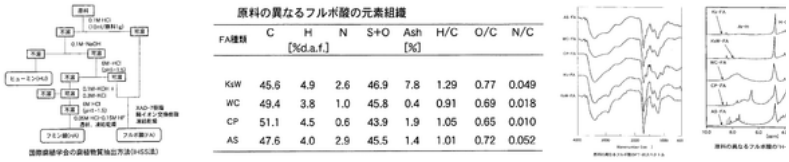
Type i allergy inhibitor using fulvic acid and method for inhibiting onset of type i allergy

Abstract

<P>PROBLEM TO BE SOLVED: To elucidate the relationship of fulvic acid contained in a humic substance with the onset mechanism of action of type I allergy and to inhibit the onset of the type I allergy with the fulvic acid. <P>SOLUTION: A type I allergy inhibitor inhibiting an antigen sensitization stage and/or an antibody sensitization stage or a degranulation stage of cells with the fulvic acid or a composition comprising at least the fulvic acid is provided. A method for inhibiting the onset of the type I allergy comprises carrying out specific desensitization with the fulvic acid or a method for inhibiting the onset of the type I allergy with the fulvic acid especially by inhibiting the antigen desensitization stage or/and the antibody sensitization stage therewith is provided or a method for inhibiting the onset of the type I allergy by carrying out non-specific desensitization with the fulvic acid is provided. <P>COPYRIGHT: (C)2006,JPO&NCIPI

Images (12)



Classifications

A23L33/10 Modifying nutritive qualities of foods; Dietetic products; Preparation or treatment thereof using additives

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Claims (13)

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translated from Japanese

An inhibitor of type I allergy that suppresses an antigen sensitization stage and / or an antibody sensitization stage with fulvic acid. An inhibitor of type I allergy that suppresses the degranulation stage of cells with fulvic acid. 4. The type I allergy inhibitor according to claim 3, wherein the degranulation step is suppressed by inhibiting calcium ion influx into cells. The type I allergy inhibitor according to any one of claims 1 to 3, wherein the fulvic acid is extracted from grass charcoal. A composition comprising at least fulvic acid that suppresses type I allergy in an antigen sensitization step and / or an antibody sensitization step. A face wash comprising the composition according to claim 5. A cosmetic comprising the composition according to claim 5. A food or drink comprising the composition according to claim 5. An external preparation for skin, comprising the composition according to claim 5. A method of suppressing the onset of type I allergy by performing specific desensitization using fulvic acid. The method according to claim 10, wherein the antigen sensitization step and / or the antibody sensitization step is suppressed by fulvic acid. A method for suppressing the onset of type I allergy by performing nonspecific desensitization using fulvic acid. 13. The method according to claim 12, wherein the onset of type I allergy is suppressed by inhibiting degranulation using fulvic acid.

Description

translated from Japanese

The present invention relates to a technique for suppressing the onset of type I allergy. More specifically, the present invention relates to a technique for suppressing the onset of type I allergy based on the specific desensitizing action of fulvic acid contained in humic substances.

"Humic substance" is an organic component produced by plant humus. According to the definition of the International Humic Substances Society (abbreviated as IHSS), this humic substance is adsorbed on the XAD resin with a fraction obtained by extracting soil with an alkali such as sodium hydroxide or with natural water, and a dilute alkaline aqueous solution. The fraction that precipitates with acid is referred to as "humic acid (humic acid)", and the fraction that does not precipitate is referred to as "fulvic acid".

Here, since the fulvic acid according to the subject of the present invention has a property of being strongly bound to iron, it can serve as an iron supply source to the sea, or can coagulate (complex-forming ability) with harmful heavy metals such as copper, and carcinogenesis. It is known to have environmental purification functions such as reduction detoxification of hexavalent chromium, which is a functional substance, and dechlorination of organic halides. However, the effects of fulvic acid, which are considered to be diverse, have not yet been elucidated in detail.

In recent years, in addition to the environmental purification function described above, research on physiological effects of fulvic acid on living bodies has also begun gradually. For example, Patent Document 1 discloses a pharmaceutical composition containing fulvic acid (particularly oxyfulvic acid), a salt, an ester or a derivative thereof, and particularly diseases such as inflammation, acne, eczema, bacterial or fungal or viral infections. An effective pharmaceutical composition is disclosed. In the examples shown in Patent Document 1, the effect of oxyfulvic acid on oxidant production by human neutrophils, the evaluation of anti-inflammatory properties by applying a cream to mouse inflammatory sites, and the purulent traumatic skin of cats and dogs Evaluations for inflammation, oral toxicity confirmation tests using laboratory animals, antimicrobial confirmation tests using a fleece agent containing oxyfulvic acid, and the like have been carried out. In general, anti-inflammatory effects have been confirmed as a topical drug for oxyfulvic acid.

Patent Document 2 discloses antibacterial, antiallergic, anti-inflammatory, and multi-component cosmetic raw materials containing fulvic acid, enzymes, vitamins, amino acids, minerals and the like, which are water-soluble humic substances eluted from humus soil, Actions such as hypoallergenicity and alleviation of itching on allergic skin and atopic skin are disclosed, and in addition, applications to cosmetic raw materials used in the mouth are also disclosed. However, the examples shown in

Patent Document 2 only disclose tests for confirming skin irritation, antibacterial activity confirmation tests, oral administration toxicity confirmation tests, tests for confirming active oxygen scavenging activity, and the like. is there.

Patent Document 3 discloses an antibacterial water that is an extract extracted from humus soil with water, dilute alcohol, or the like, and contains an extract having a pH value of 4.0 or less as an active ingredient. . This Patent Document 3 shows the growth inhibitory effect of the extract against food poisoning causative bacteria such as oral bacteria and Staphylococcus aureus that cause dental caries and periodontal disease.
JP-T-2002-526407. Japanese Patent Application Laid-Open No. 2003-267821. JP-A-6-87752.

Patent Documents 1 to 3 listed above mainly disclose the antibacterial action and anti-inflammatory action of fulvic acid, and the direct causal relationship between fulvic acid and type I allergy, Specifically, the action related to desensitization in the pathogenesis of type I allergy of fulvic acid is not disclosed or suggested at all.

Therefore, the present invention provides a technique for suppressing the onset of type I allergy based on desensitization by fulvic acid by elucidating the relationship between fulvic acid contained in humic substances and the onset mechanism of type I allergy. Main purpose.

First, “type I allergy”, which is the subject of the present invention, is a type of allergic reaction and is also called anaphylaxis type or immediate type. In this type I allergy, when an antibody such as an IgE antibody produced by exposure to an antigen binds strongly to a receptor on the surface of mast cells or basophils at the Fc portion, the receptor crosslinks and causes the cell surface to crosslink. Deformation, activation of various enzymes and inflow of calcium ions (Ca^{2+}) occur to release granules outside the cell (degranulation phenomenon), and enzymes such as histamine, serotonin, β-hexosaminidase, etc. from the granules Mediators (chemical mediators) are released. These mediators increase the permeability of capillaries and cause inflammation in the nasal mucosa, bronchial mucosa, skin and the like. Allergic rhinitis, bronchial asthma, and atopic dermatitis are caused by this type I allergy.

The present invention elucidates the relationship between the occurrence mechanism of type I allergy and fulvic acid and provides a technique for suppressing the onset of type I allergy based on specific desensitization with fulvic acid.

Specifically, the present invention relates to an inhibitor of type I allergy that suppresses the antigen sensitization stage and / or antibody sensitization stage with fulvic acid, or the suppression of type I allergy that suppresses the degranulation stage of cells with fulvic acid. An agent, particularly a type I allergy inhibitor that suppresses the degranulation step by inhibiting the inflow of calcium ions (Ca^{2+}) into cells. As the fulvic acid used in these inhibitors, for example, those extracted from grass charcoal can be suitably employed.

The present invention also provides a composition comprising at least fulvic acid that suppresses type I allergy in the antigen sensitization stage and / or antibody sensitization stage. This composition exerts its effect by, for example, blending it into cosmetics such as skin care cosmetics such as facial cleansers, creams, packs, etc., external preparations for skin, other medicines, quasi drugs, foods and drinks, etc. be able to. In addition, it is free to add components other than the fulvic acid-containing composition according to the purpose and use of the product in the compounding component of the product, and as an example, a component that adsorbs pore dirt Facial cleanser combining fulvic acid, cream or pack combining moisturizing ingredients such as collagen and fulvic acid, medicine combining other medicinal ingredients and fulvic acid, food and drink combining other functional ingredients and fulvic acid, etc. is there.

Furthermore, the present invention relates to a method for suppressing the onset of type I allergy by carrying out specific desensitization using fulvic acid, in particular, the antigen sensitization stage or / and the antibody sensitization stage of type I allergy with fulvic acid. A method of suppressing the onset is provided. Alternatively, a method for suppressing the onset of type I allergy by performing non-specific desensitization using fulvic acid, particularly a method for suppressing the onset of type I allergy by inhibiting degranulation using fulvic acid. I will provide a. The effect of this method can be obtained, for example, by administering fulvic acid in the living body as a form of medicine or food or by bringing fulvic acid into contact with the skin by a method such as application, sticking, spraying, etc. .

Here, main technical terms related to the present invention will be described.

First, “fulvic acid” means fulvic acid contained in fractions obtained from raw materials that are humic substances according to the method (described in the examples below) established by the International Society for Humic Substances (IHSS). To do.

“Peat” is a suitable raw material for extracting the fulvic acid, and in swamps and wetlands, various plants grow and die and water is relatively low in water with insufficient oxygen supply. Under these conditions, it is deposited in an incompletely decomposed state for several hundred to tens of thousands of years. This grass charcoal is not easily oxidized because the plant remains are completely immersed in water and do not touch the air. Also, it is relatively weak even if it is affected by anaerobic fungi or anaerobic fungi, and it is extremely difficult to decompose. Generated as a result of being left in the state for a long time. This process is called mud carbonization, and its essence has not been fully clarified, but it is considered that chemical hydrolysis is the main and decarboxylation is occurring. The organic component of grass charcoal is mainly composed of five elements: carbon, hydrogen, oxygen, nitrogen and sulfur. The elemental composition of grass charcoal reflects the decomposition characteristics of organic matter in the process of grass formation. Generally, as the decomposition of grass charcoal proceeds, the carbon, hydrogen, sulfur and nitrogen content of the grass charcoal organic component increases and the oxygen content decreases. Thorough research has been conducted on the elemental composition of the organic component of grass and charcoal, and it has been confirmed that it is located between wood and lignite. The elemental composition of the organic component of the grass charcoal that is weakly decomposed is close to that of wood, and the organic element composition of the grass charcoal that has been decomposed is close to that of lignite.

The “antigen sensitization stage” means the stage of antigen-antibody reaction in the above-described process of generating type I allergy.

“Antibody sensitization stage” means a stage in which IgE antibody binds to a receptor in the above-described mechanism of developing type I allergy.

“Specific desensitization” refers to inhibiting or blocking any stage in the process of transmitting a stimulus to mast cells or basophils from an antibody such as an IgE antibody produced by an antigen. .

“Non-specific desensitization” refers to suppressing or inhibiting the degranulation phenomenon of mast cells and basophils, that is, suppressing or inhibiting the release of mediators (chemical mediators) from mast cells and basophils. Say that.

According to the present invention, the onset of type I allergy can be suppressed. In particular, the antigen sensitization stage and / or antibody sensitization stage of type I allergic reaction can be suppressed by fulvic acid, or the degranulation stage of cells can be suppressed by fulvic acid.

When this fulvic acid-containing composition is used in products that affect the skin, such as facial cleansers, cosmetics, etc., in addition to the effect of suppressing the onset of type I allergy, the whitening effect, the moisturizing effect, the water penetration promoting effect of the skin water In addition, effects such as a cosmetic ingredient penetration promoting effect and a drying preventing effect can also be obtained.

<Method for extracting fulvic acid>.

In the experiment, fulvic acid of the XAD-7 resin adsorbed fraction was extracted from grass charcoal according to the method defined by the International Society of Humic Substances (IHSS) shown in FIG. Canadian grass charcoal (Sphagnum (moss) peat, Fisons Horticulture, Inc.) was used as the grass.

First, the Canadian grass charcoal as a raw material was acid-treated with 0.1 mol L^{-1} hydrochloric acid (HCL), and the insoluble components were extracted with ^{1} mol L^{-1} aqueous sodium hydroxide (NaOH), followed by centrifugation. 6 mol L^{-1} hydrochloric acid was added to the resulting supernatant to precipitate humic acid (HA).

The aqueous solution obtained in all the processes is adsorbed with XAD-7 resin (ORGANO Co.), desalted with AG MP-50 cation exchange resin (Bio-Rad Laboratories), and then lyophilized. This was designated as fulvic acid. As a result of calculating the extraction ratio of fulvic acid from the Canadian grass charcoal as a raw material from the weight ratio of the dried fulvic acid after extraction to the dry raw material, it was 6.4%.

<Analysis of fulvic acid>. In order to grasp the properties of fulvic acid according to the present invention, chemical analysis of fulvic acid obtained from each of drainage water, weathered coal, Canadian grass charcoal, and black soil was conducted.

(1) Analysis of elemental composition.

Carbon (C), hydrogen (H), and nitrogen (N) of fulvic acid were measured using a CHN analyzer (YANACO CHN CORDER MT-5), and the total ash content was subtracted (total) (composition) It was determined as a percentage by weight and oxygen was determined as the remainder. The elemental composition values are expressed on an anhydrous / ashless basis (d.a.f).

FIG. 2 shows the result of the analysis regarding the elemental composition. In FIG. 2, “KsW” represents drained water, “WC” represents weathered coal, “CP” represents Canadian grass charcoal, and “AS” represents black soil.

(2) Infrared absorption spectrum (FT-IR) analysis.

The measurement of the infrared absorption spectrum of fulvic acid was performed by JASCO FT / IR-3 spectrometer based on the known KBr tablet method. Tablets were prepared using 1 mg of the sample and 100 mg of KBr, and the absorption spectrum of 400 to 4000 cm^{-1} was measured.

FIG. 3 shows infrared absorption spectra (FT-IR) of fulvic acid (FA) extracted from different raw materials. In FIG. 3, “Ks” represents raw brine, “KsW” represents drained water, “WC” represents weathered coal, “CP” represents Canadian grass charcoal, and “AS” represents black soil.

As can be seen from FIG. 3, the absorption around 3400 cm^{-1} was derived from hydrogen-bonded OH groups and showed strong absorption in most fulvic acids, but the absorption of weathered coal FA (WC-FA) was relatively weak. Met. In the absorption around 2900 cm^{-1}, which seems to be caused by aliphatic carbon, there was no significant difference between fulvic acid of soil (AS), grass charcoal (CP), and weathered coal (WC), whereas irrigated FA (In the case of Ks-FA and KsW-FA), clear absorption is observed. Although two absorption around 1720 cm^{-1} and 1620 cm^{-1} are observed, the former mainly shows absorption by C = O stretching of carboxyl group is believed that slightly show the absorption due to a carbonyl group. In the latter case, the two structures of absorption derived from the hydrogen-bonded carbonyl group and the aromatic structure conjugate C = C are thought to contribute, respectively. Absorption near 1720 cm^{-1} showed slightly stronger absorption of weathered coal FA (WC-FA) than grass coal FA (CP-FA), and absorption of brackish water FA was remarkable. Absorption near 1620 cm^{-1} is relatively weak in most samples, which is consistent with the fact that fulvic acid has fewer quinone groups than humic acid. The absorption spectrum near 1400 cm^{-1} and the relatively broad spectrum in the 1300-1200 cm^{-1} region are thought to originate from the CO stretching vibration of the carboxyl group and the OH bending vibration. Absorption is observed in all fulvic acids, but the absorption strength of brackish water FA is relatively stronger than grass charcoal, soil and weathered coal FA. A small valley near 800 cm^{-1} is clearly seen in most samples. This is thought to be CH bending vibration on the aromatic ring. Thus, from the results of infrared absorption spectra, it is concluded that fulvic acid (FA) extracted from different raw materials is an aggregate of a series of compounds having the same basic chemical structure.

FIG. 4 shows hydrogen magnetic resonance (^{1}H-NMR) spectra of fulvic acid (FA) of different raw materials. Spectral parameters are 0-1.9ppm for aliphatic protons, 1.9-3.2ppm for hydrogen mainly leading to carboxylic carbon, 3.2-6.2ppm for hydrogen such as hydrocarbon carbon, 6.2-8.6ppm for aromatic carbon attributed hydrogen Respectively.

From the results of this measurement, since the fulvic acid derived from raw canned water (Ks-FA) and the fulvic acid derived from drained water (KsW-FA) have a remarkable peak attributable to aliphatic hydrogen appearing in the vicinity of 0-1.9 ppm. In contrast to a large amount of aliphatics, fulvic acid derived from weathered coal (WC-FA) has a relatively strong peak around 6.2-8.6 ppm indicating aromatic hydrogen, and a large amount of aromatics. (See Fig. 4).

In addition, in the case of fulvic acid derived from Canadian grass charcoal (CP-FA), a relatively large number of complex spectra were obtained as in the case of fulvic acid derived from Kuroboku soil (AS-FA). Therefore, fulvic acid derived from Canadian grass charcoal (CP-FA) is less than fulvic acid derived from discharged water (KsW-FA), but compared to fulvic acid derived from weathered coal (WC-FA). As a humic material containing a large amount of carbohydrate hydrogen, it was found to be a relatively young decomposition stage fulvic acid (see FIG. 4).

(3) ^{13}C nuclear magnetic resonance spectrum (^{13}C-NMR) analysis.

The ^{13}C nuclear magnetic resonance spectrum was measured with a BRUKER AMX-400 spectrometer using the known cross polarization / magic angle rotation (CP / MAS) method. The measurement frequency was 100.0 MHz for carbon. About 200 mg of the finely pulverized sample was placed in a dedicated cell, and accumulated at 10,000 times at a room temperature of 25 to 26 ° C. with a pulse repetition time of 5 seconds. The analysis results are shown in FIG.

FIG. 5 is a diagram showing ^{13}C-CP / MAS NMR spectra of humic acid (HA) and fulvic acid (FA) extracted from Canadian grass coal and weathered coal, respectively. In FIG. 5, in order to know the relative content of carbon species for each bond type, the range of δ0-230 of the spectrum is divided into seven regions of carbon type. That is, unsubstituted aliphatic C (alkane, fatty acid) (δ0-50), N-alkyl (amino acid, peptide, proteinaceous) + methoxy C (δ50-60), aliphatic CO (especially hydrocarbon) (δ60-110), aromatic C (δ110-150), phenolic C (δ150-160), carboxyl C (δ160-190), ketone C = O (δ190-230).

When calculating the percentage of the total area of the area enclosed by the boundary line and the base line of the spectrum curve and each of the above regions to obtain the relative carbon content, the molecules of humic acid (HA) and fulvic acid (FA) depend on the difference in raw materials. There are some differences in structure.

Canadian grass charcoal-derived humic acid (CP-HA) has more aliphatic C and less aromatic C than weathered coal-derived humic acid (WC-HA). Canadian grass charcoal-derived fulvo (CP-FA) has more aliphatic C—O and less phenolic C, carboxyl C and ketone C═O than weathered coal-derived fulvic acid (WC-FA). In addition, both humic acid and fulvic acid derived from Canadian grass charcoal have more alkyl + methoxy groups than humic acid and fulvic acid derived from weathered coal. This is thought to be due to the difference between the humus process of grass and the oxidation process of coal.

(4) Molecular weight analysis.

The molecular weight was measured using high performance liquid chromatography (HPLC, Waters 600, Millipore Co.). The column used was TOHSOH TSKgel G 2000 SW_{XL} + G3000 SW_{XL} for gel permeation chromatography, and 0.05 mol L^{-1} phosphate buffer (pH 7) +0.05 mol L^{-1} sodium chloride was used as the eluent. 10 μL of the sample dissolved in the eluent (0.1 mg mL^{-1}) was injected, and the absorbance of the fraction was measured using Waters 490E (Millipore Co.) at wavelengths of 280 nm and 400 nm. The measurement results are shown in FIG.

This FIG. 6 is a drawing substitute table summarizing the physical property evaluation analysis results for the extracted fulvic acid from raw materials of raw brine, waste brine, weathered coal, Canadian grass coal, and black soil. As shown in FIG. 6, the molecular weights of the extracted fulvic acids from the raw brine, wastewater, weathered coal, Canadian grass charcoal, and black clay were $_{740}$, $_{810}$, $_{920}$, $_{780}$, and $_{680}$, respectively.

Based on the molecular weight, elemental composition, and hydrogen and carbon assignment results for each fulvic acid of different raw materials, the average chemical structural formula was determined assuming that fulvic acid was one molecule. Since fulvic acid is a mixture and it is difficult to measure the absolute molecular weight, the molecular weight of the same fulvic acid varies depending on the measurement method, so that there is no accurate chemical structure at present. Therefore, the estimated average chemical structure (Average molecular formura) in FIG. 6 is useful for distinguishing fulvic acids with different raw materials.

(5) Carboxyl group content analysis.

The quantification of the carboxyl group of fulvic acid was performed based on the known calcium acetate method (Blom et al., 1957). As a result, the carboxyl group content of grass charcoal fulvic acid was 3.98 mmol / g.

(6) Electron spin resonance (ESR) spectrum analysis.

Fulvic acid powder was taken in a quartz tube having a diameter of 0.5 mm, and the concentration of free radicals contained in fulvic acid was measured using an electron spin resonance (ESR) apparatus (BRUKER, ESR 300E). Determination was carried out using Mn2 + standard markers, and spin concentration was measured using DPPH (1.1-Diphenil-2picrylhydrazyl, molecular weight 394, ESR 3.1×10^{20} spins / g). As a result, the free radical content of grass charcoal fulvic acid was 7.27×10^{15} spins / g.

<Verification experiment of antiallergic effect of fulvic acid>. A bioassay was conducted to elucidate the existence of anti-allergic effect of fulvic acid and its specific mechanism of action. First, characteristics of fulvic acid derived from Canadian peat charcoal used in this bioassay will be described.

First, FIG. 7 is a drawing substitute table showing the characteristics of Canadian grass charcoal fulvic acid (CP-FA) used in the following bioassay.

The elemental composition (daf%) of Canadian grass charcoal fulvic acid (CP-FA) is presented in FIG. 47.8 for carbon (C), 4.6 for hydrogen (H), 0.3 for nitrogen (N), 47.3 for oxygen (O), 0.75 mmolg $^{-1}$ for phenolic hydroxyl group (Ph-OH), 3.98 mmolg for carboxyl group (COOH) $^{-1}$.

F_{a1} and f_{a2} shown in FIG. 7 are indices representing the degree of aromatization of humic substances. f_{a1} is determined from the ^{13} C-NMR spectrum by the ratio of aromatic C to all carbons, and f_{a2} is determined by the ratio of aromatic C to carbons other than carboxyl C. Higher values of f_{a1} and f_{a2} mean that humification has progressed. As shown in FIG. 7, fulvic acid derived from Canadian grass charcoal (CP-FA) is a component in which the humification of the grass charcoal has progressed.

FIG. 8 is a graph (graph) showing ^{13} C-CP / MAS NMR spectra of humic acid (HA) and fulvic acid (FA) of Canadian grass charcoal.

From FIG. 8, it can be seen that the humic acid (HA) and fulvic acid (FA) are decomposed into a larger number of fractions compared to the raw material, Canadian peat (Peat). This is believed to be due to the decomposition action of hydrogen chloride and sodium hydroxide in the extraction stage.

In addition, fulvic acid (FA), which is a water-soluble component of grass charcoal, has more alkyl and methoxy groups, and has higher aromaticity and carbohydrate content than humic acid (HA), which is an alkali-soluble acid-insoluble component. Can be read from the spectrum. Hereinafter, the bioassay procedure for Example 3 will be described.

(1) Fulvic acid sample preparation.

It was dissolved in distilled water so that the concentration of the extracted fulvic acid was 1 mg mL $^{-1}$ and sterilized with a 0.22 μm filter (Millipore, Japan).

(2) Cells and cell culture.

Rat mast cells RBL2H3 cells were used. RBL2H3 cells were cultured in Minimum Essential Medium Eagle (MEM, Nissui) containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 60 mg L- ^{1} kanamycin.

(3) β-hexosaminidase release measurement.

In type I allergic reactions that occur in mast cells, it is known that antigen-antibody binding directly causes intracellular granule efflux such as histamine and b-hexosaminidase. For this reason, the effect of fulvic acid (CP-FA) on the binding of antigen and antibody was confirmed by measuring the release inhibition rate of β-hexosaminidase.

First, RBL2H3 cells pre-cultured in the above-mentioned MEM medium were prepared to 5.0×10^{5} cells mL $^{-1}$, and 1 mg mL $^{-1}$ mouse anti-DNP IgE antibody (Sigma Co.) was finally added thereto. It was added to a concentration of 0.3 μg mL $^{-1}$ and well suspended.

This was seeded on a 96-well plate (Falcon Co.) at 100 μL well $^{-1}$ and placed in an incubator at 37 ° C. and 5% CO_2 concentration overnight for cell sensitization. The next day, the cells were washed twice with 0.02% EDTA · 2Na / PBS (−) 100 μL well $^{-1}$ and then released with a Releasing Mixture (116.9 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$ .7H_2O, 5.6 mM Glucose, 25 mM) HEPES, 2.0 mM $CaCl_2$, 1 mg mg $^{-1}$ BSA, pH 7.7) was added at 60 μL well $^{-1}$, and the sample (adjusted to FA 0.01, 0.1, 1.0, 10.0 μg mL $^{-1}$) was exposed to 5 μL well $^{-1}$. And left in a CO_2 incubator for 10 minutes. At this time, 6.3 mM ketotifen (Sigma, Co.) was added instead of the sample as a positive control, and sterilized water was added as a negative control. After 10 minutes, 5 μL well $^{-1}$ of antigen 4 μL mL $^{-1}$ DNP-BSA (Cosmo Bio) was added, and placed in a 37 ° C., 5.0% CO_2 incubator for 1 hour. After stopping the reaction by cooling with ice for 10 minutes, 20 μL of the supernatant was transferred to a new 96-well plate, and the substrate solution (5 mM 4-Nitrophenyl N-acetyl-β-D-glucosaminide dissolved in 50 mM citrate buffer) was added to it. 80 μL well $^{-1}$ was added and reacted at 37 ° C. in a 5.0% CO_2 incubator for 30 minutes.

Stop the reaction by adding 200 μL well $^{-1}$ reaction stop solution (0.1M $NaHO_3$, pH 10.0) to this reaction solution, and then measure the absorbance at 405 nm with a microplate reader (Powerscan HT Dainippon Pharmaceutical Co., Ltd.) It was.

FIG. 9 is a diagram (graph) showing the results of the measurement and examining the effect of fulvic acid (CP-FA) on the β-hexosaminidase release inhibition rate.

The vertical axis of FIG. 9 represents the β-hexosaminidase release inhibition rate (%), and the horizontal axis represents the concentration of fulvic acid (CP-FA) (μg mL $^{-1}$). “Keto” in FIG. 9 indicates ketotifen (control group) that is used as a clinical drug for comparison.

As shown in FIG. 9, fulvic acid (CP-FA) showed a high β-hexosaminidase release inhibitory effect. In particular, when the addition amount was 10 μg mL $^{-1}$, the antiallergic effect was almost double that of ketotifen, and even when the addition concentration was higher or lower, the antiallergic effect was equal to or higher than that of ketotifen.

Therefore, fulvic acid (CP-FA) has high concentration dependency in β-hexosaminidase release inhibition, and was confirmed to be effective as an antiallergic agent that suppresses the antigen sensitization stage of type I allergic reaction. .

(4) Measurement of binding inhibition between receptor and antibody.

At the antibody sensitization stage, fulvic acid (CP-FA) was added to examine the release suppression effect of intracellular granules by inhibiting the binding between the receptor on the cell membrane and the antibody.

RBL2H3 cells pre-cultured in MEM medium were prepared to 1.0 × 10^{6} cells mL^{-1} and seeded at 50 μL $well^{-1}$ each. Samples (FA 0.01, 0.1, 1.0, 10.0 μg mL^{-1}) were exposed to 5 μL $well^{-1}$, and cell sensitization was performed in an incubator at 37 ° C. and 5% CO_2 concentration for 1 hour. Thereafter, 1 mg mL^{-1} mouse anti-DNP IgE antibody (Sigma, Co.) was added to a final concentration of 0.6 μg mL^{-1} and well suspended.

The next day, the cells were washed twice with 0.02% EDTA · 2Na / PBS (−) 100 μL $well^{-1}$ and then released with a Releasing Mixture (116.9 mM NaCl, 5.4 mM KCl, 0.8 mM $MgSO_4$ .7H_2O, 5.6 mM Glucose, 25 mM) HEPES, 2.0 mM $CaCl_2$, 1 mg mL^{-1} BSA, pH 7.7) was added at 60 μL $well^{-1}$, and left in a 37 ° C., 5.0% CO_2 incubator for 10 minutes. Antigen 4 μL mL^{-1} DNP-BSA 5 μL $well^{-1\ was}$ added and placed in a 37 ° C., 5.0% CO_2 incubator for 1 hour. After stopping the reaction by cooling with ice for 10 minutes, 20 μL of the supernatant was transferred to a new 96-well plate, and the substrate solution (5 mM 4-Nitrophenyl N-acetyl-β-D-glucosaminide dissolved in 50 mM citrate buffer) was added to it. 80 μL $well^{-1\ was}$ added, and reacted in a 37 ° C., 5.0% CO_2 incubator for 30 minutes.

Stop the reaction by adding 200 μL well-1 reaction stop solution (0.1M $NaHO_3$, pH 10.0) to this reaction solution, and then measure the absorbance at 405 nm with a microplate reader (Powerscan HT Dainippon Pharmaceutical Co., Ltd.). went.

FIG. 10 is a diagram (graph) showing the results of the absorbance measurement. As shown in FIG. 10, the release of β-hexosaminidase from mast cells was observed in fulvic acid (CP-FA), but the level was lower than that of ketotifen as a control group. No dependence was observed. From this, it was clarified that fulvic acid (CP-FA) can be expected to have some antiallergic effect even in the antibody sensitization stage of type I allergic reaction.

(5) Measurement of intracellular calcium ion (Ca^{2+}) concentration.

The intracellular Ca^{2+} concentration was measured using Calcium Kit-Fluo 3. Prepare RBL2H3 cells pre-cultured in MEM medium to 1.5 × 10^{5} cellsmL $^{-1,}$ and add ^{1} mg mL^{-1} mouse anti-DNP IgE antibody (Sigma, Co.) to a final concentration of 0.3 μL mL I added $^{-1}$ and suspended well. Cell sensitization was performed overnight at 37 ° C. in a 5% CO_2 incubator.

The next day, the cells were washed twice with PBS (−) 200 μL $well^{-1}$, 100 μL of loading buffer was added, and after placing in a 37 ° C., 5% CO_2 incubator for 1 hour, the cells were washed with PBS (−) 200 μL well $^{-}$· Washed twice with ^{1} to remove residual Fluo3-AM.

Recording medium was added to 100 μL $well^{-1}$ and samples (FA 0.01, 0.01, 0.1, 1.0, 10.0 μg mL^{-1}) were exposed to 8.3 μL $well^{-1}$ and placed in a 37 ° C., 5.0% CO_2 incubator. Left for 1 hour. At this time, 6.3 mM ketotifen (Sigma, Co.) was used instead of the sample as a positive control, and sterilized water was used as a negative control. After 1 hour, fluorescence at wavelengths of 485 nm and 530 nm was measured with a microplate reader (Powerscan HT Dainippon Pharmaceutical Co., Ltd.) while adding 4 μL mL^{-1} DNP-BSA (Cosmo Bio) of antigen 67 μL $well^{-1}$.

FIG. 11 is a diagram (graph group) showing the results of examining the influence of fulvic acid on antigen inhibition of Ca^{2+} influx. The vertical axis of FIG. 11 shows the intracellular inflow rate of Ca^{2+} by antigen stimulation with respect to the intracellular Ca^{2+} concentration of 1.0 of control (sterilized water addition), and the horizontal axis shows the time (seconds) elapsed from the antigen stimulation. ing.

It became clear that both fulvic acid additions inhibited Ca^{2+} influx induced by antigen stimulation. Furthermore, its activity is Ca^{2+} influx inhibitors ketotifen (control group) and are those equivalent, to work its Ca^{2+} influx inhibitory effect immediately antigen stimulation was confirmed. Concentration dependence was not recognized in the Ca^{2+} inflow inhibition effect by the antigen stimulation of fulvic acid.

From the results of the above examples, it was found that fulvic acid has a high antiallergic effect. FIG. 12 is a diagram for schematically explaining the relationship between the occurrence mechanism of type I allergy of fulvic acid.

Fulvic acid acts in three stages in the pathogenesis of type I allergy. First, the antigen sensitization stage indicated by the arrow X in FIG. 12, the second the antibody sensitization stage indicated by the arrow Y in FIG. 12, and the third, There is a degranulation stage (stage shown by arrow Z in FIG. 12). Fulvic acid suppresses the onset of type I allergy by inhibiting or blocking these stages.

The present invention is useful for preventing or preventing the onset of type I allergy. For example, orally administered drugs for the prevention and prevention of the onset of type I allergy, injection solutions, drugs for external use of skin, patches, etc., quasi-drugs, or functions related to the prevention and prevention of type I allergy Cosmetics such as bath preparations, facial cleansers, creams and packs, skin care cosmetics, beverages such as soft drinks, instant foods, sweets such as candy and gum, breads, noodles, baby food, food and drinks such as seasonings Widely available. In addition, fulvic acid is approved as a food additive component by the Ministry of Health, Labor and Welfare of Japan (Shujoan No. 0601001).

It is a flowchart of the extraction method of the fulvic acid which international humic substance society (IHSS) established. It is a drawing substitute table | surface which shows the analysis result regarding the elemental composition of a fulvic acid. It is a figure (graph) which shows the FT-IR spectrum of the fulvic acid (FA) from which a raw material differs. It is a graph showing the hydrogen magnetic resonance (^{1} H-NMR) spectrum of the material different fulvic acid (FA) (graph). It is a figure which shows the ^{13} C-CP / MAS NMR spectrum of humic acid (HA) extracted from each of Canadian grass coal and weathered coal, and fulvic acid (FA). It is a drawing substitute table summarizing physical property evaluation analysis results for fulvic acid extracted from raw materials of raw brine, waste water, weathered coal, Canadian grass charcoal and black soil. 6 is a drawing substitute table showing the characteristics of Canadian pulverized carbon fulvic acid (CP-FA) used in the bioassay according to Example 3. FIG. It is a figure (graph) which shows the ^{13} C-CP / MAS NMR spectrum of humic acid (HA) and fulvic acid (FA) of Canadian grass charcoal. It is a figure (graph) which shows the result of having investigated the influence which the fulvic acid (CP-FA) has on the β-hexosaminidase release inhibition rate. It is the figure (graph) which showed the result of the binding inhibition measurement of a receptor and an antibody. It is a figure (graph group) which shows the result of having investigated the influence which the antigen stimulation of fulvic acid gives to $Ca2^{+}$ inflow inhibition. It is a figure for demonstrating typically the relationship with the generation | occurrence | production mechanism of the type I allergy of a fulvic acid.

Patent Citations (11)

Publication number	Priority date	Publication date	Assignee	Title
JPH1017483A *	1996-06-26	1998-01-20	Keinzu Corp:Kk	Preventing and therapeutic agent for dermatopathy
JP2000136140A *	1998-10-29	2000-05-16	Ra Purata Koeki Kk	Aqueous solution containing substance extracted from humic soil

CN1568929A *	2003-07-16	2005-01-26	刘南凯	Cosmetics containing humic acid and application of humic acid in cosmetics
JP2006059450A *	2004-08-20	2006-03-02	Fujitsu Ltd	Wiring structure for optical pickup
JP2006087393A *	2004-09-27	2006-04-06	Global Science:Kk	Health food and medicine
Family To Family Citations				
JPH0454126A *	1990-06-21	1992-02-21	Mikio Mori	Treating liquid and sanitary product containing the same treating liquid
WO1992016216A1 *	1991-03-16	1992-10-01	Torf Establishment	Peat-derived bioactive products and pharmaceutical and cosmetic compositions containing them
PL167664B1 *	1991-03-16	1995-10-31	Torf Ets	Peat extraction method and apparatus
DE4226553A1 *	1992-08-11	1994-02-17	Teves Gmbh Alfred	Brush holders for electric motors
EP1700599B1 *	1998-10-08	2008-10-08	Pfeinsmith Limited	Fulvic acid and its use in the treatment of candida infections
JP2006089450A *	2004-09-22	2006-04-06	Crescendo Corporation	Fulvic acid

* Cited by examiner, † Cited by third party

Cited By (6)

Publication number	Priority date	Publication date	Assignee	Title
KR101532939B1 *	2013-09-13	2015-07-02	주식회사 코리아나화장품	Cosmetic Composition for the Skin Whitening Comprising the Fulvic acid
JP5970112B1 *	2015-06-23	2016-08-17	譲 平野	Noodles containing dietary fiber and method for producing the same
JP2017160447A *	2012-03-30	2017-09-14	エルデーイノバショーン アンパルトセルスカブ R D I n n o v a t i o n A p S	Benzene polycarboxylic acid compound and use thereof as agent
KR101810300B1	2015-06-23	2017-12-18	유즈루 히라노	Dietary-fiber containing noodles and soup stock and manufacturing methods for same
Family To Family Citations				
JP5970118B1 *	2015-08-18	2016-08-17	譲 平野	Production containing dietary fiber and method for producing the same
WO2017106859A1	2015-12-18	2017-06-22	University Of Notre Dame Du Lac	Covalent heterobivalent antibody inhibitors and ligands

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

Similar Documents

Publication	Publication Date	Title
Vasudevan et al.	2007	Antinociceptive and anti-inflammatory effects of Thespesia populnea bark extract
Rotelli et al.	2003	Comparative study of flavonoids in experimental models of inflammation
Suresh et al.	2012	Effect of ethanol extract of Trigonella foenum graecum (Fenugreek) seeds on Freund's adjuvant-induced arthritis in albino rats
JP2006232785A	2006-09-07	Type i allergy inhibitor using fulvic acid and method for inhibiting onset of type i allergy
KR20160119703A	2016-10-14	Composition for diagnosing damages of skin cells by microdust and composition comprising galangin as an effective ingredient
Singh et al.	2013	Phlorotannins
Gu et al.	2013	4-methoxycarbonyl curcumin: a unique inhibitor of both inflammatory mediators and periodontal inflammation
KR101627001B1	2016-06-03	Cognitive disorder-ameliorating agent
JP2002526407A	2002-08-20	Fulvic acid and its use in treating various conditions
Yin et al.	2018	Inhibitory activity of allergic contact dermatitis and atopic dermatitis-like skin in BALB/c mouse through oral administration of fermented barks of Alnus sibirica
Mosquera et al.	2020	Antiurolithiatic activity of Boldoa purpurascens aqueous extract: An in vitro and in vivo study
TW200831106A	2008-08-01	Antiinflammatory agent comprising 2-aminophenol or its derivatives as an effective ingredient(s)
CN102481327A	2012-05-30	The preparation and uses of a longan seed extract
JP6440631B2	2018-12-19	Algal extracts containing sulfated and non-sulfated polyanionic polysaccharides and uses thereof
Reis et al.	2019	Modulation of COX-2, INF-γ, glutamatergic and opioid systems contributes to antinociceptive, anti-inflammatory and anti-hyperalgesic effects of bis (3-amino-2-pyridine) diselenide

AU2010202232A1	2010-07-08	Use of Yessotoxins in the Treatment of Allergic and Asthmatic Processes
FR2848851A1	2004-06-25	Skin soothing active agent for cosmetic use, effective e.g. in reducing release of interleukin-1 alpha or prostaglandin E2, obtained by enzymatic hydrolysis of peppermint powder in aqueous solution
JP2008013553A	2008-01-24	Cosmetic composition containing limnocitrus littoralis extract
WO2003059366A1	2003-07-24	Skin perparation for external use containing purpuricenus temminckii frass as the active ingredient
Chanaj-Kaczmarek et al.	2021	Development and Evaluation of Thermosensitive Hydrogels with Binary Mixture of Scutellariae baicalensis radix Extract and Chitosan for Periodontal Diseases Treatment
Hyperuricemia	2016	Kinetics of inhibition of xanthine oxidase by globularia alypum and its protective effect against oxonate-induced hyperuricemia and renal dysfunction in mice
CN107801402B	2022-03-11	Composition for diagnosing skin damage caused by mote and composition comprising galangin as effective ingredient
Assiry et al.	2022	Evaluation of In Vitro Antiprotease Activity of Selected Traditional Medicinal Herbs in Dentistry and Its In Silico PASS Prediction
Janardhanan et al.	2009	Anti-inflammatory and Free Radical Scavenging Activities of Polysaccharide– Protein Complex Isolated from Phellinus rimosus (Berk.) Pilát (Aphyllophoromycetideae)
Shahrestani et al.	2021	Behavioral and molecular analysis of antioxidative potential of rosmarinic acid against methamphetamine-induced augmentation of Casp3a mRNA in the zebrafish brain

Priority And Related Applications

Priority Applications (3)

Application	Priority date	Filing date	Title
JP2005053656A	2005-02-28	2005-02-28	Type i allergy inhibitor using fulvic acid and method for inhibiting onset of type i allergy
US11/885,217	2005-02-28	2006-01-05	Type I Allergy Inhibitor and Methods of Inhibiting the Onset of Type I Allergy Using Fulvic Acid
PCT/JP2006/300026	2005-02-28	2006-01-05	Type i allergy inhibitor and methods of inhibiting the onset of type i allergy using fulvic acid

Applications Claiming Priority (1)

Application	Filing date	Title
JP2005053656A	2005-02-28	Type i allergy inhibitor using fulvic acid and method for inhibiting onset of type i allergy

Legal Events

Date	Code	Title	Description
2006-02-01	A521	Written amendment	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20060104
2007-12-12	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621 **Effective date**: 20071211
2007-12-17	A621	Written request for application examination	**Free format text**: JAPANESE INTERMEDIATE CODE: A621 **Effective date**: 20071211
2011-04-13	A131	Notification of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A131 **Effective date**: 20110412
2011-06-10	A521	Written amendment	**Free format text**: JAPANESE INTERMEDIATE CODE: A523 **Effective date**: 20110609
2011-06-28	A521	Written amendment	**Free format text**: JAPANESE INTERMEDIATE CODE: A821 **Effective date**: 20110609
2012-03-14	A131	Notification of reasons for refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A131 **Effective date**: 20120313
2012-07-11	A02	Decision of refusal	**Free format text**: JAPANESE INTERMEDIATE CODE: A02 **Effective date**: 20120710

Concepts

machine-extracted

Download Filter table

Name	Image	Sections	Count	Query match
fulvic acid		title,claims,abstract,description	154	0.000
3,7,8-trihydroxy-3-methyl-10-oxo-1,4-dihydropyrano[4,3-b]chromene-9-carboxylic acid		title,claims,abstract,description	150	0.000
fulvic acid		title,claims,abstract,description	150	0.000
Hypersensitivity		title,claims,abstract,description	51	0.000
allergic hypersensitivity disease		title,claims,abstract,description	46	0.000
inhibitory effect		title,claims,abstract,description	38	0.000
inhibitor		title,claims,abstract,description	10	0.000
Sensitisation		claims,abstract,description	27	0.000
sensitization		claims,abstract,description	27	0.000
sensitizing		claims,abstract,description	27	0.000
mixture		claims,abstract,description	24	0.000
antigen		claims,abstract,description	23	0.000
antigens		claims,abstract,description	23	0.000
antigens		claims,abstract,description	23	0.000
antibodies		claims,abstract,description	19	0.000
antibodies		claims,abstract,description	19	0.000
desensitisation		claims,abstract,description	12	0.000
Cannabis sativa		claims,description	42	0.000
charcoal		claims,description	36	0.000
suppression		claims,description	21	0.000
Skin		claims,description	12	0.000
cosmetic		claims,description	8	0.000
food		claims,description	6	0.000
influx		claims,description	5	0.000
calcium cation		claims,description	4	0.000
calcium ion		claims,description	4	0.000
preparation method		claims,description	3	0.000
substance		abstract,description	20	0.000

Show all concepts from the description section