptive resin of XAD type (product of Rohm & Haas), preferably of a XAD-2 or XAD-4 type. During the contact with such an adsorbent, it is the hydrophobic substances which are most strongly adsorbed while the weakest adsorption takes place for hydrophilic substances such as sugars. The liquid passing through the column is collected. The column is then eluted with distilled water, and the eluate is separately collected in fractions. Each fraction is chromatographically analysed and/or tested for its activity with biological assays. The liquid that initially passed through the column is then combined with chosen eluate fractions selected for their desired activity in order to obtain the product reaching a predetermined ratio of activities measured in different tests and biological assays. The biological assays are described in details below. Next, the most strongly adsorbed substances are eluted with a diluted alkali solution until the eluate is still neutral. Further fractions are discarded since they contain substances strongly inhibiting biological activity of the final preparation.

The solution obtained in the previous step is sterilized by means of membrane filtration and dried in a spray dryer under sterile conditions with an air inlet temperature of 180-185°C and an air outlet temperature of approx. 80°C.

The obtained powder is beige in colour and when stored - at room temperature, in an air-tight container in dark - maintains its biological activity for more than a year.

The activity of the above described final product has been evaluated by performing the following tests showing the effect of the preparation on individual reactions of the immunological system:

1. Determination of the percentage of splenocytes forming E-rosettes according to Bach and Dardenn (Cell. Immunol. 3,1-16,1972)
2. Determination of the number of splenocytes producing hemolytic antibodies of the IgM type according to Jerne, as modified by Mishell and Dutton (J. Exp. Med. 126,423-442,1967)
3. Determination of hemagglutination 19S + 7S and 7S antibodies in an active hemagglutination test according to Adler (J. Immunol. 95,26-38,39-47,1965)
4. Tests for survival of mouse thymocytes in 20 hours' cultures with hydrocortisone (cytotoxic test)
5. Interferon and TNF induction test (PCT/EP93/00327) In all the above listed tests the product obtained according to the present invention has proved - in comparison with blind controls - to be stimulating already in concentrations of from 0,05 to 1 mg/kg (tests 1 to 3), 0,05 to 10 µg/ml (test 4) and 10 to 100 µg/ml (test 5).

As far as the other properties of the products obtained according to the invention are concerned it has to be underlined that the respective properties fall within the ranges determined by the Polish Health Ministry in the approval certificate covering TTP (Torf Preparation) specified in the analytical standard for that preparation.

The present process for obtaining a bioactive product out of peat results in a 10 times higher yield than traditional methods according to Polish patent No. 124 110. This means that less of the valuable peat is used as a starting raw material. Furthermore the post-extraction peat is actually washed with water after the alkaline extraction process and therefore finally is neutral (not contaminated with alkali or acids) and environmentally safe; it is not ecologically dangerous and may be used directly in agriculture.

Moreover, in the process according to the present invention, any use of flammable organic solvents such as ethyl ether is eliminated. Due to the higher concentration of active substances - also those of low molecular weight - the preparation is resorbed more easily when administered orally, than a TTP obtained in a traditional way.

The present invention is illustrated in more detail in the following examples: Example 1 :

45 kg of peat of 64-65% moisture content, comminuted, sieved through a grid with 14 mm openings and filled into a semi- technical scale extractor specially designed for the ex- traction of peat. A 0,4 % sodium hydroxide was fed from a 180 l feeding tank as the extracting medium in a very slow flow throughout the peat bed, so that the level of liquid in the extractor increased by approximately 1 cm/min. When the whole bed of peat was covered with extraction medium, the flow speed was increased up to 500 l/min. The extract was recirculated for 27 hours at temperature 40 ± 2°C. After that time the feeding pump was stopped; the feeding valves were operated to establish communication with 120 l of distilled water in another tank and circulation of the wash- ing liquid was continued for further 16 hours at the same temperature.

Alkaline and water extracts were combined; the extract was decanted and resulted in a total volume of 240 l of liquids of pH = 12,1. The whole lot was further processed separately in two portions.

Example 2:

120 l of the extract obtained by the procedure described in Example 1 was acidified to a pH value of 2,1 using 1,6 l of 6N hydrochloric acid. The acidified mixture was left for se- dimentation during 24 hours. Then 75 liters of clear solu- tion was decanted, and further 25 liters were recovered by means of filtration. The clear solutions were combined and concentrated under reduced pressure in a rotary glass evapo- rator (SIMAX) to a volume of 19 l. The pH-value of the con- centrated solution was brought to 6,8 by adding sodium hydroxide. The resulting solution was nearly neutral and diluted with distilled water to a total volume of 50 l and subjected to nano-filtration in Lab Unit M20 (product of Dow Separation System, Denmark) equipped with flat membranes of a HC50PP type. The total filtration area of such membranes was 0,36 m2 at a pressure of 20 atm and at a temperature of 21 °C. The final volume of the filtrate was 7 liters. The concentrated extract had a pH-value of 6,6 and therefore did not require any adjusting.

The concentrated extract as obtained above was charged into a vacuum rotary evaporator (BUCHI, Switzerland) and further concentrated under reduced pressure to a volume of 1,3 l. Next, the solution was brought to atmospheric pressure and to a temperature of 80°C; heating was continued under con- tinuous stirring by means of rotation. The progress of the desired reaction was monitored by means of determining the intensity of colour in the samples taken after the test re- duction of potassium ferricyanide. After 135 min the colour intensity was stable or even slightly decreased. The reac- tion was stopped by cooling it down to a temperature of 20° C. The post- reaction mixture was now placed in a smaller . (laboratory scale) evaporator and further concentrated under reduced pressure to a volume of 800 ml having a dry solids content of 13,25 g/100 ml). The concentrated solution was filtered and passed at 0,8 ml/min through a column of 26 mm diameter that was filled with 270 ml of the adsorbent XAD-2. The liquid was collected and the column eluated with dis- tilled water. Three consecutive fractions (1 to 3) were collected, each having a volume of 160 ml, 240 ml and 400 ml, resp. Next, a 0,1% solution of sodium hydroxide was used as eluant and the liquid collected until the pH-value of the eluate exceeded 7,0; this fourth 220 ml fraction had a pH- value of 7,0. The composition of the fractions was determin- ed chromatographically (HPLC). Fractions No. 1, 2, 300 ml of fraction No. 3 and 150 ml of fraction No. 4 were combined, concentrated to 100 ml and then combined with the passing portion of the liquid introduced on the column. The final solution was sterilized by means of filtration under sterile conditions through a membrane with 0,22 µm pores and then dried under sterile conditions in a spray-dryer; the inlet air temperature was 185°C, the outlet air temperature 81 °C. Total yield of the resulting powder was 96 g.

Example 3:

Another portion of 120 l of the extract was acidified (as in Example 2 above) to pH 2,3; the humus precipitate was separ- ated as described in Example 2 above, and 98 l of acidified clear solution were obtained. The solution was neutralized to pH 6,7 with 4N NaOH and left for 12 hours. Then it was filtered and subjected to nano-filtration under the same conditions as described in Example 2. The final filtrate volume was 10 l. Next, 20 l of distilled water were added and the resulting solution concentrated to 10 l. Then once again 20 l of distilled water were added; the resulting so- lution was finally concentrated to 7 l. Further processing was carried out in the same way as in Example 2, except that the formation of Amadori rearrangement compounds and pro- ducts lasted 110 min. The final powder yield was 84 g.

Patent Citations (3)

Publication number	Priority date	Publication date	Assignee	Title
DE2846482A1 *	1977-10-25	1979-04-26	Akad Wroclawiu Rolnicza	A PROCESS FOR THE OBTAINING A PREPARATION COUNTERACTING NEOPLASMS FROM ACIDIFIED, ALKALINE HYDROLYSATE FROM PEAT
WO1992016216A1 *	1991-03-16	1992-10-01	Torf Establishment	Peat-derived bioactive products and pharmaceutical and cosmetic compositions containing them
WO1992016600A1 *	1991-03-16	1992-10-01	Torf Establishment	Process for the extraction of peat and apparatus for carrying out the process
Family To Family Citations				

* Cited by examiner, † Cited by third party

Non-Patent Citations (2)

Title
A.D. INGLOT ET AL.: "NEW NATURAL CLINICALLY USEFUL ORAL IMMUNOMODULATOR TOLPA R EXTRACTED FROM PEAT IS INTERFERON INDUCER IN HUMAN LEUKOCYTES", JOURNAL OF INTERFERON RESEARCH, vol. 11, no. S. 1, November 1991 (1991-11-01), pages S264 *
ANNA D. INGLOT ET AL.: "TOLPA* TORF PREPARATION (TTP*) INDUCES INTERFERON AND TUMOR NECROSIS FACTOR PRODUCTION IN HUMAN PERIPHERAL BLOOD LEUKOCYTES", ARCHIVUM IMMUNOLOGIAE ET THERAPIAE EXPERIMENTALIS, vol. 41, no. 1, 1993, pages 73 - 80 *

* Cited by examiner, † Cited by third party

Cited By (1)

Publication number	Priority date	Publication date	Assignee	Title
FR2818967A1 *	2001-01-04	2002-07-05	Evatex	Recycling system for water, containing brine and organic pollutants from textile dyeing, has auxiliary circuit with tangential nanofiltration unit
Family To Family Citations				

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

Similar Documents

Publication	Publication Date	Title
CA1333779C	1995-01-03	Method for producing galactooligosaccharide
Morris et al.	1962	The isolation and characterization of γ -l-glutamyl-l-tyrosine and γ -l-glutamyl-l-phenylalanine from soybeans
SK111193A3	1994-03-09	Bacterial macromolecule extract, method for preparing same and pharmaceutical composition containing said extract
WO1994012197A1	1994-06-09	Process for the manufacture of a preparation having immunomodulating activity and stimulating cytokine formation by extracting plants and plant residues
JP3347822B2	2002-11-20	Extraction and purification method of quinic acid
WO2000042066A1	2000-07-20	Method of purifying whey of lactic acid fermentation by electrodialysis
KR19990068441A	1999-09-06	The extraction and purification of polysaccharides from Panax ginseng
CN111233658B	2022-06-07	Method for extracting shikimic acid and quinic acid from folium ginkgo
JPH1135591A	1999-02-09	Production of l-fucose from fucoidan separated from cladosiphon okamuranus tokida and its production
SU957835A1	1982-09-15	Method of producing aminoacid and lower peptide mixture
SU654612A1	1979-03-30	Method isolating c cytochrome
RU2114173C1	1998-06-27	Method of crystalline tylosin preparing
CN111848735B	2022-03-04	Immunoregulation active peptide and preparation method and application thereof
RU2025488C1	1994-12-30	Method for preparation of mixture of amino acids and nuclein components
RU2091491C1	1997-09-27	Method of preparing nucleic acid from bacteria
DE3045910C2	1987-08-27	
RU1821213C	1993-06-15	Process for producing vituperate
DE1492047C	1971-10-07	Process for the production of a polypeptide having an immunizing effect
CN1105569A	1995-07-26	Process for the manufacture of a preparation having immunomodulating activity and stimulating cytokine formation by extracting plants and plant residues
RU2110522C1	1998-05-10	Method of preparing the triterpene glycoside sum (variants)
SU786856A3	1980-12-07	Method of preparing escine
RU2139068C1	1999-10-10	Method of isolation of dna sodium salt from animal tissues
CN110904178A	2020-03-24	Preparation method of protein polypeptide
RU2153346C1	2000-07-27	Method of preparing ecdysteroids

Priority And Related Applications

Priority Applications (1)

Application	Priority date	Filing date	Title
AU56295/94A	1992-12-02	1993-12-01	Process for the manufacture of a preparation having immunomodulating activity and stimulating cytokine formation by extracting plants and plant residues

Applications Claiming Priority (2)

Application	Filing date	Title
PLP.296811	1992-12-02	
PL92296811A	1992-12-02	Method of obtaining from peat a preparation exhibiting immunomodulating and cytokinins formation stimulating action

Legal Events

Date	Code	Title	Description
1994-06-09	AK	Designated states	Kind code of ref document: A1 Designated state(s): AT AU BB BG BR BY CA CH CZ DE DK ES FI GB HU JP KP KR KZ LK LU LV

			MG MN MW NL NO NZ PL PT RO RU SD SE SK UA US UZ
1994-06-09	AL	Designated countries for regional patents	Kind code of ref document: A1 Designated state(s): AT BE CH DE DK ES FR GB GR IE IT LU MC NL PT SE BF BJ CF CG CI CM GA GN ML MR NE SN TD TG
1994-09-14	121	Ep: the epo has been informed by wipo that ep was designated in this application	
1994-09-15	DFPE	Request for preliminary examination filed prior to expiration of 19th month from priority date (pct application filed before 20040101)	
1995-10-05	REG	Reference to national code	Ref country code: DE Ref legal event code: 8642
1996-08-02	NENP	Non-entry into the national phase	Ref country code: CA
1996-12-27	122	Ep: pct application non-entry in european phase	

Concepts

machine-extracted

[Download](#) [Filter table](#)

Name	Image	Sections	Count	Query match
■ preparation method		title,claims,abstract,description	14	0.000
■ stimulating		title,claims,abstract,description	6	0.000
■ method		title,claims,description	27	0.000
■ manufacturing process		title,claims,description	7	0.000
■ biosynthetic process		title,abstract,description	9	0.000
■ formation reaction		title,abstract,description	8	0.000
■ immonomodulatory		title,abstract,description	5	0.000
■ extract		claims,abstract,description	39	0.000
■ peat		claims,abstract,description	34	0.000
■ substance		claims,abstract,description	25	0.000
■ chemical reaction		claims,abstract,description	20	0.000
■ mixture		claims,abstract,description	14	0.000
■ nano-filtration		claims,abstract,description	11	0.000
■ sodium chloride		claims,abstract,description	11	0.000
■ HCl		claims,abstract,description	10	0.000
■ Amadori rearrangement reaction		claims,abstract,description	9	0.000
■ reduced		claims,abstract,description	9	0.000
■ amino acids		claims,abstract,description	8	0.000
■ glycans		claims,abstract,description	8	0.000
■ polysaccharide		claims,abstract,description	8	0.000
■ polysaccharide		claims,abstract,description	8	0.000
■ polysaccharides		claims,abstract,description	8	0.000
■ salts		claims,abstract,description	8	0.000
■ resin		claims,abstract,description	6	0.000

➤ resin	claims,abstract,description	6	0.000
➤ cooling	claims,abstract,description	5	0.000
➤ oligosaccharides	claims,abstract,description	5	0.000
➤ pH reduction	claims,abstract,description	5	0.000
➤ Metazoa	claims,abstract,description	4	0.000
➤ elimination reaction	claims,abstract,description	4	0.000
➤ hydrophobic	claims,abstract,description	4	0.000
➤ processed proteins & peptides	claims,abstract,description	4	0.000
➤ processed proteins & peptides	claims,abstract,description	4	0.000
➤ separation method	claims,abstract,description	4	0.000
➤ therapeutic	claims,abstract,description	4	0.000
➤ evaporation	claims,abstract,description	3	0.000
➤ preventive	claims,abstract,description	3	0.000
➤ simple sugar	claims,abstract,description	3	0.000
➤ syrup	claims,abstract,description	3	0.000
➤ syrup	claims,abstract,description	3	0.000
➤ solution	claims,description	36	0.000
➤ product	claims,description	23	0.000
➤ effects	claims,description	13	0.000
➤ distilled water	claims,description	10	0.000
➤ water	claims,description	10	0.000
➤ amino acid	claims,description	8	0.000
➤ acidificating	claims,description	7	0.000
➤ neutralization	claims,description	7	0.000
➤ aqueous solution	claims,description	6	0.000
➤ final product	claims,description	6	0.000
➤ precipitate	claims,description	4	0.000
➤ Chelating resin	claims,description	3	0.000
➤ adsorptive	claims,description	3	0.000
➤ alkali	claims,description	3	0.000
➤ acidic solution	claims,description	2	0.000
➤ Precis	claims	1	0.000
➤ simultaneous	claims	1	0.000
➤ sterilising	claims	1	0.000
➤ sterilization and disinfection	claims	1	0.000
➤ immunotropic	abstract,description	7	0.000
➤ solid	abstract,description	6	0.000
➤ Interferons	abstract,description	4	0.000
➤ Interferons	abstract,description	4	0.000
➤ nontoxic	abstract,description	4	0.000
➤ nontoxic	abstract,description	4	0.000
➤ Interferons	abstract,description	3	0.000
➤ Tumor Necrosis Factor-alpha	abstract,description	3	0.000

■ spray drying	abstract,description	2	0.000
■ Tumor Necrosis Factor-alpha	abstract	1	0.000
Show all concepts from the description section			

Data provided by IFI CLAIMS Patent Services