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EXTRACT OF THE FAMILY DIOSCOREACEAE AND COMPOSITION FOR PREVENTING OR TREATING PERIPHERAL NEUROPATHY COMPRISING THE SAME

EXTRAKT AUS DER FAMILIE DER DIOSCOREACEAE UND DIESEN ENTHALTENDE ZUSAMMENSETZUNG ZUR PRÄVENTION ODER BEHANDLUNG VON PERIPHERER NEUROPATHIE

EXTRAIT DE LA FAMILLE DIOSCOREACEAE ET COMPOSITION LE COMPRENANT POUR PREVENIR OU TRAITER LA NEUROPATHIE PERIPHERIQUE

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Proprietor: University-Industry Cooperation Group of Kyung Hee University Gyeonggi-do 17104 (KR)

Inventors:
- KIM, Sun-Yeou Seoul 137-030 (KR)
- KANG, Tong-Ho Seoul 121-854 (KR)
- PARK, Ji-Ho Seoul 133-758 (KR)

Representative: Turner, Craig Robert et al. A.A. Thornton & Co. 10 Old Bailey London EC4M 7NG (GB)

References cited:
- WO-A2-03/082893
- KR-A- 20000008 809
- KR-A- 20050 111 400

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• KANG T. ET AL.: "Diosgenin from Dioscorea nipponica Ameliorates Diabetic Neuropathy by Inducing Nerve Growth Factor", BIOL. PHARM. BULL., vol. 34, no. 9, September 2011 (2011-09), pages 1493-1498, XP000002658349,
• MA C. ET AL.: 'Neuroprotective and antioxidant activity of compounds from th aerial parts of Dioscorea opposita' J. NAT. PROD. vol. 68, no. 8, August 2005, pages 1259 - 1261, XP003004147


Remarks:
The file contains technical information submitted after the application was filed and not included in this specification
The present invention relates to an extract of the family Dioscoreaceae for treating the peripheral neuropathy; and a pharmaceutical composition or food composition comprising the extract or a compound isolated from the extract.

Neuropathy is a disease caused by structural or functional abnormalities of the nervous system. The nervous system is divided into the central nervous system which is distributed in the brain and the spinal cord and involved in controlling their functions, and the peripheral nervous system which is distributed in almost all organs excluding the brain and the spinal cord and involved in controlling their functions. The peripheral nervous system is subdivided into the motor nervous system, the sensory nervous system, the autonomic nervous system. A peripheral nerve, where neurites branch out beyond the brain and the spinal cord to the body, arms, and legs, transmits sensation felt at arms and legs to the central nerve (brain and spinal cord), and transmits orders of the central nerve to muscles.

Neuropathy can be induced by various causes, such as metabolic diseases (e.g., diabetes, renal failure, hypothyroidism), drugs (e.g., antitumor agents, antituberculosis drugs) or toxic substance intoxication (e.g., Pb, organic solvents), malnutrition (e.g., vitamin deficiency, alcoholism), connective tissue disorders (e.g., rheumatoid arthritis, systemic lupus erythematosus), inflammatory diseases (Guillain-Barre syndrome), or genetically determined neuropathy. In addition, the multiple neuropathy can be caused by cancers.

The multiple neuropathy can be induced by various causes, such as metabolic diseases (e.g., diabetes, renal failure, hypothyroidism), drugs (e.g., antitumor agents, antituberculosis drugs) or toxic substance intoxication (e.g., Pb, organic solvents), malnutrition (e.g., vitamin deficiency, alcoholism), connective tissue disorders (e.g., rheumatoid arthritis, systemic lupus erythematosus), inflammatory diseases (Guillain-Barre syndrome), or genetically determined neuropathy. In addition, the multiple neuropathy can be caused by cancers.

Until now, the neuropathy has been treated with drugs used as a symptomatic therapy that improves symptoms only and there are almost no fundamental remedies for the neuropathy. Only epalrestat, an aldose reductase inhibitor, was approved by US Food and Drugs Administration (FDA), with respect to diabetic peripheral neuropathy, one of the multiple neuropathies, but epalrestat is not used due to its low therapeutic effects (Foster DW., Harrison’s Principles of Internal Medicine 13, p1979, 1999; Stephen LD, Applied Therapeutics: the clinical use of drugs. 6, p48.1-48.62, 1996).

Meanwhile, a protein that affects the growth, differentiation, and survival of neurons in the central nervous system (CNS) and the peripheral nervous system (PNS) is called collectively as a neurotrophic factor (NF), which is one of neuron control factors that regulate the growth, differentiation, and death of neurons. Examples of the NF include a brain-induced neurotrophic factor (BDNF), neurotrophin-3 (NT-3), NT-4, and NT-5. These NFs are synthesized at different areas, and have different differentiation, different expression, and different target regions.
Disclosure

Technical Problem

[0011] The present invention provides herbal extracts and compounds isolated therefrom useful for treating the peripheral neuropathy induced by various causes.

[0012] That is, the present invention provides an extract of the family Dioscoreaceae for treating the peripheral neuropathy.

[0013] The present invention also provides a pharmaceutical composition or food

[0014] According to an aspect of the present invention, there is provided an extract of a family Dioscoreaceae for use in treating the peripheral neuropathy, the family Dioscoreaceae being at least one selected from the group consisting of Dioscorea nipponica, Dioscorea septemloba, Dioscorea quinquelobata, Dioscorea batatas, Dioscorea japonica, Dioscorea bulbifera, Dioscorea tokoro, and Dioscorea tenuipes wherein the extract is obtained by performing an extraction process which comprises extracting a root of the family Dioscoreaceae with a first extraction solvent selected from the group consisting of water, C_1-C_4 alcohol, and a mixture of water and a C_1-C_4 alcohol, and wherein the extract contains the compound of Formula 1

[Formula 1]

wherein R is a hydrogen atom, a C_1-C_4 alkyl group, or a saccharide.

[0015] According to an aspect of the present invention, there is provided a health functional food product for use in treating the peripheral neuropathy comprising an extract according to any one of claims 1 through 8.

[0016] Disclosed herein is a pharmaceutical composition for preventing or treating the peripheral neuropathy, which comprises a therapeutically effective amount of the extract; and a pharmaceutically acceptable carrier.

[0017] Disclosed herein is a food composition for preventing or treating the peripheral neuropathy, which comprises the extract as an active ingredient.

[0018] Disclosed herein is a pharmaceutical composition for preventing or treating the peripheral neuropathy, which comprises a therapeutically effective amount of a compound represented by Formula 1 or salt thereof; and a pharmaceutically acceptable carrier.

[Formula 1]
wherein R is a hydrogen atom, a C_1-C_4 alkyl group, or a saccharide.

[0019] Disclosed herein is a food composition for preventing or treating the peripheral neuropathy, which comprises the compound represented by Formula 1 or salt thereof as an active ingredient.

Advantageous Effects

[0020] An extract of the family Dioscoreaceae and/or a compound isolated from the extract induces an endogenous nerve growth factor in an organism so that it can be used in a wide range of applications for treating peripheral neuropathy.

Description of Drawings

[0021] FIG. 1 and FIG. 2 shows the effect of the compound isolated from an extract according to an embodiment of the present invention on neurite outgrowth.

FIG. 3 is a graph illustrating a change in the level of a nerve growth factor (NGF) in normal mouse serum resulting from administration of a compound isolated from an extract according to an embodiment of the present invention and a compound isolated from the extract.

FIG. 5 and FIG. 6 illustrates the effect of a compound isolated from an extract according to an embodiment of the present invention on the transmission speed of the motor nerve and sensory nerve in a sciatic nerve of diabetes-induced mice (FIG. 5: DG treatment for one-month, FIG. 6: DG treatment for two-month).

FIG. 7 is a graph illustrating a change in the level of a NGF in the sciatic nerve of a diabetes-induced mouse resulting from administration of a compound isolated from an extract according to an embodiment of the present invention.

FIG. 8 is a graph illustrating a change in the level of sorbitol in the sciatic nerve of a diabetes-induced mouse resulting from administration of an extract according to an embodiment of the present invention and a compound isolated from the extract.

BEST MODE

[0022] In the present specification, the term "peripheral neuropathy" refers to a condition of peripheral nerves (motor nerves, sensory nerves, and autonomic nerves) injured by various causes. The peripheral neuropathy may be subdivided into the mono-neuropathy and the poly-neuropathy (also called as multiple neuropathy). The multiple neuropathy includes any neuropathy caused by metabolic diseases (e.g., diabetes, renal failure, hypothyroidism), drugs (e.g., antitumor agents, antibacterial drugs) or toxic substance intoxication (e.g., Pb, organic solvents), malnutrition (e.g., vitamin deficiency, alcoholism), connective tissue disorders (e.g., rheumatoid arthritis, systemic lupus erythematosus), inflammatory diseases (Guillain- Barre syndrome), or genetically determined neuropathy. In addition, the multiple neuropathy may include a neuropathy caused by genetic factors and cancers.

[0023] An extract of the family Dioscoreaceae according to an embodiment of the present invention or a compound isolated from the extract derives neurite outgrowth and increases the amount of an endogenous nerve growth factor secreted, so that nerves of the peripheral nervous system can be effectively differentiated, protected, and reinnervated. In particular, the extract and/or the compound allow oral administration, which improve patients' medication compliance.

[0024] The present invention provides an extract of the family Dioscoreaceae for treating the peripheral neuropathy.

[0025] The family Dioscoreaceae is at least one selected from Dioscorea nipponica, Dioscorea septemloba, Dioscorea quinqueoloba, Dioscorea batatas, Dioscorea japonica, Dioscorea bulbifera, Dioscorea tokoro, and Dioscorea tenuipes. Preferably, the family Dioscoreaceae is Dioscorea nipponica, Dioscorea quinqueoloba, and/or Dioscorea tokoro. More preferably, the family Dioscoreaceae is Dioscorea nipponica.

[0026] The extract according to the present invention can be obtained through an extraction process which includes extracting a whole part, root, or aerial part (for example, leave or stem) of the family Dioscoreaceae with an extraction solvent (first extraction solvent) selected from the group consisting of water, C_1-C_4 alcohol, and a mixture of water and a C_1-C_4 alcohol. For example, the extract of the family Dioscoreaceae can be obtained by extracting the root of the family Dioscoreaceae with the first extraction solvent. The first extraction solvent may be a mixture solvent of water and methanol or a mixture solvent of water and ethanol.

[0027] In the extraction process, the whole part, root, or aerial part, preferably, the root of the family Dioscoreaceae is cut into small sections, and then extracted with the first extraction solvent. At this time, the amount of the first extraction
solvent may be 1 to 20 times, preferably about 3 to 10 times, greater than that of the family Dioscoreaceae. The first extract solvent may be a mixture of water and methanol (for example, about 85% methanol solution) or a mixture of water and ethanol (for example, about 85% ethanol solution). The extraction is not affected by temperature, and can be performed at various temperature ranges, such as a temperature of 15°C to 100°C. The extraction can be performed by cold extraction, hot extraction, superfluid extraction, centrifugal extraction, ultrasonic extraction, or reflux cooling extraction. The extraction time may vary according to the extraction method. For example, the extraction can be performed once or multiple times for about 1 hour to 10 days. Preferably, the extraction can be performed twice or three times at room temperature for about 2 days using the first extraction. The extract obtained by extraction with the first extraction solvent can be a liquid form in which impurities in the extract are removed using a conventional method, e.g., filtration, or a powder form obtained by concentrating under reduced pressure or drying the liquid extract using a conventional method.

[0028] In addition, when needed, the extraction process may further include obtaining a fraction having higher contents of active ingredients. That is, the extraction process further includes: dispersing the extract obtained by the extraction with the first extraction solvent in water; and extracting the resultant solution with water saturated C1-C4 alcohol (second extraction solvent), thereby increasing the contents of active ingredients in the obtained extract.

[0029] When the extract obtained by extracting with the first extraction solvent is dispersed in water, a liquid form per se obtained by the extraction with the first extraction solvent may be dispersed in water, or a powder form obtained by concentrating the liquid extract under reduced pressure and/or drying the liquid extract using a conventional method may be dispersed in water.

[0030] The water saturated C1-C4 alcohol (second extraction solvent) may be water saturated butanol.

[0031] The present invention includes, within its scope, a composition comprising a compound isolated from the extract, i.e., a steroidal saponin or steroidal sapogenin. That is, the present invention includes a pharmaceutical composition for treating the peripheral neuropathy, which comprises a therapeutically effective amount of a compound represented by Formula 1 or salt thereof; and a pharmaceutically acceptable carrier:

\[
\text{Formula 1}
\]

wherein R is a hydrogen atom, a C1-C4 alkyl group, or a saccharide.

[0032] In the compound of Formula 1, R may be hydrogen or methyl, preferably hydrogen. That is, the compound of Formula 1 can be 3-β, 25R-spirost-5-en-3-ol.

[0033] The saccharide can be monosaccharide, disaccharide, or polysaccharide, such as glucose, fructose, mannose, galactose, ribose, cellulose, glycogen, sucrose, maltose, and lactose.

[0034] The salt of the compound of Formula 1 can be a conventional inorganic acid and/or organic acid addition salt prepared from steroidal saponin or sapogenin compounds. Examples of the salt of the compound of Formula 1 include salts disclosed in International Laid-open Patent Publication No. WO2003/082893. These salts can be prepared in situ during final separating and purifying processes of a compound. In particular, an acid addition salt can be prepared by reacting a refined compound in a free base form with a suitable organic or inorganic acid and then separating the produced salt (see S. M. Berge, et al., Pharmaceutical Salts, J. Pharm. Sci., 66: p.1-19(1977)). International Laid-open Patent Publication No. WO2003/082893 and the journal of M. Berge, et al. are used as a reference in the present invention. A base addition salt can be prepared by reacting a refined compound in an acid form with a suitable organic or inorganic base and separating the produced salt. The base addition salt can be a pharmaceutically acceptable metal or amine salt. The acid addition salt can be a salt prepared from an acid selected from hydrochloric acid, sulfuric acid, phosphoric acid, and nitric acid. The base addition salt can be a salt prepared from a base selected from sodium hydroxide, potassium hydroxide, and ammonium hydroxide.

[0035] The compound of Formula 1 can be isolated from the extract according to an embodiment of the present invention, synthesized using a known method (see Herbert O. House, Modern Synthetic Reactions, The Benjamin
The present invention provides a pharmaceutical composition for treating the peripheral neuropathy, which comprises a therapeutically effective amount of an extract of the family Dioscoreaceae or a compound of Formula 1 or salt thereof; or a pharmaceutically acceptable carrier.

The pharmaceutical composition according to the present invention includes a pharmaceutically acceptable carrier, and can be formulated into oral dosage form, external dosage form, suppository, and sterile injection solution, such as powders, granules, tablets, capsules, suspensions, emulsions, syrups, or aerosols. The pharmaceutically acceptable carrier can be lactose, dextrose, sucrose, sorbitol, mannitol, xylitol, erythritol, maltitol, starch, acacia rubber, alginate, gelatin, calcium phosphate, calcium silicate, cellulose, methyl cellulose, microcrystalline cellulose, polyvinyl pyrrolidone, water, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate, or mineral oil. The pharmaceutical composition may further include a diluent or an excipient, such as filler, expander, binder, humectant, disintegrant, or surfactant. A solid oral formulation can be a tablet, a pill, a powder, a granule, or a capsule. Such solid formulations may include at least one excipient selected from, for example, starch, calcium carbonate, sucrose, lactose, and gelatin. In addition, such solid formulations may further include a lubricant, such as magnesium stearate or talc. A liquid oral formulation can be a suspension, a solution, an emulsion, or syrup. In addition, the liquid oral formulation may include a diluent, such as water, liquid paraffine; humectant; sweetening agent; odorant; or preservative. A parenteral formulation can be a sterile aqueous solution, a non-aqueous solution, a suspension, an emulsion, a lyophilized formulation, or a suppository. Non-aqueous solvents or suspending agents can be propylene glycol, polyethylene glycol, natural oil, such as olive oil, or injectable esters, such as ethylolate. Vehicles for suppository can be witepsol, macrogol, Tween 61, cacao butter, Laurin, or glycergelatine.

The pharmaceutical composition according to the present invention, a dose of the extract of the family Dioscoreaceae or the compound of Formula 1 may vary depending on patient's state or body weight, seriousness of disease, dosage forms, administration routes, and the period of administration, and can be appropriately determined by a person having ordinary skill in the art. For example, the extract of the family Dioscoreaceae or the compound of Formula 1 can be administered in an amount of 0.0001 to 1000 mg/kg, preferably 0.001 to 1000 mg/kg, per day. The administration can be completed once or through several times per day. In the pharmaceutical composition according to the present invention, the amount of the extract of the family Dioscoreaceae or the compound of Formula 1 may be in the range of 0.001 to 50 % by weight based on 100 % by weight of the pharmaceutical composition.

The pharmaceutical composition can be administered to mammals, such as rats, mouse, livestock, or human beings, through various routes, e.g., orally, rectally, intravenously, intramuscularly, subcutaneously, through intrauterine dura mater injection, or through intracerebroventricular injection.

The present invention includes, within its scope, a food composition for treating the peripheral neuropathy, which comprises an extract of the family Dioscoreaceae or the compound of Formula 1 as an active ingredient.

The food composition according to the present invention can be used as a health functional food. According to Article 6727 of Korean Health Functional Food law, the "health functional food" refers to a food which is produced and processed using a source or component that carries out good functions on the human body. The "function" refers to an intake purporting to attain good health effects, that is, a nutrient control with respect to the structure and function of the human body or a physiological operation.

The food composition according to the present invention can include a conventional food additive. The conformity of the "food additive" is determined, as long as there are no other regulations, in consideration with the standard and criteria of the corresponding item according to the general rule of the food additives codex and general tests approved by Korea Food & Drug Administration.

The items listed on the "food additives codex" include a chemically synthesized substance, such as ketone, glycine, potassium citrate, nicotinic acid, or cinnamic acid; natural additives, such as persimmon color, an extract of licorice, crystalline cellulose, caoliang color, or guar gum; or mixed formulation, such as L-sodium glutamate formulation, alkali additives for noodles, preservatives, or tar color formulation.

The food composition according to the present invention may include the extract of the family Dioscoreaceae.
or the compound of Formula 1 in an amount of 0.01 to 95 % by weight, preferably 1 to 80 % by weight, based on 100 % by weight of the food composition, in order to prevent and/or treat the peripheral neuropathy. In addition, in order to prevent and/or treat the peripheral neuropathy, the food composition can be produced and processed into tablets, capsules, powder, granule, liquid phase, or pills.

[0046] For example, in order to produce a health functional food in a tablet form, a mixture of the extract of the family Dioscoreaceae or the compound of Formula 1, an excipient, a binder, a disintegrant, and other additives can be granulated using a conventional method, and then compression molding process is preformed with a lubricant. Alternatively, the mixture can be directly subjected to the compression molding process. In addition, when needed, the health formulated food in a tablet form may include sweetening agents, and when needed, the health formulated food in a tablet form can be coated with coating materials.

[0047] Among health functional foods in a capsule form, a hard capsule formulation can be produced by filling a conventional hard capsule with a mixture of the extract of family Dioscoreaceae or the compound of Formula 1 and an additive, such as an excipient, or granules of the mixture, or coated granules of the mixture; and a soft capsule formulation can be produced by filling a capsule support of gelatin with a mixture of the extract of family Dioscoreaceae or the compound of Formula 1 and an additive, such as an excipient. When needed, the soft capsule formulation can include plasticizer, such as glycerin or sorbitol, a coloring agent, and a preservative.

[0048] A health functional food in a pill form can be produced by molding a mixture of the extract of family Dioscoreaceae or the compound of Formula 1, an excipient, a binder, and a disintegrant using a suitable method. When needed, the health functional food in a pill form can be coated with white sugar or other coating materials, or can be covered with starch, talc, or other materials.

[0049] A health functional food in a granule form can be produced by granulating a mixture of the extract of family Dioscoreaceae or the compound of Formula 1, an excipient, a binder, and a disintegrant using a suitable method. When needed, the health functional food in a granule form can include a flavoring agent and a sweetening agent.

[0050] The excipient, the binder, the disintegrant, the lubricant, the sweetening agent, and the flavoring agent used in the present invention can be defined as corresponding materials having the same or similar functions disclosed in references known in the art (The Korean pharmacopoeia review, Moonsungsa Publication Co., Korea Pharmaceutical University Association, Fifth edition, p33-48, 1989).

Mode for Invention

[0051] The present invention will be described in further detail with reference to the following examples. These examples are for illustrative purposes only and are not intended to limit the scope of the present invention.

Example 1: Preparation of Extract

[0052] Dioscorea nipponica was dried and the root thereof was cut into small sections. The 500 g of the sample was added to 10 of 85 % methanol solution and then extracted three times (each for 2 hours) at room temperature. Such an extraction process was repeated twice. The resultant supernatants were collected and concentrated under reduced pressure, thereby obtaining 74 g of a crude extract.

[0053] The 74 g of the crude extract was suspended in 1 of distilled water, 1 of water-saturated butanol was added thereto, and then, the generated organic layer was separated, which was repeated five times. The obtained organic layers were collected altogether and dried under reduced pressure. As a result, 17 g of the extract of Dioscorea nipponica was obtained.

Example 2: Preparation of Extract

[0054] Dioscorea quinqueoloba was dried and the root thereof was cut into small sections. The 500 g of the sample was added to 5 of 85 % ethanol solution and then extracted three times (each for 2 hours) at room temperature. Such an extraction process was repeated twice. The resultant supernatants were collected and concentrated under reduced pressure, thereby obtaining 90 g of a crude extract.

[0055] The 90 g of the crude extract was suspended in 1 of distilled water, 1 of water-saturated butanol was added thereto, and then, the generated organic layer was separated, which was repeated five times. The obtained organic layers were collected altogether and dried under reduced pressure. As a result, 26 g of the extract of Dioscorea quinqueoloba was obtained.

Example 3: Preparation of Extract

[0056] Dioscorea tokoro was dried and the root thereof was cut into small sections. The 300 g of the sample was
added to 3 ℓ of 85 % ethanol solution and then extracted three times (each for 2 hours) at room temperature. Such an extraction process was repeated twice. The resultant supernatants were collected and concentrated under reduced pressure, thereby obtaining 35 g of a crude extract.

The 35 g of the crude extract was suspended in 0.5 ℓ of distilled water, 0.5 ℓ of water-saturated butanol was added thereto, and then, the generated organic layer was separated, which was repeated five times. The obtained organic layers were collected altogether and dried under reduced pressure. As a result, 11 g of the extract of Dioscorea tokoro was obtained.

Example 4: Separation of Active Compound

1 g of the extract of Dioscorea nipponica obtained in Example 1 was hydrolyzed at 94 °C for four hours by adding 10 ml of 2.5N HCl 10 ml thereto. Then, the resultant hydrolysate was extracted with 10 ml of chloroform for 15 minutes. The chloroform layer was separated, filtered, and then concentrated under reduced pressure at a temperature of 30-35 °C. The obtained residue was recrystallized at 4 °C using 5 ml of 95 % ethanol solution. The recrystallized precipitate was filtered, washed with water, recrystallized at 4 °C using 3 ml of acetone, and then filtered to obtain about 100 mg of the precipitate. The precipitate was identified as 3beta, 25R-spirost-5-en-3-ol represented by Formula 2.

\[
\text{[Formula 2]}
\]

(1) Formula: C_{27}H_{42}O_{3}
(2) Molecular Weight: 414.62
(3) Melting Point: 204-207 °C
(4) [α]_{D}^{25°} = -129°
(5) NMR Data: refer to Table 1

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**Experimental Example 1: Measurement of Neurite Outgrowth**

In an incubator with conditions including 5% CO₂ and a temperature of 37°C, PC 12 cell (pheochromocytoma, ATCC Number: CRL-1721) was cultured in a RPMI 1640 medium supplemented with horse serum (10%, v/v), fetal bovine serum (5%, v/v), and 1% penicillin-streptomycin.

In order to find the effect of the compound of Formula 2 on the neurite outgrowth, the mediums supplemented with 2% horse serum, 1% fetal bovine serum, and 1% penicillin-streptomycin were added to each 6-well plate coated with poly-d-lysine and then the PC12 cells were inoculated in 5 × 10⁴ cells each well. After 24 hours, these wells were treated with 10 μg/ml ethanol, 10 μg/ml of the compound of Formula 2, and 50 ng/ml of a nerve growth factor (R&D system, USA), respectively. Then, after 48 hours, the length of the neurite was measured using an inverted image contrast microscope (CK-2, Olympus, USA) (See FIG. 1 and FIG. 2). Referring to FIGS. 1 and 2, the neurite outgrowth was not observed in the ethanol-injected group (control), but the neurite outgrowth was induced both in the compound of Formula 2-treated group (DG) and in the nerve growth factor-treated group (NGF). Accordingly, it was found that the compound of Formula 2 induced differentiation of the PC 12 cell by inducing the neurite outgrowth.

**Experimental Example 2: Measurement of Level of Nerve Growth Factor in Mouse Serum**

The compound of Formula 2 was dissolved in 0.2 ml of a solution of dimethyl sulfoxide:ethanol (3:1 and then orally administered once to 7-week old male ICR mice (n=7) in an amount of 10 mg/kg. After 24 hours, the amount of an endogenous nerve growth factor was measured by ELISA. As a control group, an ICR mouse was orally administered once with 0.2 ml of the dimethylsulfoxide:ethanol (3:1). Then, the amount of a nerve growth factor in the control group was measured in the same manner as described above.

Referring to FIG. 3, the compound of Formula 2-administered group (DG 10 mg/kg, P.O) showed a nerve growth factor in the serum about 2.5 times greater than the control group. Such results show that the compound of Formula 2 can treat the neuropathy by suppressing the degeneration and death of neurons in a mouse and thus preventing a decrease in the number of neurons.

### Table: ¹³C Chemical shift (δ) and ¹H Chemical shift (δ)

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<tr>
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<th>¹³C Chemical shift (δ)</th>
<th>¹H Chemical shift (δ)</th>
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<td>C-13</td>
<td>40.6-40.7</td>
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</tr>
<tr>
<td>C-14</td>
<td>56.8-56.8</td>
<td>H-14 1.01(m,o)</td>
</tr>
<tr>
<td>C-15</td>
<td>32.4-32.5</td>
<td>H-15 1.95-1.98(m, J=5.9-6.1 Hz); 1.31-1.35(m,o)</td>
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<td>81.3-81.3</td>
<td>H-16 4.45-4.48(m, J=7.0-7.4 Hz)</td>
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<tr>
<td>C-17</td>
<td>63.0-63.1</td>
<td>H-17 1.72-1.75(m,o)</td>
</tr>
<tr>
<td>C-18</td>
<td>16.5-16.6</td>
<td>H-18 0.73-0.76(s)</td>
</tr>
<tr>
<td>C-19</td>
<td>19.6-19.6</td>
<td>H-19 0.80-1.00(s)</td>
</tr>
<tr>
<td>C-20</td>
<td>42.1-42.2</td>
<td>H-20 1.88(m)</td>
</tr>
<tr>
<td>C-21</td>
<td>15.2-15.2</td>
<td>H-21 1.05-1.07(d, J=6.8-7.2 Hz)</td>
</tr>
<tr>
<td>C-22</td>
<td>109.4-109.5</td>
<td></td>
</tr>
<tr>
<td>C-23</td>
<td>32.0-32.0</td>
<td>H-23 1.57-1.63(m,o)</td>
</tr>
<tr>
<td>C-24</td>
<td>29.4-29.5</td>
<td>H-24 1.48-1.52(m,o)</td>
</tr>
<tr>
<td>C-25</td>
<td>30.8-30.8</td>
<td>H-25 1.50(m,o)</td>
</tr>
<tr>
<td>C-26</td>
<td>67.0-67.1</td>
<td>H-26 3.50-3.40(m, J=10.5; 3.0; 10.5 Hz)</td>
</tr>
<tr>
<td>C-27</td>
<td>17.5-17.5</td>
<td>H-27 0.60-0.63(d, J=4.7-5.8 Hz)</td>
</tr>
</tbody>
</table>
Experimental Example 3: Measurement of Level of Nerve Growth Factor in Serum of Diabetes-induced Mouse

[0064] An alloxan-induced diabetic mouse was prepared as an animal model having diabetic neuropathy, one of the multiple neuropathies. 7-week old male ICR mice underwent alimentary abstinence for 18 hours, and then alloxan dissolved in a physiological saline was once injected to them by intraperitoneal injection in an amount of 160 mg/kg to induce diabetes. Mice that maintained their fasting blood sugar to 200 mg/dl or more for one week, that is, diabetes-induced mice were selected and then divided into a control group (n=10), an extract-administered group (n=10), and a compound-administered group (n=10). The control group was administered with 0.2 ml of a solution of dimethylsulfoxide:ethanol(3:1), the extract-administered group was administered with the extract obtained according to Example 1 dissolved in a solution of dimethylsulfoxide:ethanol (3:1) in an amount of 100 mg/kg, and the compound-administered group was administered with the compound of Formula 2 dissolved in a solution of dimethylsulfoxide:ethanol (3:1) in an amount of 10 mg/kg. The all groups were orally administered three times per one week, and the entire administration period was one month. The amount of an endogenous nerve growth factor in the serum was measured by ELISA.

[0065] Referring to FIG. 4, the amounts of the endogenous nerve growth factor in the serum of both the extract-administered group (DN 100 mg/kg p.o) and the compound-administered group (DG 10 mg/kg p.o) were three and four times higher than the control group to which only a vehicle was injected, respectively. Such results show that the extract and compound according to the present invention can treat the diabetic neuropathy by suppressing the degeneration and death of neurons in nerve-injured diseases caused by diabetes and thus preventing a decrease in the number of neurons.

Experimental Example 4: Measurement of Transmission Speed of Motor Nerve and Sensory Nerve of Sciatic Nerve in Diabetes-induced Mouse

[0067] The therapeutic effect of the compound according to the present invention on the diabetic neuropathy, one of the multiple neuropathies, was identified by measuring the effect of the compound according to the present invention on the transmission speed of the motor nerve and sensory nerve in the sciatic nerve. 7-week old male ICR mice underwent alimentary abstinence for 18 hours, and then alloxan dissolved in a physiological saline was once injected to them by intraperitoneal injection in an amount of 160 mg/kg to induce diabetes. Mice that maintained their fasting blood sugar to 200 mg/dl or more for one week, that is, diabetes-induced mice were selected and then divided into a control group (n=7), and a compound-administered group (n=7). The control group was administered with 0.2 ml of a solution of dimethylsulfoxide:ethanol (3:1 and the compound-administered group was administered with the compound of Formula 2 dissolved in a solution of dimethylsulfoxide:ethanol (3:1 in an amount of 10 mg/kg. 7-week old male ICR mice in which diabetes was not induced were grouped as a normal group (n=7), and the normal group was administered with 0.2 ml of a solution of dimethylsulfoxide:ethanol(3:1). The all groups were orally administered three times per one week, and the entire administration period was 2 months. Administration-completed mice were sacrificed by cervical dislocation, and then the skin and muscle in the femoral region were quickly removed. Then, left and right sciatic nerves were respectively separated in a length of 20 mm or more and stored in a physiological saline while air flows through the physiological saline. The separated sciatic nerves were placed on a 20 mm round measurement plate. Then, a sensor and a stimulating probe were connected to respective neuroterminals and the electrical conductivity was measured using a digital storage oscilloscope to assess the nerve transmission speed (see FIGS. 5 and 6.)

[0068] In general, the myelin is a phospholipid membrane surrounding axons with several layers, and also called as myelin sheath. Like the plastic coating of an electric wire, the myelin, through a white lipid material, prevents the electrical signals transmitted by neurons from leaking or dispersing. The myelin is regularly spaced between nodes of ranvier (a portion that forms nodes of myelin) at which the myelin is not formed, and surrounds axons. The electrical signal is transmitted along the space, impulses are quickly transmitted along neurons, and the myelin increases the electrical impulse speed. Accordingly, when the myelin is destructed by nerve injury due to the neuropathy induced by diabetes, axons stops their function and the nerve transmission speed decreases.

[0069] During one month of a diabetes-induced period, the compound-administered group (DM-DG) showed a transmission speed of the sensory nerve 25% higher than the diabetes-induced control group (DM) to which only the vehicle was administered (see FIG. 5). During two months of a diabetes-induced period, the compound-administered group (DM-DG) showed a transmission speed of the sensory nerve 45% higher than the control group, and showed a transmission speed of the motor nerve 40% higher than the control group (see FIG. 6). Accordingly, it was found that the compound according to the present invention has a therapeutic effect on nerve injury due to the diabetic neuropathy by increasing the transmission speed of the sensory nerve and motor nerve of a diabetes-induced mouse.
Experimental Example 5: Measurement of Level of Nerve Growth Factor in Sciatic Nerve in Diabetes-induced Mouse

[0071] 7-week old male ICR mice underwent alimentary abstinence for 18 hours, and then alloxan dissolved in a physiological saline was once injected to them by intraperitoneal injection in an amount of 160 mg/kg to induce diabetes. Mice that maintained their fasting blood sugar to 200 mg/dl or more for one week, that is, diabetes-induced mice were selected and then divided into a control group (n=5), and a compound-administered group (n=5). The control group was administered with 0.2 ml of a solution of dimethylsulfoxide:ethanol (3:1 and the compound-administered group was administered with the compound of Formula 2 dissolved in a solution of dimethylsulfoxide:ethanol (3:1) in an amount of 10 mg/kg. The all groups were orally administered three times per one week, and the entire administration period was one month. The amount of an endogenous nerve growth factor in the sciatic nerve was measured by ELISA (see FIG. 7)

[0072] Referring to FIG. 7, it was found that the nerve growth factor in the sciatic nerve of the compound-administered group (DM-DG 10 mg/kg P.O) was about 30% higher than that of the control group to which only the vehicle was injected. Accordingly, it is considered that the results shown in FIGS. 5 and 6 result from the nerve protecting function of the nerve growth factor.

Experimental Example 6: Measurement of Change in Amount of Sorbitol in Sciatic Nerve of Diabetes-Induced Mouse

[0073] The therapeutic effect of the compound according to the present invention on the neuropathy was identified by measuring a change in the amount of sorbitol in the sciatic nerve.

[0074] 7-week old male ICR mice underwent alimentary abstinence for 18 hours, and then alloxan dissolved in a physiological saline was once injected to them by intraperitoneal injection in an amount of 160 mg/kg to induce diabetes. Mice that maintained their fasting blood sugar to 200 mg/dl or more for one week, that is, diabetes-induced mice were selected and then divided into a control group (n=3), and a compound-administered group (n=3). The control group was administered with 0.2 ml of a solution of dimethylsulfoxide:ethanol (3:1), and the compound-administered group was administered with the compound of Formula 2 dissolved in a solution of dimethylsulfoxide:ethanol (3:1) in an amount of 10 mg/kg. 7-week old male ICR mice in which diabetes was not induced were grouped as a normal group (n=3), and the normal group was administered with 0.2 ml of a solution of dimethylsulfoxide:ethanol (3:1). The normal group, the control group, and the compound-administered group were orally administered three times per one week, and the entire administration period was 2 weeks. Administration-completed mice were sacrificed by cervical dislocation, and then the amount of the sorbitol in the sciatic nerve was measured by HPLC (see FIG. 8).

[0075] A polyol pathway refers to a process in which glucose is converted to sorbitol by aldose reductase and the sorbitol is changed into fructose by sorbitol dehydrogenase. In a hyperglycemic state, such as diabetes, excess glucose enters cells and sorbitol is generated and accumulated by the polyol pathway. At this time, nerves can be injured by the osmotic operation drawing water into cells. Accordingly, as a result of measuring the effect of the compound according to the present invention on the sorbitol accumulation in the sciatic nerve of the diabetes-induced mouse, as shown in FIG. 8, it was found that the sorbitol in the sciatic nerve of the diabetes-induced control group (DM-CON) was about 40% higher than that of the normal group, but the sorbitol in the sciatic nerve of the compound-administered group (DM-DG) was 10% lower than that of the diabetes-induced control group (DM-CON). Such results show that the compound according to the present invention can partially decrease aggressive factors causing nerve injury.

Experimental Example 7: Histological Comparison of Sciatic Nerve of Diabetes-induced Mouse

[0076] The therapeutic effect of the compound according to the present invention on the neuropathy was identified by measuring a histological change of the sciatic nerve.

[0077] 7-week old male ICR mice underwent alimentary abstinence for 18 hours, and then alloxan dissolved in a physiological saline was once injected to them by intraperitoneal injection in an amount of 160 mg/kg to induce diabetes. Mice that maintained their fasting blood sugar to 200 mg/dl or more for one week, that is, diabetes-induced mice were selected and then divided into a control group (n=5), an extract-administered group (n=5), and a compound-administered group (n=3). The control group was injected with 0.2 ml of a solution of dimethylsulfoxide:ethanol (3:1), the extract-administered group was administered with the extract obtained according to Example 1 dissolved in a solution of dimethylsulfoxide:ethanol (3:1) in an amount of 100 mg/kg, and the compound-administered group was administered with the compound of Formula 2 dissolved in a solution of dimethylsulfoxide:ethanol (3:1) in an amount of 10 mg/kg. All groups were treated three times by oral administration per one week, and the entire administration period was 2 months. Administration-completed mice were sacrificed by cervical dislocation, and then the sciatic nerve was separated, dyed according to the following conditions, and then observed using a 600x, 1200x confocal microscope.
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(1) prefixing: 2.5% glutaraldehyde, 12 or more hours
(2) washing: pH 7.4, 0.1 M phosphate buffer solution, 15 minutes/twice
(3) postfixing: 1% OsO4 osmium tetroxide, 60 minutes
(4) washing: pH 7.4, 0.1 M phosphate buffer solution, 5 minutes/twice
(5) dehydrating:
   50, 70, 80, 90 % alcohol: 10 minutes/each
   100 % alcohol: 15 minutes/twice
(6) substituting: propylene oxide, 15 minutes/twice
(7) permeating: propylene oxide (1), EPOK 812 (2), 12 or more hours
(8) embedding: refined EPOK 812 (80°C polymerization): 12 or more hours
(9) sectioning: semi-thin section, 35-95 µm
(10) dyeing: 1% toluidine blue

[0078] FIG. 9A and 9B show the results obtained with 600 magnification (9A) and 1200 magnification (9B), respectively. In the diabetes-induced group (DM), the axon and the myelin in the central part of the sciatic nerve were significantly destructed. However, in the compound-administered group (DM-DG) and the extract-administered group (DM-DN), the axon and the myelin in the central part of the sciatic nerve were clearly observed. Such results show that the extract or compound according to the present invention can protect and treat nerves injured by the neuropathy.

[0079] The compound according to the present invention was formulated into the following forms. However, these formulation examples are for illustrative purposes only and are not intended to limit the scope of the present invention.

Formulation Example 1: Tablet Formation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound of Formula 2</td>
<td>200 mg</td>
</tr>
<tr>
<td>Lactose</td>
<td>100 mg</td>
</tr>
<tr>
<td>Starch</td>
<td>100 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>proper</td>
</tr>
</tbody>
</table>

Formulation Example 2: Liquid Formulation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound of Formula 2</td>
<td>1000 mg</td>
</tr>
<tr>
<td>CMC-Na</td>
<td>20 g</td>
</tr>
<tr>
<td>Isomerized sugar</td>
<td>20 g</td>
</tr>
<tr>
<td>Lemon flavor</td>
<td>proper</td>
</tr>
</tbody>
</table>

Formulation Example 3: Capsule Formulation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound of Formula 2</td>
<td>300 mg</td>
</tr>
<tr>
<td>Crystalline cellulose</td>
<td>3 mg</td>
</tr>
<tr>
<td>Lactose</td>
<td>14.8 mg</td>
</tr>
<tr>
<td>Magnesium stearate</td>
<td>0.2 mg</td>
</tr>
</tbody>
</table>

Formulation Example 4: Injection Formulation

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compound of Formula 2</td>
<td>300 mg</td>
</tr>
<tr>
<td>Mannitol</td>
<td>180 mg</td>
</tr>
</tbody>
</table>

[0080] These components were mixed and compressed into tablets according to a conventional tablet formulating method.

[0081] Purified water was added so that the volume of the entire solution was 1000 mL. These components were mixed according to a conventional liquid formulation method, filled into a brown bottle, and sterilized, thereby producing the liquid formulation.

[0082] These components were mixed according to a conventional capsule formulating method, and then filled to a gelatin capsule, thereby producing the capsule formulation.
An injection containing the components having amounts described above per one ample (2 ml) was manufactured according to a conventional injection manufacturing process.

**Claims**

1. An extract of a family **Dioscoreaceae** for use in treating a peripheral neuropathy, the family **Dioscoreaceae** being at least one selected from the group consisting of **Dioscorea nipponica**, **Dioscorea septemloba**, **Dioscorea quinqueoloba**, **Dioscorea batatas**, **Dioscorea japonica**, **Dioscorea bulbifera**, **Dioscorea tokoro**, and **Dioscorea tenuipes**, wherein the extract is obtained by performing an extraction process which comprises extracting a root of the family **Dioscoreaceae** with a first extraction solvent selected from the group consisting of water, C₁-C₄ alcohol, and a mixture of water and a C₁-C₄ alcohol, and wherein the extract contains the compound of Formula 1

   ![Formula 1](image)

   wherein R is a hydrogen atom, a C₁-C₄ alkyl group, or a saccharide.

2. The extract for use according to claim 1, wherein the family **Dioscoreaceae** is **Dioscorea nipponica**, **Dioscorea quinqueoloba**, or **Dioscorea tokoro**.

3. The extract for use according to claim 1, wherein the peripheral neuropathy is the multiple neuropathy.

4. The extract for use according to claim 1, wherein the first extraction solvent is a mixture of water and methanol or a mixture of water and ethanol.

5. The extract for use according to claim 1, wherein the extraction process further comprises dispersing the extract obtained by the extraction with the first extraction solvent in water, and extracting with a second solvent of water-saturated C₁-C₄ alcohol.

6. The extract for use according to claim 5, wherein the second extraction solvent is water-saturated butanol.

7. The extract for use according to any one of claims 1 through 6, wherein R is a hydrogen atom.

8. The extract for use according to any one of claims 1 through 7, wherein the saccharide is selected from the group consisting of glucose, fructose, mannose, galactose, ribose, cellulose, glycogen, sucrose, maltose, and lactose.

9. A pharmaceutical composition for use in treating the peripheral neuropathy, which comprises a therapeutically effective amount of the extract according to any one of claims 1 through 8, and a pharmaceutically acceptable carrier.

10. A health functional food product for use in treating the peripheral neuropathy comprising an extract according to any one of claims 1 through 8.
Patentansprüche

1. Extrakt aus der Familie der Dioscoreaceae zur Verwendung bei der Behandlung einer peripheren Neuropathie, wobei die Familie der Dioscoreaceae mindestens eine ist, die aus der Gruppe ausgewählt ist, die besteht aus: Dioscorea nipponica, Dioscorea septemloba, Dioscorea quinqueoloba, Dioscorea batatas, Dioscorea japonica, Dioscorea bulbifera, Dioscorea tokoro und Dioscorea tenuipes, wobei der Extrakt gewonnen wird, indem ein Extraktionsprozess ausgeführt wird, der das Extrahieren einer Wurzel der Familie der Dioscoreaceae mit einem ersten Extraktionslösemittel umfasst, das aus der Gruppe ausgewählt ist, die aus Wasser, C1-C4-Alkohol und einer Mischung aus Wasser und C1-C4-Alkohol besteht, und wobei der Extrakt die Verbindung der Formel 1 enthält:

\[
\text{[Formel 1]}
\]

wobei R ein Wasserstoffatom, eine C1-C4-Alkylgruppe oder ein Saccharid ist.

2. Extrakt zur Verwendung nach Anspruch 1, wobei die Familie der Dioscoreaceae Dioscorea nipponica, Dioscorea quinqueoloba oder Dioscorea tokoro ist.

3. Extrakt zur Verwendung nach Anspruch 1, wobei die periphere Neuropathie multiple Neuropathie ist.

4. Extrakt zur Verwendung nach Anspruch 1, wobei das erste Extraktionslösemittel eine Mischung aus Wasser und Methanol oder eine Mischung aus Wasser und Ethanol ist.

5. Extrakt zur Verwendung nach Anspruch 1, wobei der Extraktionsprozess ferner das Dispergieren des durch Extraktion mit dem ersten Extraktionslösemittel in Wasser gewonnenen Extrakts und das Extrahieren mit einem zweiten Lösemittel aus wassergesättigtem C1-C4-Alkohol umfasst.

6. Extrakt zur Verwendung nach Anspruch 5, wobei das zweite Extraktionslösemittel wassergesättigtes Butanol ist.

7. Extrakt zur Verwendung nach einem der Ansprüche 1 bis 6, wobei R ein Wasserstoffatom ist.

8. Extrakt zur Verwendung nach einem der Ansprüche 1 bis 7, wobei das Saccharid aus der Gruppe ausgewählt ist, die besteht aus: Glucose, Fructose, Mannose, Galactose, Ribose, Cellulose, Glycogen, Sucrose, Maltose und Lactose.

9. Pharmazeutische Zusammensetzung zur Verwendung bei der Behandlung der peripheren Neuropathie, die eine therapeutisch wirksame Menge des Extrakts nach einem der Ansprüche 1 bis 8 und einen pharmazeutisch unbebedenklichen Träger umfasst.

10. Funktionelles Gesundheitslebensmittelprodukt zur Verwendung bei der Behandlung der peripheren Neuropathie, einen Extrakt nach einem der Ansprüche 1 bis 8 umfassend.

Revendications

1. Extrait d’une famille de dioscoréacées destiné à être utilisé dans le traitement d’une neuropathie périphérique, la famille de dioscoréacées étant l’une au moins choisie dans le groupe constitué par Dioscorea nipponica, Dioscorea septemloba, Dioscorea quinqueoloba, Dioscorea batatas, Dioscorea japonica, Dioscorea bulbifera, Dioscorea tokoro et Dioscorea tenuipes, dans lequel l’extrait est obtenu en effectuant un processus d’extraction qui comprend
l’extraction d’une racine de la famille de dioscoréacées avec un premier solvant d’extraction choisi dans le groupe constitué par l’eau, un alcool en C₁-C₄, et un mélange d’eau et d’un alcool en C₁-C₄, et dans lequel l’extrait contient le composé de formule 1

\[ \text{Formule 1} \]

où R est un atome d’hydrogène, un groupe alkyle en C₁-C₄ ou un saccharide.

2. Extrait destiné à être utilisé selon la revendication 1, dans lequel la famille de dioscoréacées est *Dioscorea nipponica*, *Dioscorea unguiculata* ou *Dioscorea tokoro*.

3. Extrait destiné à être utilisé selon la revendication 1, dans lequel la neuropathie périphérique est la neuropathie multiple.

4. Extrait destiné à être utilisé selon la revendication 1, dans lequel le premier solvant d’extraction est un mélange d’eau et de méthanol ou un mélange d’eau et d’éthanol.

5. Extrait destiné à être utilisé selon la revendication 1, dans lequel le processus d’extraction comprend en outre la dispersion de l’extrait obtenu par l’extraction avec le premier solvant d’extraction dans de l’eau et l’extraction avec un deuxième solvant à base d’alcool en C₁-C₄ saturé d’eau.

6. Extrait destiné à être utilisé selon la revendication 5, dans lequel le deuxième solvant d’extraction est du butanol saturé d’eau.

7. Extrait destiné à être utilisé selon l’une quelconque des revendications 1 à 6, dans lequel R est un atome d’hydrogène.

8. Extrait destiné à être utilisé selon l’une quelconque des revendications 1 à 7, dans lequel le saccharide est choisi dans le groupe constitué par le glucose, le fructose, le mannose, le galactose, le ribose, la cellulose, le glycogène, le saccharose, le maltose et le lactose.

9. Composition pharmaceutique destinée à être utilisée dans le traitement de la neuropathie périphérique, qui comprend une quantité thérapeutiquement efficace de l’extrait selon l’une quelconque des revendications 1 à 8 et un véhicule pharmaceutiquement acceptable.

10. Produit alimentaire à fonction de santé, destiné à être utilisé dans le traitement de la neuropathie périphérique, comprenant un extrait selon l’une quelconque des revendications 1 à 8.
FIG. 1
FIG. 4

Nerve Growth Factor (pg/ml)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM-Control</td>
<td>500</td>
</tr>
<tr>
<td>DM-DG 10mg/kg p.o</td>
<td>2000</td>
</tr>
<tr>
<td>DM-DN 100mg/kg p.o</td>
<td>2500</td>
</tr>
</tbody>
</table>

FIG. 5

V (m/sec)

<table>
<thead>
<tr>
<th>Condition</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>25</td>
</tr>
<tr>
<td>DM</td>
<td>10</td>
</tr>
<tr>
<td>DM-DG</td>
<td>25</td>
</tr>
<tr>
<td>Normal</td>
<td>25</td>
</tr>
<tr>
<td>DM</td>
<td>25</td>
</tr>
<tr>
<td>DM-DG</td>
<td>25</td>
</tr>
</tbody>
</table>

Sensory Nerve          Motor Nerve
FIG. 8

[Bar chart showing sorbitol levels in Normal, DM-CON, and DM-DG groups.]

FIG. 9

[Images showing different conditions labeled as DM, DM-DG, and DM-DN, with magnifications x600 and x1200.]
REFERENCES CITED IN THE DESCRIPTION

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