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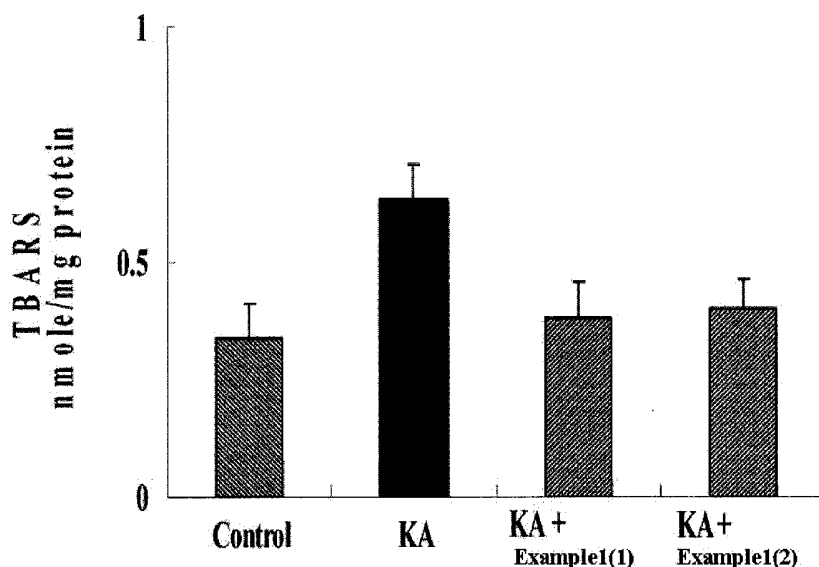
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(54) Title: NOVEL LIGNAN COMPOUNDS, THEIR PREPARATION METHODS AND THEIR USES



(57) Abstract: The present invention relates to novel lignan compounds having antioxidative and neuroprotective activity, more particularly, to a lignan compound having Chemical Formula 1 or a pharmaceutically acceptable salt or hydrate thereof and health food and pharmaceutical compositions comprising the same. The lignan compounds according to the present invention have excellent antioxidant activity, relief of neurotoxicity and neuroprotective effect and thus, can be usefully used in the prevention and treatment of brain diseases such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, hypoxic-ischemic injury and chronic spinal cord injury.

NOVEL LIGNAN COMPOUNDS, THEIR PREPARATION METHODS AND THEIR USES

Technical Field

5 The present invention relates to a novel lignan compound having anti-oxidative and neuroprotective activity, more particularly to a novel lignan compound having anti-oxidative and neuroprotective activity that is isolated from an extract of *Petasites japonicus* and health foods and pharmaceutical compositions comprising the same.
10

Background Art

Generally, the excitatory neurotransmitter, such as L-kainic acid, L-glutamic acid
15 and domoic acid, is an excitatory transmitter playing an important role in brain nerves. Especially, it is disclosed that the L-kainic acid stimulates the incorporation of cation such as sodium and calcium through an ion channel of brain nerve cell and causes polarization between membranes of nerve cells in order to excite the nerve cell.

In addition, it is reported that excito-toxicity, caused by the L-kainic acid, makes
20 endogenous glutamic acids or other excitatory neurotransmitters discharged in an excessive amount at a time so as to damage nerve tissues and affect neuropathogenesis (Choi, *et al.*, *Ann. Rev. Neurosci.*, 13, 171-182, 1990).

Besides, it is illustrated that if administered to the whole body, the L-kainic acid kills pyramidal neurons located in C1 and CA3 domains of hippocampus selectively so
25 as to cause epilepsies following limbic motor seizure or convulsion (Ben-Ari, Y., *Limbic seizure and brain damage produced by kainic acid: mechanisms and relevance to human temporal lobe epilepsy. Neuroscience*, 14(2): 375-403, 1985).

For this reason, the kainic acid has been used as a therapeutic agent to induce temporal lobe epilepsy in experimental animals. Further, in practice, it is demonstrated
30 as a model system to develop a drug inhibiting neural toxicity selectively in the hippocampus.

Extracts from *Petasites* plants (Compositae) have been used for thousands of

years for therapeutic purposes in folk medicine. The extract of *Petasites hybridus*, a native European perennial, has been found to contain spasmolytic (Ziolo and Samochowiec, 1988) and analgesic (Grossman and Schmidramsl, 2001) compounds such as petasin or isopetasin. *Petasites formosanus*, an indigenous species of *Petasites* in Taiwan, has been used for the treatment of hypertension; one of hypotensive constituents was identified to be S-petasin, a sesquiterpene (Wang *et al.*, 2001). Additionally, phenolic compounds such as phenylpropanoyl derivatives were isolated from the leaves of this species (Lin *et al.*, 2001; Lin *et al.*, 2004). Separately, the leaves of *Petasites japonicus* (Sieb. et Zucc.) Maxim. (Compositae), a perennial plant widely grown in Japan and Korea, is used as an edible vegetable. Recent studies showed that petasinophenol (Mizushina *et al.*, 2002) and flavonoid glycosides (Mizushina *et al.*, 2003), isolated from *P. japonicus*, inhibited eukaryotic DNA polymerase γ and DNA polymerase α , respectively. In the course of screening antioxidant compounds from the extract of leaves of *P. japonicus*, the butanol-soluble fraction was found to contain a remarkable antioxidative action in DPPH radical scavenging assay.

In the present invention, we isolated a new lignan compound having neuroprotective activity against oxidative damage in the brain of mice treated with kainic acid, from the butanol-soluble fraction of leaves of *P. japonicus* and completed the present invention.

Disclosure Of Invention

Therefore, it is an object of the present invention to provide novel lignan compounds having neuroprotective activity.

It is another object of the present invention to provide a health food comprising the lignan compounds as an active ingredient.

It is another object of the present invention to provide a pharmaceutical composition having neuroprotective activity comprising the lignan compounds as an active ingredient.

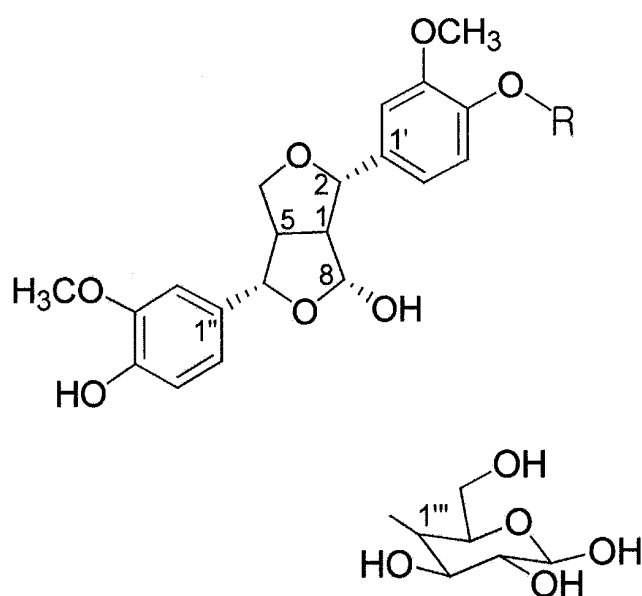
It is another object of the present invention to provide a method for isolating the

lignan compounds from *Petasites japonicus*.

Hereinafter, the present invention will be described more clearly as follows.

In order to achieve the above object, the present invention provides a lignan compound having the Chemical Formula 1, or a pharmaceutically acceptable salt or hydrate thereof:

[Chemical Formula 1]



wherein, R is hydrogen or

In order to achieve the above object, the present invention provides a pharmaceutical composition having neuroprotective activity, which comprises an effective amount of the lignan compound according to the present invention as an active ingredient, together with a pharmaceutically acceptable carrier.

In order to achieve the above object, the present invention provides a health food comprising the lignan compound according to the present invention as an active ingredient.

In order to achieve the above object, the present invention provides a method for isolating a lignan compound comprising the following steps: 1) extracting *Petasites japonicus* with methanol to obtain a solid extract; 2) extracting the solid extract with butanol after suspending it in water; 3) eluting the butanol extract with a stepwise gradient of chloroform and methanol on a silica gel column to separate multiple fractions; 4) eluting the separated fractions with methanol to separate multiple fractions; and, 5)

obtaining a lignan compound of the present invention from the separated fractions.

Now, the present invention will be described in detail.

As used herein, the term "pharmaceutically acceptable salt" means alkali metal salts, alkaline earth metal salts, salts of inorganic acids, salts of organic acids, salts with
5 amino acids, etc. The concrete examples of the pharmaceutical salts include salts of inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid or sulfuric acid; and salts of organic carboxylic acids, such as acetic acid, trifluoroacetic acid, citric acid, formic acid, maleic acid, oxalic acid, succinic acid, benzoic acid, tartaric acid, fumaric acid, mandelic acid, ascorbic acid or malic acid; and the like. These acid
10 addition salts can be prepared by known methods on the basis of the said Chemical Formula 1.

As used herein, the term "hydrate" means a compound of the present invention or a salt thereof, that further includes a stoichiometric or non-stoichiometric amount of water bound thereto by non-covalent intermolecular forces.

15 Other terms used herein can be interpreted as having their usual meanings in the art to which the present invention pertains, unless their definitions are explained in the description.

The lignan compounds of the present invention are novel Furofuran lignan compounds isolated from *Petasites japonicus*. The lignan compounds have
20 antioxidative and neurotoxicity inhibitory activity and therefore can protect the brain against oxidative neurotoxicity that disrupt or degenerate neuronal cells.

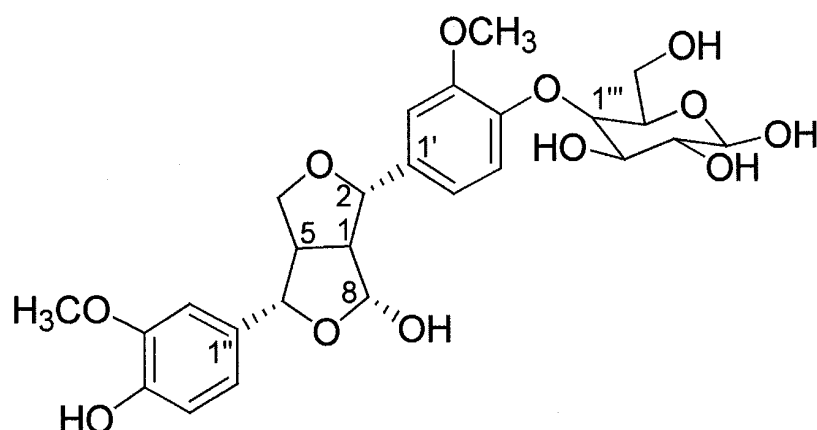
Besides, the lignan compound of the present invention has strong antioxidative activity and suppresses oxidative activity of kainic acid that is an excitatory neurotransmitter, which can prevent failure of neuronal tissue, generation of neuronal
25 disease, death of pyramidal neurons in hippocampus and therefore alleviate epilepsy by suppressing excessive secretion of the excitatory transmitter.

Therefore, the lignan compounds according to the present invention have excellent antioxidant activity, relief of neurotoxicity and neuroprotective effect and thus, can be usefully used in the prevention and treatment of brain diseases such as
30 Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, hypoxic-ischemic injury, and chronic spinal cord injury.

According to the present invention, the BuOH-soluble fraction of the leaves of *P. japonicus* was subjected to repeated column chromatographies on normal and reverse phase silica gels and HPLC, and finally the lignan compound was obtained as an amorphous powder with a negative optical rotation, $[\alpha]_D -10.0^\circ$. The molecular formula of lignan compound of the present invention was found to be $C_{26}H_{32}O_{12}$, based on a quasi-molecular ion at m/z 535 $[M - H]^-$ in the negative API-MS.

The active neuroprotective compound, which was isolated as above, was identified by LC/MS and NMR to be the following Chemical Formula 2 and named as 2-(4'-hydroxy-3'-methoxyphenyl)-6-(4''-hydroxy-3''-methoxyphenyl)-8-hydroxy-3,7-dioxabicyclo[3.3.0]octane 4'-O-(D-glucopyranoside) according to IUPAC nomenclature.

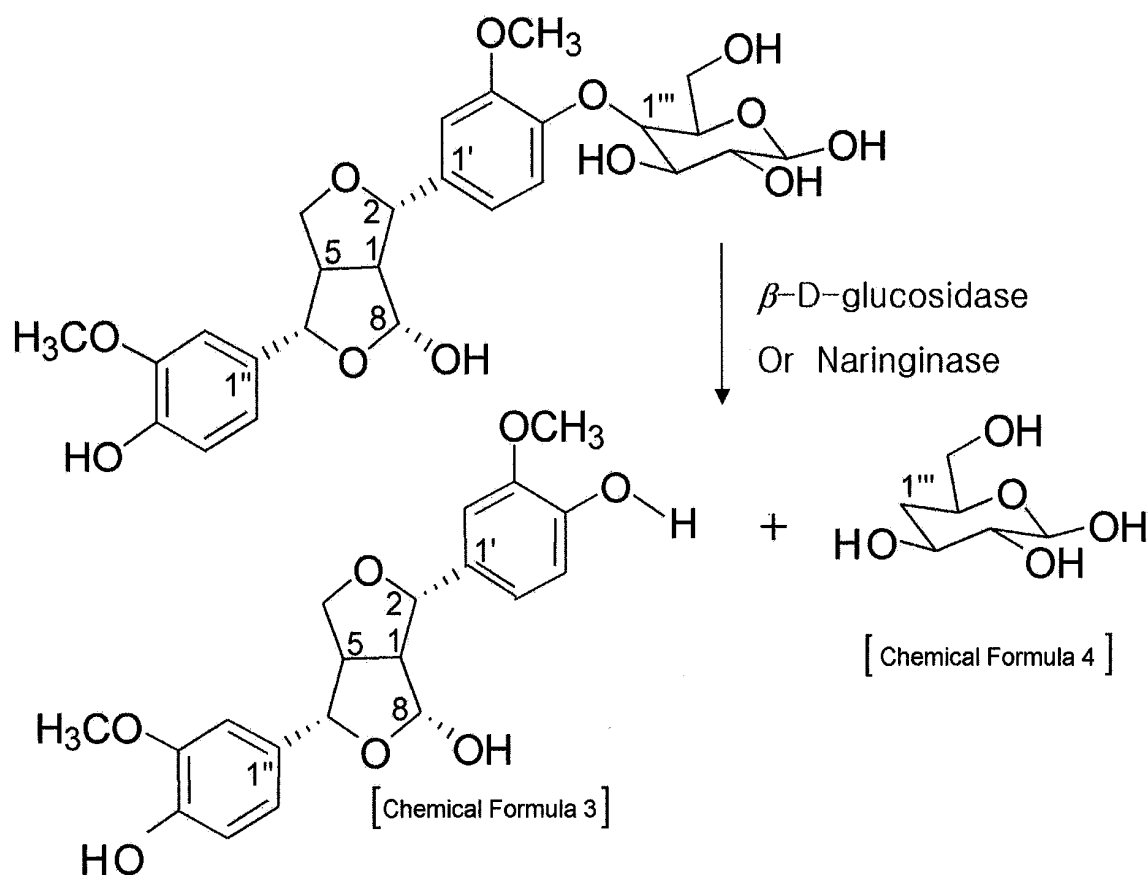
[Chemical Formula 2]



However, the natural source of the lignan compound is not limited to *P. japonicus* but can be any natural plant which contains the lignan compound. Further, the method for extracting and isolating the lignan compound is not limited to the method specifically disclosed in the description, but any conventional methods which can extract and isolate the lignan compound can be used.

According to the following Reaction Formula 1, the compound of Chemical Formula 2 can be hydrolysed to obtain Aglycone of Chemical Formula 3 and D-glucose (β -type D-glucose) of Chemical Formula 4. Reaction Formula 1 is one example for preparing the compound of Chemical Formula 3.

[Reaction Formula 1]



However, except for the above enzymatic hydrolysis method, any method can be used to obtain Aglycone from the lignan compound of Chemical Formula 2 which can hydrolyze the compound. Therefore, any enzyme can be used, which have the same activity as naringinase.

Further, the Aglycone of Chemical Formula 3 has the same activity as the lignan compound of Chemical Formula 2 and therefore can be used in place of the lignan compound of Chemical Formula 2.

When formulating a pharmaceutical composition of the present invention, the lignan compound of the present invention and its pharmaceutically acceptable salts or hydrates are combined with pharmaceutically acceptable carriers and depending upon use, may be formulated into oral dosage forms, such as tablets, soft or hard capsules, chewing capsules, powder, liquid agents or suspension, and parenteral dosage forms such as injectable liquid agents or suspension.

When formulating the lignan compound of the present invention into oral dosage forms such as tablets, soft or hard capsules, chewing capsules, powder, liquid agents or suspension, binders such as Arabia gum, corn starch, minute crystalline cellulose or gelatin; excipients such as calcium diphosphate or lactose; disintegrants such as alginic acid, corn starch or potato starch; lubricants such as magnesium stearic acid; sweetening agents such as sucrose or saccharin; and flavoring agents such as peppermint, methyl salicylic acid or fruit flavor may be included. When formulating unit dosage form, liquid carriers such as polyethylene glycol or fat oil in addition to above-mentioned components may be included.

In addition, the injectable agent for parenteral dosage forms, such as solution or suspension, may be administered parenterally, for example, subcutaneously, intravenously, intramuscularly or peritoneally. In general, the injectable solution or suspension may be prepared by blending active ingredients of pharmaceutically acceptable carriers such as water, salt water, soluble dextrose, and other sucrose solution, non-volatile oil, ethanol, glycerin, polyethylene glycol and propylene glycol in effective amounts homogeneously. Besides depending upon requirements, other additives such as anti-bacterial agents, chelates, buffering agents may be included.

Furthermore, the pharmaceutically acceptable carriers can be any additives if they are pharmaceutically pure, substantially non-toxic and not affecting the action of the active components.

The pharmaceutical compositions comprising the lignan compound of the present invention in an effective amount can be administered orally in 25 to 100 mg/kg weight per day once or several times and preferably, administered separately three times to four times per day.

The term "effective amount" means an amount of active ingredients effective to alleviate, ameliorate or prevent symptoms of disease or decrease or delay the onset of clinical markers or symptoms of disease. The individual patient with a particular body weight and life style may readily determine the proper dosage by starting out with the general dosage level set forth above and adjust the dosage as necessary to alleviate the disease.

Further, the lignan compound of the present invention was extracted from *Petasites japonicus* which have been used as a traditional food and therefore is edible

without an adverse effect to the human body. The lignan compound can be used as an active ingredient in health food, such as solid food or liquid drink.

For example, the lignan compound of the present invention can be mixed with conventional sweetening agents, vitamins, amino acids, minerals organic acids, odorants, preservatives, etc. to formulate solution, suspension or any conventional formulation form, which may be a good health food that can prevent adult diseases such as neuronal disease due to its antioxidative activity. Therefore, the present invention can provide a new health food which comprises the lignan compound as an active ingredient, and, if required, additionally contains assitant components selected from other galenical extracts, vitamins, amino acids, sweetening agents, minerals, organic acids, odorant, fruit juices or preservatives, etc.

The vitamins used in the present invention can be vitamin A, vitamin B₁, B₂, B₆, B₁₂, vitamin C, vitamin D, vitamin E, vitamin F, vitamin G, vitamin H, vitamin K, or conventional vitamins required in the human body. In the case of lipid-soluble vitamin, it is preferable to add a sutable amount of emulsifier.

The amino acids used in the present invention can be glycine, alanine, valine, glutamic acid, lycin, arginin, histidine, phenylalanine, tyrosine, tryptophan, proline, oxyproline, etc.

The organic acids used in the present invention can be citric acid, tartaric acid, malic acid, acetic acid, or other edible organic acid.

The odorant used in the present invention can be mint water, mint oil, orange oil, strawberry essence, or other conventional fruit odor or plant essence.

The fruit juice used in the present invention can be apple juice, strawberry juice, orange juice, lemon juice, peach juice, pineapple juice, banana juice, paraya juice, plum juice or other conventional fruit juice.

The above assitant components selected from other galenical extract, vitamins, amino acids, sweetening agent, minerals, organic acids, odorant, fruit juice or preservative can be added to the health food in a conventional amount.

Brief Description of the Drawings

The above and other objects, features and other advantages of the present invention will be more clearly understood from the following detailed description taken in conjunction with the accompanying drawings, in which;

5 Fig. 1 shows a schematic draw of extraction, fractionation, and isolation of a lignan compound of the present invention from *Petasites japonicus*;

Fig. 2 shows a Mass spectrum of a lignan compound of the present invention;

Fig. 3 is a graph showing the effect of a lignan compound of the present invention on TBARS in brain tissue of mice administered with kainic acid;

10 Fig. 4 is a graph showing the effect of a lignan compound of the present invention on total glutathione level in the brain tissue of mice administered with kainic acid;

Fig. 5 is a graph showing the change of glutathione peroxidase activity in the brain tissue of mice administered with kainic acid;

15 Fig. 6 is a graph showing the change of glutathione reductase activity in the brain tissue of mice administered with kainic acid; and

Fig. 7 is a graph showing the effect of a lignan compound of the present invention on Evans blue uptake into the brain of mice administered with kainic acid.

20 **Best Mode for Carrying Out the Invention**

Practical and presently preferred embodiments of the present invention are illustrated as shown in the following Examples. However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and
25 improvements within the spirit and scope of the present invention.

<Example 1> Materials

The leaves of *Petasites japonicus* were collected from agricultural fields in Kongju, Korea in May, 2004, and verified by Dr. Young Jin Choi, Herbarium of Wild
30 Vegetable Experiment Station, Kangwon-Do, Korea.. A voucher specimen was deposited in the Herbarium of Wild Vegetable Experiment Station, Kangwon-Do, Korea.

Kainic acid, glutathione reductase (Type III from bakers yeast), reduced glutathione (GSH), oxidized glutathione (GSSG), 5,5'-dithio-2-nitrobenzoic acid (DTNB), NADP, NADPH, thiobarbituric acid (TBA) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) were products of Sigma Chemical Co. (St. Louis, MO).

5

<Example 2> Preparation of lignan compounds of the present invention

(1) Preparation of 2-(4-hydroxy-3-methoxyphenyl)-6-(4-hydroxy-3-methoxyphenyl)-8-hydroxy-3,7-dioxabicyclo[3.3.0]octane-4-O-(-D-glucopyranoside)

The leaves of *P. japonicus* (1.5 kg) were extracted with MeOH at room
10 temperature to obtain 220 g of the solid extract. The MeOH extract was suspended in H₂O and extracted successively with hexane (3 L), CHCl₃ (3 L), EtOAc (3 L), and BuOH (3 L) to give the hexane (8.1 g), CHCl₃ (7.6 g), EtOAc (8.9 g), and BuOH-soluble fractions (26.6 g), respectively. The extraction was performed three times. The BuOH-soluble fraction (78 g) was chromatographed on a silica gel column (5 x 60 cm) eluted
15 with a stepwise gradient of CHCl₃ and MeOH to yield four fractions: 10% MeOH 3000 ml (A), 30% MeOH 2,500 ml (B), 50% MeOH 4000 ml (C) and 100% MeOH 3000 ml (D). The four fractions were dried into 2.2g(A), 5.3g(B), 7.0g (C) and 3.3g (D). The fraction (B) was chromatographed on a RP C-18 column (3.5 cm x 60 cm) eluted with MeOH-H₂O (1 : 4) to obtain four subfractions: 5000 ml (1), 1000 ml (2), 1000 ml (3) and
20 1000 ml (4). The four subfraction were dried into B-1: 70mg, B-2: 291.8mg, B-3:220.7mg and B-4: 238.5mg. The fraction B-2 was precipitated from MeOH at 4 °C to yield 88 mg of amorphous powder. The amorphous powder was identified by LC/MS and NMR as Example 3(1) to be 2-(4-hydroxy-3-methoxyphenyl)-6-(4-hydroxy-3-methoxyphenyl)-8-hydroxy-3,7-dioxabicyclo[3.3.0]octane-4-O-(-D-glucopyranoside).
25 The schematic draw of extraction, fractionation, and isolation of lignan compound of the present invention from *Petasites japonicus* is depicted in Fig. 1.

(2) Preparation of Aglicone

Naringinase (100 mg, from *Penicillium decumbens*) was added to a suspension
30 of lignan compound of Example 1(1) (8 mg) in 50 mM acetate buffer (pH 5.5) and the mixture was stirred at 37 °C for 5 h. The reaction mixture was extracted with EtOAc (10

mL × 3). The extract was isolated by silica gel TLC (EtOAc-MeOH-H₂O-AcOH, 65 : 20 : 15 : 15). The spot on the TLC plate was visualized by an anisaldehyde-H₂SO₄ reagent. The stereochemistry of glucose was determined by GC method as described in Example 3(2).

<Example 3> Analysis of physical properties of lignan compounds of the present invention

Optical rotation was measured with a JASCO DIP-370 digital polarimeter in MeOH. UV spectrum was recorded on a UV-2450 spectrometer. ¹H and ¹³C NMR spectra were recorded on Varian Unity Inova 400 spectrometer with the tetramethylsilane as an internal standard. Chemical shifts were presented as ppm. API-MS was measured with a Perkin-Elmer SCIEX API III Biomolecular Mass Analyzer. The molecular formula of the present lignan compound was found to be C₂₆H₃₂O₁₂, based on quasi-molecular ion at *m/z* 535 [M - H]⁻ in the negative API-MS. The Mass spectrum of lignan compound of the present invention is shown in Fig. 2..

(1) lignan compound of the present invention

Molecular formula: C₂₆H₃₂O₁₂ API-MS *m/z*: 535 [M - H]⁻, 373 [M - Glc - H]⁻

Optical rotation: [α]_D: -10.0° (c 0.23, MeOH);

UV (MeOH) λ_{max} (log ε): 204 (4.73), 229 (4.19), 279 (3.75) nm

¹H-NMR (400 MHz, pyridine-*d*₅) δ: 7.10 (1H, d, *J* = 2 Hz, H-2''), 7.06 (1H, d, *J* = 8.4 Hz, H-5'), 6.95 (1H, d, *J* = 2 Hz, H-2'), 6.84 (2H, d, *J* = 2 Hz, H6'6''), 6.71 (1H, d, *J* = 8.4 Hz, H-5''), 5.41 (1H, d, *J* = 4 Hz, H-8), 4.89 (1H, d, *J* = 7.2 Hz, H-1'''), 4.76 (2H, d, *J* = 7.2 Hz, H-2,6), 4.12 (1H, dd, *J* = 8.8, 5.6 Hz, H_{ax}-4), 3.91 (1H, dd, *J* = 8.8, 2.6 Hz, H_{equa}-4), 3.77 (3H, s, OCH₃-3'), 3.76 (3H, s, OCH₃-3''), 3.67 (1H, dd, *J* = 11.6, 4.8 Hz, H-6'''), 3.44 (1H, dd, *J* = 11.6, 5.6 Hz, H-6'''), 3.30 (1H, m, H-5'''), 3.26 (2H, m, H-2''',3'''), 3.16 (1H, m, H-4'''), 3.03 (1H, m, H-5), 2.73 (1H, d, *J* = 7.2 Hz, H-1).

¹³C-NMR (100 MHz, pyridine-*d*₅) δ: 149.0 (C-3'), 147.4 (C-3''), 145.9 (C-4'), 145.7 (C-4''), 135.9 (C-1'), 134.0 (C-1''), 118.8 (C-6'), 118.0 (C-6''), 115.3 (C-5'), 114.9 (C-5''), 110.7 (C-2''), 110.3 (C-2'), 100.9 (C-8), 100.1 (C-1'''), 86.1 (C-6), 82.5 (C-2), 77.0 (C-3'''), 76.8 (C-5'''), 73.2 (C-2'''), 71.4 (C-4), 69.6 (C-4'''), 62.0 (C-1), 60.6 (C-6'''), 55.7 (OCH₃-

3'), 55.5 (OCH₃-3"), 53.3 (C-5).

(2) Aglycone

The enzymatic hydrolysis of the present lignan compound with naringinase
5 yielded a monosaccharide unit, which was identified by co-TLC, as described in
Example 2(2). Its absolute configuration was determined by gas chromatography to be
D-glucose (Min *et al.*, 2000). The configuration of the glycosidic linkage for the
glucopyranoside unit was determined to be β form, based on the $J_{1,2}$ value of the
anomeric proton at 7.2 Hz (δ 4.89). The linkage of D-glucose moiety was determined,
10 based on the HMBC correlation between at δ_{H} 4.89 (H-1''') and δ_{C} 145.9 (C-4'). The
stereochemistry of glucose separated from aglycone was determined by GC method.
The sugar derivative thus obtained showed a retention time of 21.30 min, identical to
that of authentic D-glucose. Therefore, it is noted that the enzyme can hydrolyze D-
glucose, which is bound to the present lignan compound as a glucoside, to give
15 Aglycone.

<Example 4> Effect of lignan compounds of the present invention on suppression of kainic acid

To examine the neuroprotective activity of the present lignan compounds
20 suppressing the neurotoxicity of kainic acid, an oxidative-stress generator, in brain
tissue, the effect of the present lignan compounds on suppression of seizure and
mortality caused by kainic acid was analyzed using mice. ICR Mice were administered
orally with the compounds of Example 1(1) and (2) (50 mg/kg and 100 mg/kg),
suspended in saline, using an esophagus needle for 4 days consecutively before kainic
25 acid injection. Then, the mice were injected intraperitoneally (50 mg/kg) with kainic acid,
dissolved in saline, and control mice were administered with the same volume of saline.
Following the challenge with kainic acid, the onset time of seizure and the viability
(mortality) of the animals were monitored for 60 min.

30 [Table 1]

Effect of the compounds of the present invention on seizure in mice injected with

kainic acid

Group	Onset time of seizure (min)
Kainic acid only	12.5 ± 4.0
Kainic acid + Example 1(1) compound	29.0 ± 5.7
Kainic acid + Example 1(2) compound	27.5 ± 4.3

[Table 2]

Effect of the compounds of the present invention on viability in mice injected with

5 kainic acid

Group	viability (%)
Kainic acid only	30%
Kainic acid + Example 1(1) compound	70%
Kainic acid + Example 1(2) compound	80%

As a result, it was found that the oral administration of the present lignan compounds delayed the onset time of seizure more than 2 X and increased the viability more than 2 X compared with the control group. Under the same condition, quercetin, despite its antioxidant action, showed no significant effect on the seizure and mortality caused by kainic acid. Based on these observations, it is suggested that the present lignan compounds have examine the neuroprotective activity suppressing the neurotoxicity of kainic acid.

15 <Example 5> DPPH radical scavenging assay

To examine the antioxidant effect of the present lignan compounds, scavenging activity of the present lignan compounds on DPPH radicals was measured as described previously (Sok D.-E., Oh S. H., Kim, Y.-B., Kim M. R., *J. Agri. Food Chem.*, 51, 4570-4575, 2003; Blois M. S., *Nature*, 181, 1199-2000, 1958). 0.135 ml of DPPH radical solution (0.15 mM) in methanol and 0.015 ml of various concentrations of the lignan compounds of Example 1(1) and (2) in DMSO were mixed in a microplate reader well. The mixture was kept at room temperature for 30 min, and the absorbance at 535 nm was measured. IC₅₀ value was expressed as the concentration of compound to show

50 % inhibition of DPPH radicals.

[Table 3]

DPPH free radical scavenging activity of compounds

Compound	IC ₅₀ (μg/mL)
Example 1(1) compound	113.04 ± 4.21
Example 1(2) compound	25.52 ± 1.42
Alpha-tocopherol	25.65 ± 0.49
Quercetin	6.83 ± 1.60

As a result, it was found that DPPH scavenging activity is proportional to the concentration of the present lignan compounds and the lignan compound of Example 1(2), which is enzymatically deglycosylated, has more than 4 X of DPPH scavenging activity than the lignan compound of Example 1(1), which is almost equal with that of alpha-tocopherol.

<Example 6> Measurement of lipid peroxidation in Brain Tissue

Brain tissue, rinsed with 0.15 M KCl solution containing 2 mM EDTA, was homogenized in 9 volumes of 10 mM phosphate buffer (pH 7.4) using a tissue homogenizer with a Teflon pestle. To the brain homogenate (1.0 ml) was added 1.0 ml of 8.1 % SDS, 2 ml of 20% acetic acid and 1 ml of 0.75% thiobarbituric acid (TBA). The mixture was boiled for 30 min and then centrifuged (14,000 rpm, 10 min), and then the absorbance of the supernatant was measured at 533 nm.

To see whether the neuroprotective action of Example 1(1) and 1(2) compounds were related to the prevention against oxidative stress in the brain tissue of mice intoxicated with kainic acid, we examined the effect of the compounds on the level of TBARS, a biochemical marker of oxidative stress, in the brain of mice administered with kainic acid (50 mg/kg). Figure 3 shows that TBARS value was increased to 168 % of control value ($P < 0.05$) in the homogenate of whole brain of mice treated with kainic acid. Meanwhile, Example 1(1)(40 mg/kg) reduced the TBARS value, which was enhanced by kainic acid challenge, to the level of control group ($P < 0.05$). Also, a

similar results was observed with Example 1(2) compound(40 mg/kg). These results suggest that the neuroprotective action of lignan compounds of the present invention may be in part due to its preventive action against oxidative stress in the brain.

5 <Example 7> Determination of total GSH

Brain tissue (about 0.27-0.30 g wet wt) was pulverized in a cooled ceramic percussion mortar with 6 % metaphosphoric acid, and the mixture was centrifuged (27,000g, 20 min) at 4 °C. Total GSH was determined enzymatically according to a published procedure with a slight modification. To 0.02 ml of supernatant was added
10 0.39 mL of 100 mM phosphate buffer (pH 7.4) containing 5 mM EDTA, 0.025 ml of 10 mM DTNB and 0.08 ml of 5 mM NADPH. After 3 min equilibration at 25°C, the reaction was started by adding 1 unit of GSH reductase. The formation of 2-nitro-5-thiobenzoic acid was continuously recorded at 412 nm with a UV/VIS spectrophotometer. The total amount of GSH in the samples was determined from a standard curve obtained by
15 plotting the known amount of GSH versus.

We examined the effect of Example 1(1) and 1(2) compounds on the level of total glutathione, another biomarker of oxidative stress, in the brain of mice administered with kainic acid (50 mg/kg). As shown in Fig. 4, the administration of kainic acid (50 mg/kg) reduced the level of total glutathione in the cytosol fraction of brain homogenate to
20 approximately 73 % of control level. Then, the administration of Example 1(1) and 1(2) (40 mg/kg) elevated the level of total GSH in the brain cytosol from 73 % in kainic acid-treated group to 97 % of control group, further confirming the notion that the neuroprotective effect of lignan compounds of the present invention may be related to its prevention against oxidative stress in the brain.

25

<Example 8> Assay of GSH peroxidase and reductase

Brain tissue was homogenized in 9 volumes of 20 mM phosphate buffer containing 0.1 M KCl, 1 mM EDTA and 0.5% Triton X-100. The homogenate was centrifuged (15,000g, 15 min), and the supernatant was re-centrifuged (105,000g, 30
30 min). The last supernatant was retained for enzymatic assays. The assay of GSH peroxidase activity was carried out as described previously. A mixture containing 0.1 M

phosphate buffer (pH 7.0), 3 mM EDTA, 1 mM GSH, 0.1 mM NADPH, 2 units of GSH reductase and 0.05 ml of supernatant was incubated for 3 min, and 0.01 ml of 10 mM cumene hydroperoxide was then added to the reaction mixture. GSH reductase activity was measured in a mixture containing 0.1 M phosphate buffer (pH 7.0), 0.5 mM EDTA, 1 mM GSSG, 0.1 mM NADPH, and 0.05 ml of the supernatant.

In order to elucidate the mechanism responsible for the restoration of GSH level by Example 1(1) and 1(2) (40 mg/kg), the change in glutathione-related enzymes such as GSH peroxidase and GSH reductase in lignan compound/kainic acid-treated group was examined. Fig. 5 indicates that the treatment with Example 1(1) and 1(2) appeared to restore the loss of the GSH peroxidase activity, which was caused by kainic acid. However, there was no significant change of GSH-reductase activity among vehicle-treated group, kainic acid-treated group, and lignan compound/kainic acid-treated group (Fig. 6), suggesting that under the experimental condition used, the neurotoxicity of kainic acid may not be sufficient to alter the level of GSH-reductase activity.

<Example 9> Blood brain barrier breakdown during kainic acid-induced seizures

Animals were immobilized in a loose-fitting plaster cast, and then 3ml/kg of 2 % Evan-blue in saline solution was injected from tail vein as a visual indicator. Thirty min later, seizures were intraperitoneally induced by 50 mg/kg kainic acid administration. At the end of experiments, 60 min after the injection of kainic acid, mice were killed and the brains perfused transaortically with a saline solution and removed. Thereafter, the brain section was selected and cut. Sections were viewed under the video microscope. Separately, the brain tissue was homogenized with 50% trichloroacetic acid in fourfold, and the homogenate was centrifuged at 30,000 rpm for 20 min and the absorbance was measured at 615 nm. Values were calculated as Evans Blue mg/g tissue.

Separately, the protective effect of Example 1(1) and 1(2) on damage of blood brain barrier caused by kainic acid (50 mg/kg) was examined. For the purpose, Evans blue (3 mL/kg, 2% in saline) was iv administered to mice, either the lignan compound-treated group or the vehicle-treated group, 30 min before KA administration, and the brain tissue was analyzed for Evans blue retaining. When the tissue was homogenized, and the absorbance of the supernatant of the brain homogenate was measured at 615

nm (Figure 7), the amount of Evans blue retained in the brain following KA intoxication was significantly higher as compared to control. Meanwhile, pretreatment with Example 1(1) and 1(2) significantly reduced the Evans blue level in the KA-treated group compared to the control level. Thus, the lignan compounds of the present invention is assumed to prevent against oxidative damage of the blood-brain barrier in KA-treated mice.

<Example 10> Preparation of formulations containing lignan compounds of the present invention

1. Preparation of Syrup

The syrup containing 2% (m/v) of lignan compounds of the present invention or its pharmaceutically acceptable salt was prepared as follows. An acid-added salt of the present lignan compounds, saccharin and sugar were dissolved in 80g of warm water. The solution was cooled and mixed with a solution comprising glycerin, saccharin, flavor, ethanol, sorbic acid and distilled water. The mixture was added by water into 100 ml. The acid-added salt may be replaced with another pharmaceutically acceptable salt.

[Table 4] Composition of the Syrup

Hydrochloride of the present lignan compounds.....	2 g
Saccharin.....	0.8 g
Glycerin.....	8.0 g
Flavor	0.04 g
Ethanol.....	4.0 g
Sorbic acid.....	0.4 g
Distilled water.....	Balance

2. Preparation of Tablet

The tablet containing lignan compounds of the present invention or its pharmaceutically acceptable salt was prepared as follows. Hydrochloride of the present lignan compounds 250 g was mixed with lactose 175.9 g, potato starch 180 g and

colloidal silicic acid 32 g. The mixture was added with 10% gelatin solution, pulverized and passed through 14 mesh sieve. The resultant was dried and added with potato 160 g, talc 50 g and Mg. stearate 5 g. The final mixture was compressed into tablets by common techniques.

5

[Table 5] Composition of the Tablet

	Hydrochloride of the present lignan compounds.....	250 g
	Lactose.....	175.9 g
	Potato starch.....	180 g
10	Colloidal silicic acid.....	32 g
	10% Gelatin solution.....	
	Potato starch.....	160 g
	Talc.....	50 g
	Mg. Stearate	5 g

15

3. Preparation of Injection

The injection containing lignan compounds of the present invention or its pharmaceutically acceptable salt was prepared as follows. Hydrochloride of the present lignan compounds 1 g, NaCl 0.6 g and ascorbic acid 0.1 g were dissolved in distilled water into 100 mL. The solution was inputted in a vial and sterilized by heating at 200°C in 30 min.

20

[Table 6] Composition of the Injection

	Hydrochloride of the present lignan compounds.....	1 g
25	NaCl.....	0.6 g
	Ascorbic Acid.....	0.1 g
	Distilled water.....	Balance

30

Industrial Applicability

As illustrated and confirmed above, the lignan compounds or their pharmaceutically acceptable salt or hydrate according to the present invention have excellent antioxidant activity, relief of neurotoxicity and neuroprotective effect and thus, can be usefully used in the prevention and treatment of brain diseases such as
5 Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, hypoxic-ischemic injury and chronic spinal cord injury.

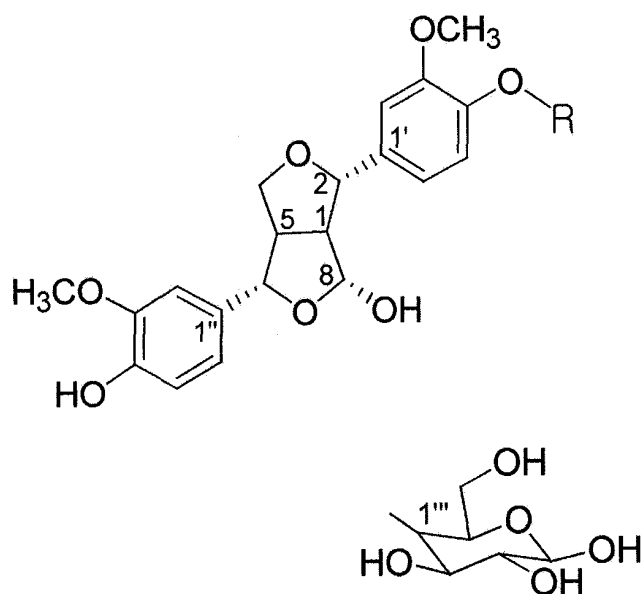
Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis
10 for modifying or designing other embodiments for carrying out the same purposes of the present invention.

Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

CLAIMS

1. A lignan compound having the Chemical Formula 1, or a pharmaceutically acceptable salt or hydrate thereof:

5 [Chemical Formula 1]



wherein, R is hydrogen or

2. A health food comprising the lignan compound according to claim 1 as an active ingredient.

3. The health food according to claim 2, which additionally comprises one or more of assistant foods selected from the group consisting of galenical extracts, vitamins, amino acids, sweetening agents, minerals, organic acids, odorants and fruit juices and one or more of assistant agents selected from the group consisting of excipient, diluent and antioxidant, and is formulated into liquid drug, soft capsule, tablet, granule or capsule.

4. A pharmaceutical composition having neuroprotective activity, which comprises an effective amount of the lignan compound according to claim 1 as an active ingredient, together with a pharmaceutically acceptable carrier.

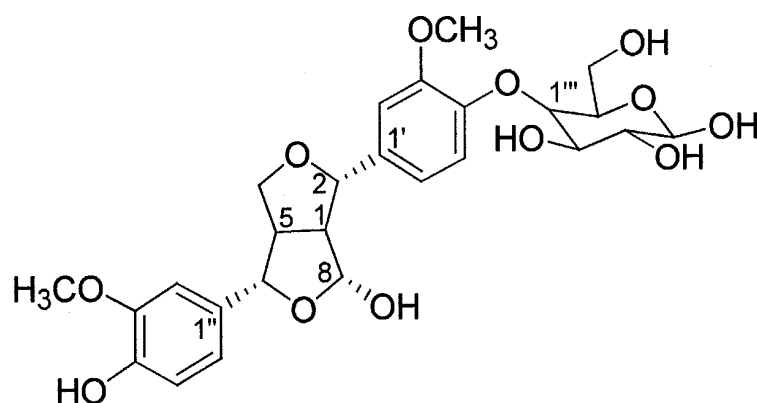
5. The pharmaceutical composition according to claim 4, wherein the neuroprotective activity is performed by suppressing neurotoxicity of excitatory neurotransmitter.

5 6. The pharmaceutical composition according to claim 5, wherein the excitatory neurotransmitter is kainic acid.

7. A method for isolating a lignan compound comprising the following steps:

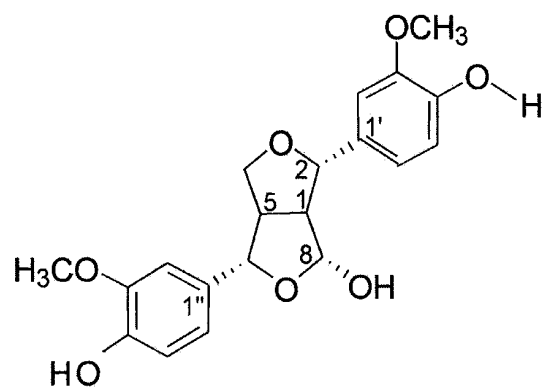
- 1) extracting *Petasites japonicus* with methanol to obtain a solid extract;
- 10 2) extracting the solid extract with butanol after suspending it in water;
- 3) eluting the butanol extract with a stepwise gradient of chloroform and methanol on a silica gel column to separate multiple fractions;
- 4) eluting the separated fractions with methanol to separate multiple fractions; and,
- 5) obtaining a lignan compound having the Chemical Formula 2 from the separated
- 15 fractions.

[Chemical Formula 2]

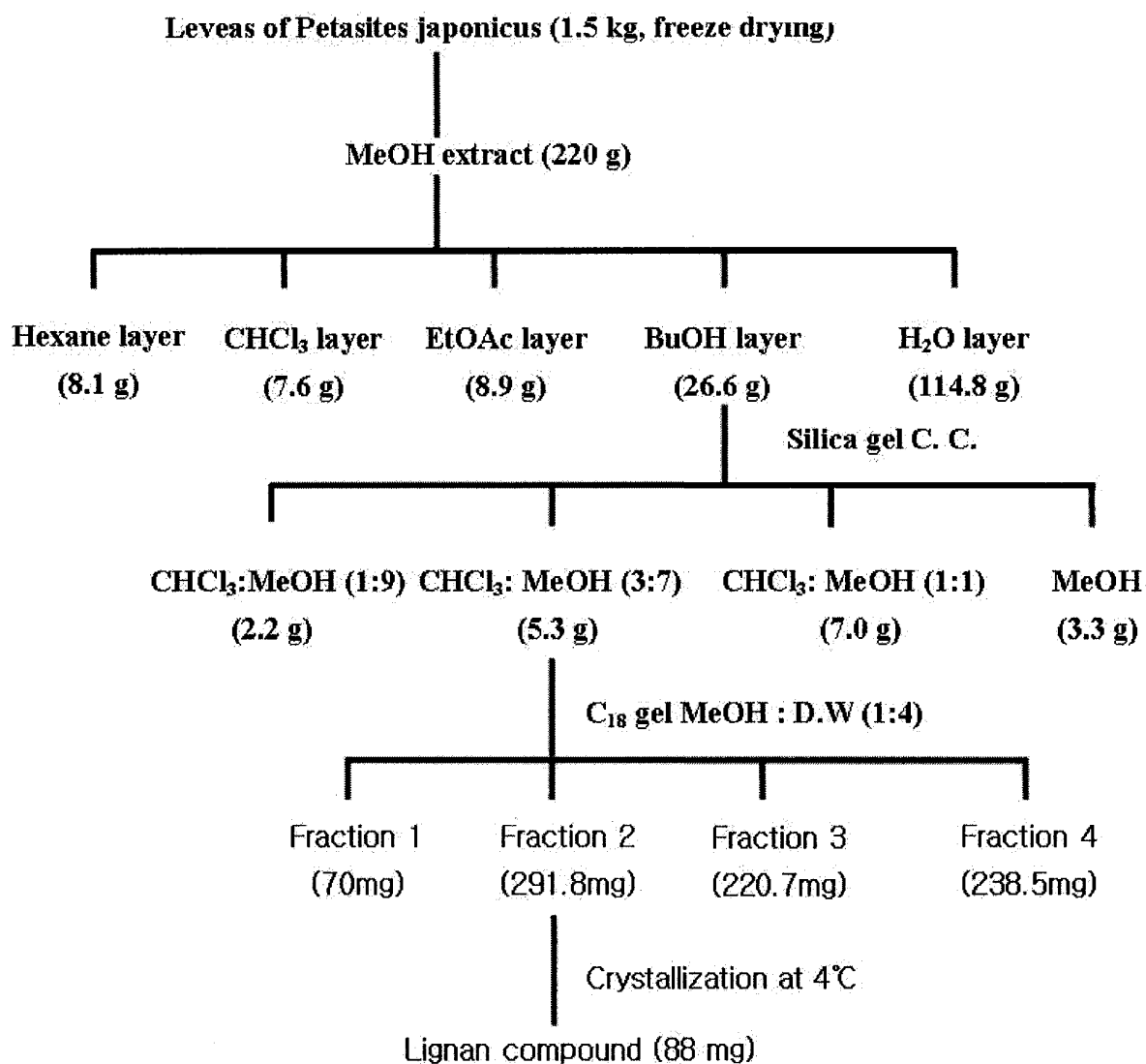


20 8. The method for isolating a lignan compound according to claim 7, which has an additional step for leaving out β -D-glucose from the lignan compound having the Chemical Formula by naringinase or β -D-glucosidase to obtain a lignan compound having the Chemical Formula 3.

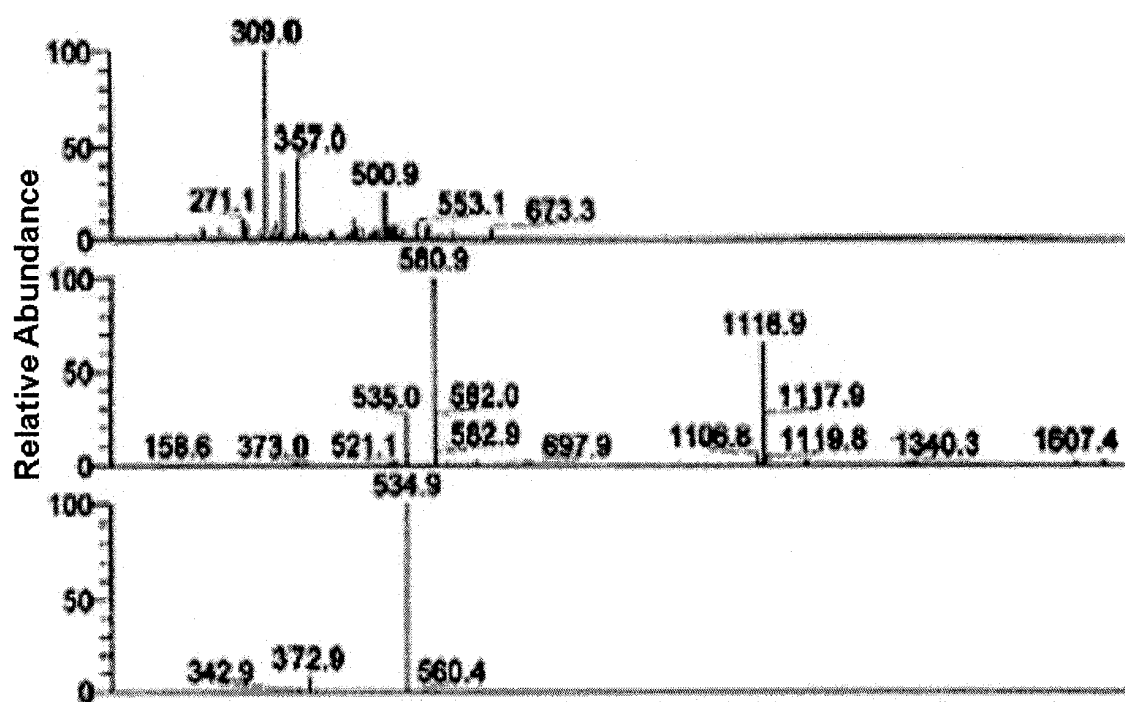
[Chemical Formula 3]



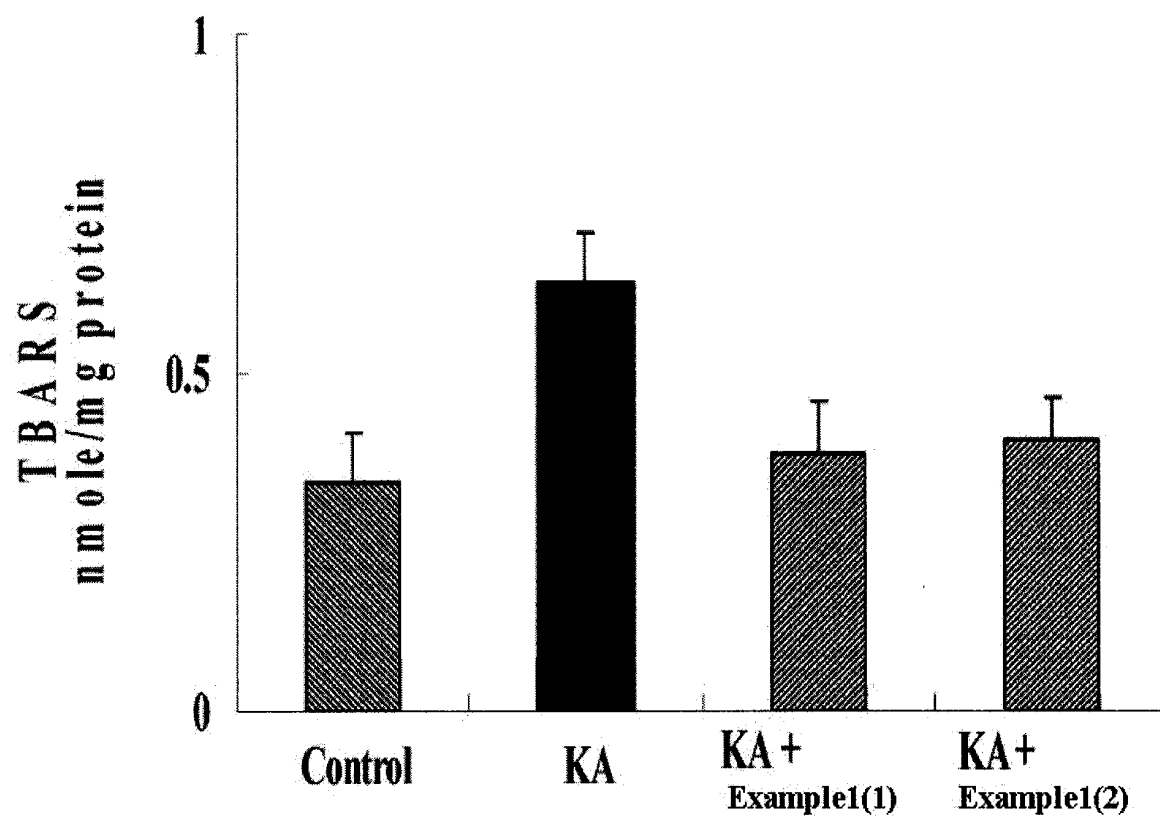
[Fig. 1]



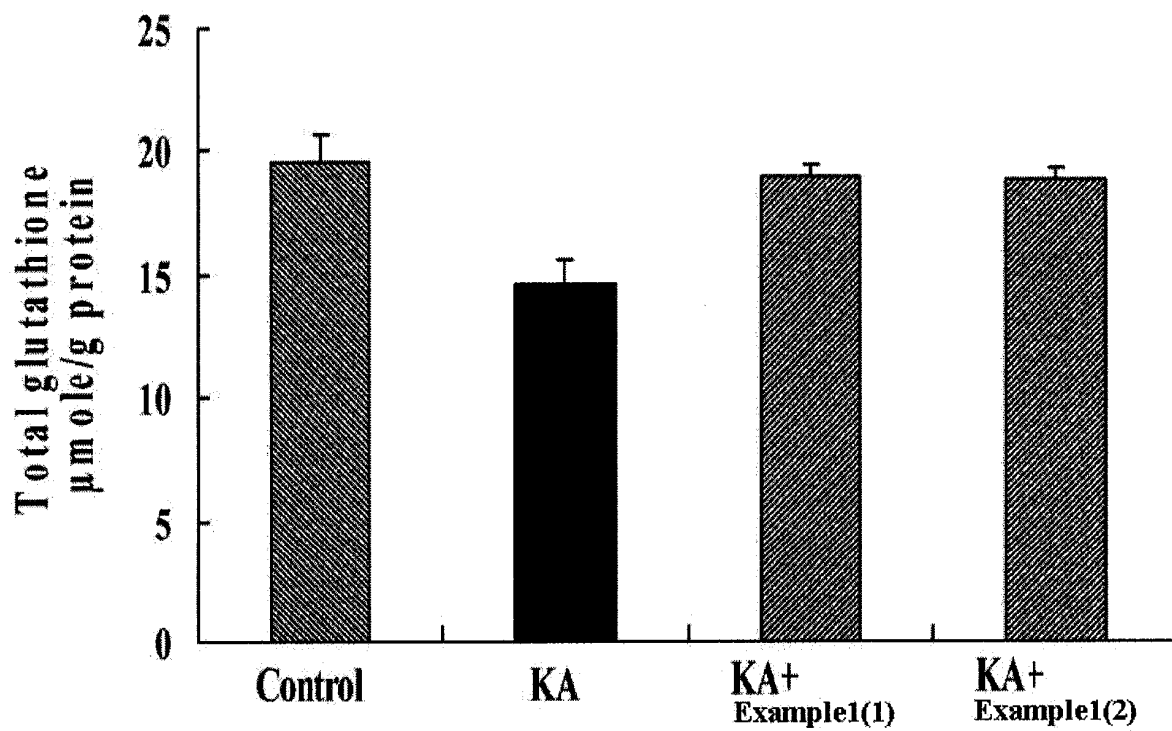
[Fig. 2]



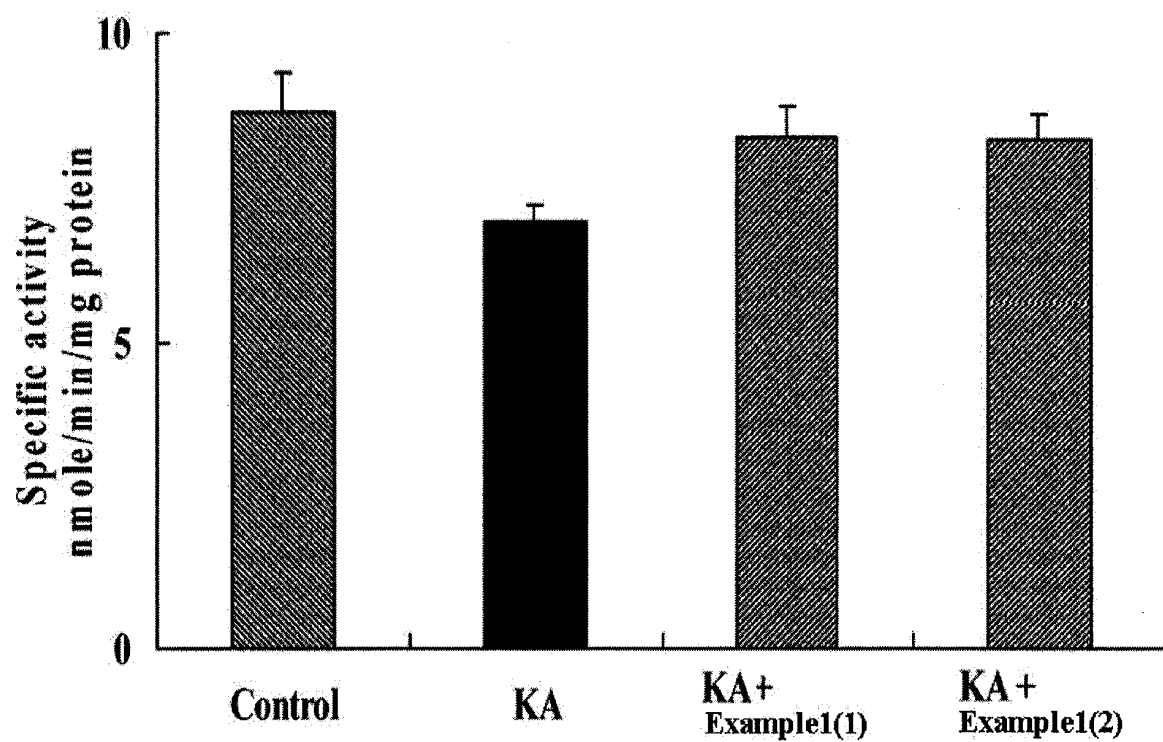
[Fig. 3]



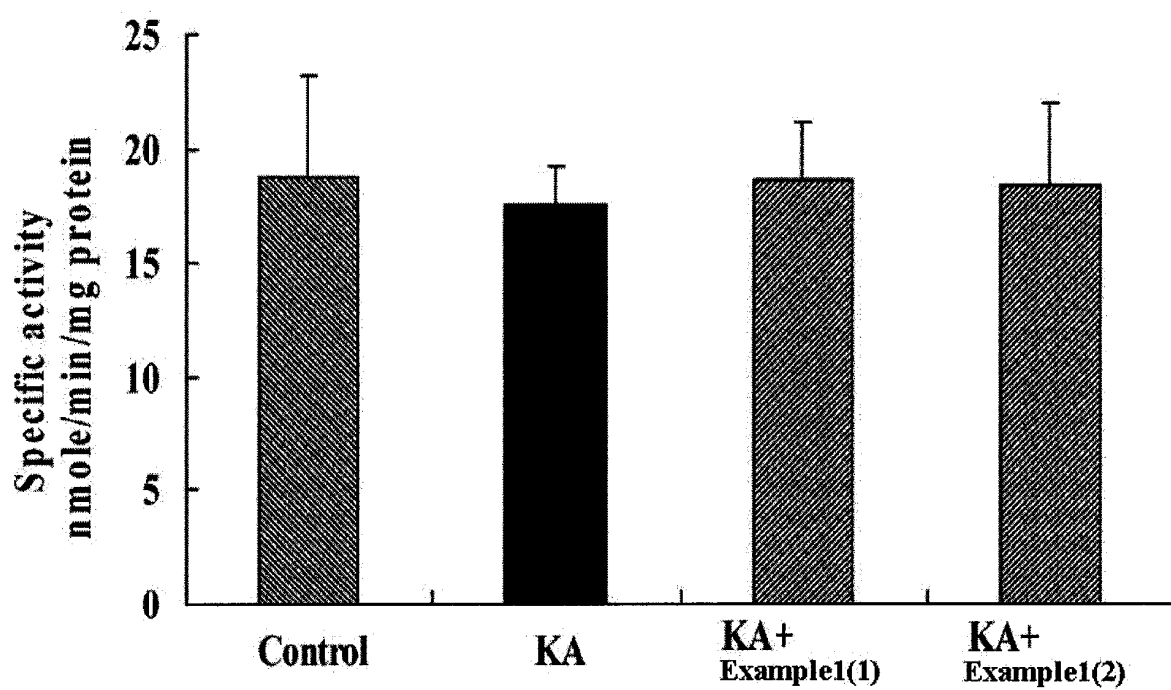
[Fig. 4]



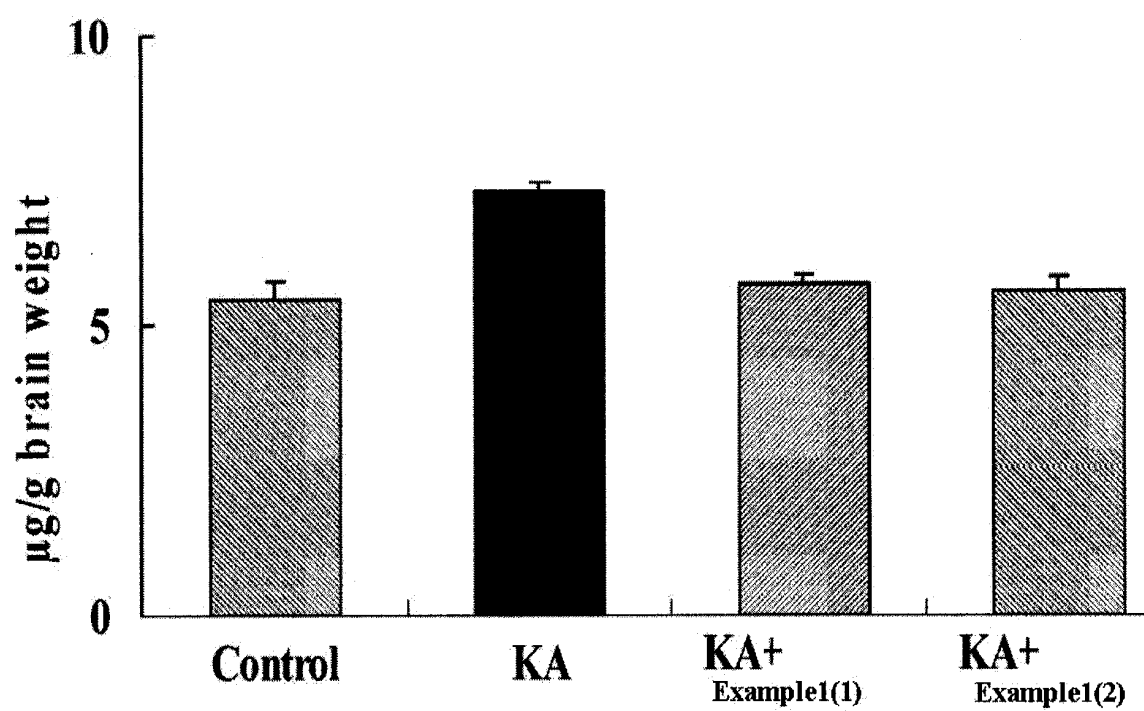
[Fig. 5]



[Fig. 6]



[Fig. 7]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2006/001524**A. CLASSIFICATION OF SUBJECT MATTER***C07D 493/04(2006.01)i*

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D 493/04

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean Patents and applications for inventions since 1975.

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

STN(Caslink)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	STEFFAN et. al. 'Polyphenols from plants used in traditional indonesian medicine (Jamu): uptake and antioxidative effects in rat H4IIE hepatoma cells' In: Journal of pharmacy and pharmacology, 2005, 57(2), p.233-240	1 - 6, 8 7
X Y	LI et. al. 'Study on chemical constituents of Ligularia kanaitzensis Hand.-Mazz.' In: Zhongguo yaoke daxue xuebao, 2001, 32(5), p.342-345	1 2 - 8
X Y	ABE et. al. '9 α -Hydroxypinoresinol, 9 α -Hydroxymedioresinol and related lignans from Allamanda neriifolia' In: Phytochemistry, 1988, 27(2), p.575-577	1 2 - 8
X Y	KHAN et. al. 'A lignan from Lonicera hypoleuca' In: Phytochemistry, 1985, 24(3), p.628-630	1 2 - 8
A	GAN et. al. 'Lignans and their degraded derivatives from Sarcostemma acidum' In: Journal of natural products, 2005, 68(2), p.221-225	1 - 8

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 JULY 2006 (18.07.2006)

Date of mailing of the international search report

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