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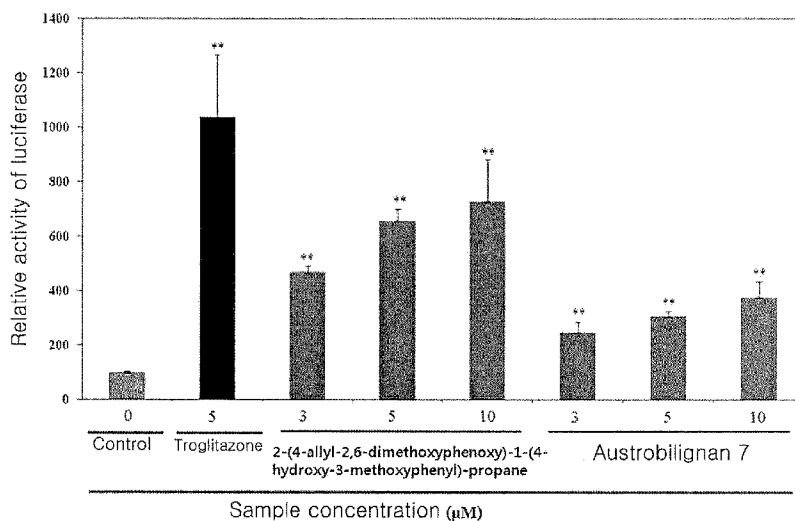
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[Continued on next page]

(54) Title: NOVEL USE OF LIGNAN COMPOUNDS

FIG. 12



(57) Abstract: The present invention relates to novel use of lignan compounds, and more particularly, the present invention relates to novel use of an extract of the aril of nutmeg, 2-(4-allyl-2, 6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austroblignan 7 or licarin E, for preventing or treating diabetes or PPAR γ -mediated diseases. The said compound and extract of the aril of nutmeg act as ligands for PPAR γ , and they may be used for preventing or treating PPAR γ -mediated diseases, not limited thereto, such as diabetes and diabetic complication. Accordingly, 2-(4-allyl-2, 6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austroblignan 7 or licarin E and extract of the aril of nutmeg may be useful as preventing or treating agent for PPAR γ -mediated diseases, not limited thereto, such as diabetes and diabetic complication.



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Invention Title

NOVEL USE OF LIGNAN COMPOUNDS

Technical Field

The present invention relates to novel use of
5 lignan compounds, and more particularly, the present
invention relates to novel use of an extract of the aril
of nutmeg, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-
3-methoxyphenyl)-propane, austrobailignan 7 or licarin E,
for preventing or treating diabetes or PPAR γ -mediated
10 diseases.

Background Art

PPAR(peroxisome proliferator activated receptor)is
nuclear receptor which bind with the compound increasing
15 number of peroxisome, named as peroxisome proliferator as
a ligand, and PPAR α , PPAR δ , isoform of PPAR γ has been
known (*J. Steroid Biochem. Molec. Biol.*, 51, 157, 1994;
Gene Expression, 4, 281, 1995; *Biochem. Biophys. Res.*
Commun., 224, 431, 1996).

20

PPAR mainly regulates expression of genes which are
related to lipid metabolism or differentiation of
adipocyte (*J. Invest. Dermatol.* 111, 1116-1121, 1998; *J.*
Med. Chem., 43, 527-550, 2000),and PPAR α which is an
25 isoform of PPAR mainly expressed in liver, retina,
adipose tissue, and related to oxidation of fatty acid or
neutralization of toxic materials, and inflammation.

PPAR γ is expressed mainly in fat cell, immune cell, adrenal gland, spleen, and intestine, and it is known as core regulator of fat cell differentiation. PPAR δ is not expressed in the manner of tissue-specific, rather it is
5 expressed broadly and its function is not identified. (*Endocrinology.*, 137, 354, 1996).

Since PPAR plays an important role in fat metabolism, a lot of researches about developing
10 treatment reagent for PPAR mediated metabolic diseases are in progress. If PPAR γ is activated, sugar acceptability of fat cell become maximized, and differentiation of fat cell is promoted, and then insulin resistance is reduced (*Tren. Pharmacol. Sci.*, 25: 331-336,
15 2004). Accordingly, ligand of PPAR γ may play a role as a treating reagent for diabetes which is caused by insulin resistance. The TZD reagents such as pioglitazone and rosiglitazone are typical ligands of PPAR γ and used as treating reagents for diabetes mellitus.

20

The diabetes may be classified into type 1 and type 2. The type 1 diabetes is autoimmune disease which pancreas induce immunological reaction by genetic
25 susceptibility and viral infection, and β -cells are selectively destroyed. The type 2 diabetes is the type which insulin resistance is priority occurred and reported

that caused by complicated factors such as obesity, reduction of insulin receptor, reduction of tyrosine kinase activity, reduction of muscle/adipose tissue type transporter in muscle and fat tissues, deficiency of
5 insulin receptor substrate-1(IRS-1) in the cells. The type 2 diabetes is mainly occurred in adult and it occupies 90% of diabetes (*Drugs*, 65: 433-445, 2005).

10 Meanwhile, the nutmeg is a nut of *Myristica fragrans* which is a perennial plant cultured in tropical regions, and has been used as spices for a long time. In addition, the extract of the aril of nutmeg is peel region of the said fruit of *Myristica fragrans* and is
15 reported that it has inhibition of proliferation of *Helicobacter pylori*(*In Vivo.*, 17;541-4, 2003), activation of detoxification in liver(*Food Chem. Toxicol.*, 31;517-21, 1993), chemical prevention of skin verruca(*Cancer Lett.*, 56;59-63, 1991), anti-inflammation activity(*Jpn. J. Pharmacol.*, 49;155-63, 1999). However, the relationship
20 between the nutmeg or the extract of the aril of nutmeg and PPAR or anti-diabetic use thereof have never been reported as yet.

25 Therefore, the inventors of the present invention have screened many kind of natural materials, to find active component which preventing or treating insulin

resistance diabetes, and then identified that 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan 7, licarin E which are isolated from the extract of the aril of nutmeg have activity in preventing or treating diabetes or PPAR γ mediated diseases, thereby completing the present invention.

Disclosure

Technical Problem

Accordingly, it is an object of the present invention to provide a novel use of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan 7, and licarin E which are isolated from the aril of nutmeg for preventing or treating diabetes or PPAR γ mediated diseases.

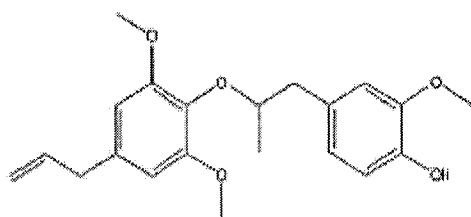
Technical Solution

To achieve the above objects, the present invention provides a pharmaceutical composition comprising a C1-C6 organic solvent extract of the aril of nutmeg as an effective component for preventing or treating diabetes.

In another aspect, the present invention provides a pharmaceutical composition comprising one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or

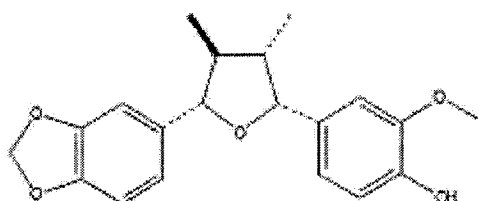
their pharmaceutically acceptable salts as an effective component for preventing or treating diabetes.

<Formula 1>

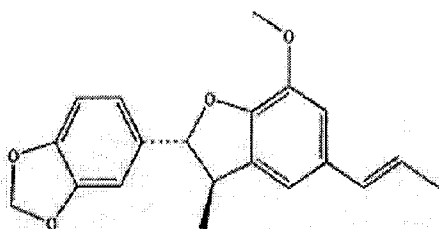


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<Formula 2>



<Formula 3>



10 In another aspect, the present invention provides a pharmaceutical composition comprising one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or
15 their pharmaceutically acceptable salts as an effective component for preventing or treating PPARγ(peroxisome

proliferator activated receptor γ) mediated diseases.

In another aspect, the present invention provides a use of a C1-C6 organic solvent extract of the aril of
5 nutmeg for preparing a treating reagent of diabetes.

In another aspect, the present invention provides method comprising administering an effective amount of C1-C6 organic solvent extract of the aril of nutmeg to a
10 subject in need thereof.

In another aspect, the present invention provides a use of one which is selected from the group consisting of a compound represented by Formula 1, a compound
15 represented by Formula 2, and a compound represented by Formula 3, or their pharmaceutically acceptable salts for preparing a treating reagent of diabetes.

In another aspect, the present invention provides a
20 method comprising administering an effective amount of one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or their pharmaceutically acceptable salts for preventing or
25 treating diabetes.

In another aspect, the present invention provides a

use of one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or their pharmaceutically acceptable salts for preparing a treating reagent of PPAR γ mediated diseases.

In another aspect, the present invention provides a method comprising administering an effective amount of one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or their pharmaceutically acceptable salts for preventing or treating PPAR γ mediated diseases.

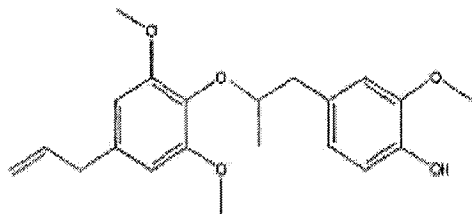
Hereafter, the present invention will be described in detail.

The present invention relates to novel use of an extract of the aril of nutmeg, and more particularly to novel use of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E, separated from the extract of the aril of nutmeg.

The inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E, which have antidiabetic activity, are compounds having structures of the following formulas 1,

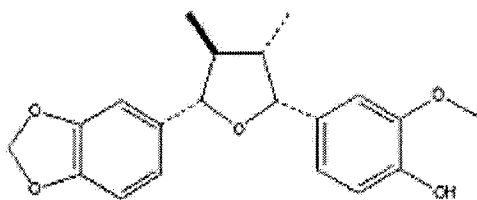
2 and 3, respectively, and can be separated and purified from nutmeg or the aril of nutmeg using a prior method for extracting and separating material:

<Formula 1>

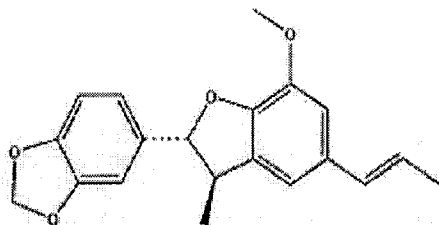


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<Formula 2>



<Formula 3>



10

Preferably, the 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E of the present invention can be obtained using a conventional extraction and separation method.

15 For example, an extract of the aril of nutmeg is obtained by placing the dried aril of nutmeg in an extractor

together with a 4-10 fold volume of an extraction solvent, allowing the plant to stand for 2 days to obtain an extract, and concentrating and drying the extract in a concentrator. As the extraction solvent for separation
5 in the present invention, water or a C1-C6 organic solvent may be used. Preferably, various solvents, including purified water, methanol, ethanol, propanol, isopropanol, butanol, acetone, ether, benzene, chloroform, ethyl acetate, methylene chloride, hexane, cyclohexane,
10 petroleum ether and the like, may be used alone or in a mixture thereof. The extract obtained as described above is subjected to silica gel column chromatography to obtain fractions according to polarity, and the separated specific fraction is subjected to reverse phase column
15 chromatography and high-performance liquid chromatography (HPLC), thus separating 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E from the fraction. However, the method for extracting and separating the active ingredients is not
20 necessarily limited to the above-described method.

The aril of nutmeg that is used in the present invention indicates the aril of the fruit of *Myristica fragrans*, and the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane,
25 austrobailignan-7 and licarin E, separated from the extract of the aril of nutmeg, act as ligands for PPAR γ

to activate PPAR γ . This fact has been found for the first time by the present inventors.

In one Example of the present invention, a known method for finding a ligand, which activates a gene
5 having a PPAR response element (PPRE), by binding to PPAR γ , was used to confirm that the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E bind to PPAR, particularly PPAR γ , to activate PPAR (FIGS. 12 and 20).

10 When PPAR γ is activated, it binds to a DNA sequence, PPRE (PPAR response element), to regulate the expression of the target genes of PPAR γ , and the expression of the target genes of PPAR γ , which are mainly involved in lipid metabolism, is increased. Thus, in another Example of
15 the present invention, it was examined whether the expression of aP2, LPL, PEPCK and GLUT4, known as the target genes of PPAR, is increased. As a result, it could be seen that the expression of the target genes of PPAR γ was significantly increased upon treatment with 2-
20 (4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E (see FIGS. 13 and 21).

Thus, the present inventors have found for the first time that the inventive 2-(4-allyl-2,6-
25 dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7, licarin E, or pharmaceutically acceptable salts thereof, have an activity to prevent or

treat PPAR γ -mediated diseases. Accordingly, the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7, licarin E, or pharmaceutically acceptable salts thereof, can be effectively used as active ingredients in pharmaceutical compositions for preventing or treating PPAR γ -mediated diseases.

As used herein, PPAR γ -mediated diseases means the diseases which symptoms are prevented, treated, or relieved by activation of PPAR γ . Preferably, the said PPAR γ -mediated diseases may comprise, but not limited thereto, type 2 diabetes(NIDDM; non-insulin-dependent diabetes mellitus), hyperinsulinemia, obesity, hyperglycemia, hyperlipidemia, syndrome X, hypercholesterolemia, hyperlipoproteinemia, atherosclerosis, hypertension, insulin resistance, dysmetabolic syndrome, diabetic complications, impaired glucose homeostasis, impaired glucose tolerance, hypertriglyceridemia, osteoporosis(*J. Biol. Chem.* 275: 14388-14393, 2000), glomerulonephritis(*Kidney Int.*, 60: 14-30, 2001) or kidney diseases caused by diabetes(*Kidney Int.*, 60: 14-30, 2001).

Meanwhile, it is known that, as PPAR γ becomes activated, the sugar absorption of adipocytes is maximized and adipocyte differentiation is stimulated

(*Trends in Pharmacological Sciences*, 25: 331-336, 2004). Thus, in another Example of the present invention, it was examined whether the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 activate PPAR γ to stimulate adipocyte differentiation. As a result, it was seen that the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 stimulated the differentiation of preadipocytes 3T3-L1 cells into adipocytes (see FIG. 14).

Meanwhile, ob/ob mice continually ingest excessive food because their appetite is not regulated due to the deletion of a leptin gene. As a result, fat is excessively accumulated in the body, and at about 3 months after birth, the mice maintain a weight of about 50 g, reaching about 2 times the weight of wild-type mice. Also, the mice become type 2 diabetic models having a blood glucose level higher than that of wild-type mice (*Exp. Clin. Endocrinol. Diabetes*, 109: 307-319, 2001). Such ob/ob mice are typical experimental animal models which are used to investigate anti-obesity agents and anti-diabetic agents or to evaluate anti-obesity and anti-diabetic effects. Thus, the action of the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E was examined in an ob/ob mouse model. As a result, the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-

3-methoxyphenyl)-propane, austrobailignan-7 and licarin E significantly reduced a blood glucose level in the ob/ob mouse model, suggesting that they have the effect of preventing diabetes, particularly type 2 diabetes.

5 Accordingly, it could be found that the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E were PPAR ligands, particularly PPAR γ ligands increasing insulin sensitivity, had the effect of preventing obesity, 10 that is a PPAR γ -mediated disease, metabolic diseases hyperlipidemia and cardiovascular diseases by significantly reducing weight and blood triglyceride levels in the ob/ob mice model, and had the effect of preventing diabetes, particularly type 2 diabetes, by 15 reducing blood glucose levels.

Accordingly, the present invention provides a pharmaceutical composition comprising, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, 20 austrobailignan-7 and licarin E or their pharmaceutically acceptable salts as an effective component for preventing or treating diabetes.

The compounds according to the present invention 25 may be used in the form of itself or a salt, and preferably a pharmaceutically acceptable salt. As used herein, the term "pharmaceutically acceptable" means what

is physiologically acceptable and, when administered to human beings, generally does not cause allergic reactions, or similar reactions thereto. Preferably, the salt is the acid-addition salt formed by a pharmaceutically acceptable free acid. The free acid used in the present invention may be organic acids and inorganic acids. The organic acids include, but are not limited to, citric acid, acetic acid, lactic acid, tartar acid, maleic acid, fumaric acid, formic acid, propionic acid, oxalic acid, trifluoroacetic acid, benzoic acid, gluconic acid, methane sulfonic acid, glycolic acid, succinic acid, 4-toluene sulfonic acid, glutamic acid and aspartic acid. Also, the inorganic acids include, but are not limited to, hydrochloric acid, bromic acid, sulfuric acid and phosphoric acid.

As used herein, diabetes means, but not limited thereto, type 2 diabetes, and when it administered for the purpose of treating diabetes, it may be administered by oral or parenteral routes with proper types of individual dose composition. The common drug formulations may be prepared using diluents such as fillers, thickeners, binders, wetting agents, disintegrants, or excipients. Solid formulations for oral administration include tablets, pills, powders, granules, and capsules and the said Solid formulations are manufactured by mixing one or more excipient, for example, starch,

calcium carbonate, sucrose or lactose and gelatin, with 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 or licarin E. Also, except the simple excipient, lubricant such as magnesium stearate or talc may be used. Examples of liquid formulations for oral administration include suspensions, liquids, emulsions and syrups. The liquid formulations may comprise a simple diluent such as water, liquid paraffin, and various excipients, for example, humectants, sweet agents, aromatic agents and preservatives. Examples of pharmaceutical formulations for parenteral administration include sterilized aqueous solutions, non-aqueous solutions, suspensions, emulsions, freeze-dried preparations, ointments and creams. The non-aqueous solutions and suspensions may be prepared using propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable esters such as ethyl oleate.

Also, the composition of the present invention may be administered via the parenteral administration and the parenteral administration includes subcutaneous, intravenous, intramuscular or intraperitoneal injection. For parenteral administration, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 or licarin E of the present invention may be mixed with a stabilizer or buffer in water to

prepare a solution or suspension, which may then be provided as ampoules or vials each containing a unit dosage form. The dosage units can contain, for example, 1, 2, 3, or 4 times of an individual dose or 1/2, 1/3 or 1/4 times of an individual dose. An individual dose preferably contains the amount of an effective drug which is given in one administration and which usually corresponds to all, 1/2, 1/3 or 1/4 times a daily dose. Suitable administration dose may be changed by considering various factors, such as body weight, age, sex, health condition, diet, administration time, administration route, excretion of a subject and mixture of component, and disease severity.

The said pharmaceutical compositions are well described in Remington's Pharmaceutical Science, 15th Edition, 1975. Mack Publishing Company, Easton, Pennsylvania 18042, Chapter 87: Blaug, Seymour which is general prescription book in chemical pharmacology.

20

2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 or licarin E of the present invention are orally administered to rats for toxicology test, and it is shown that 50% Lethal dose(LD 50) is 2,000mg/kg.

25

In another aspect, this invention provides a use of

C1-C6 organic solvent extracts of the aril of nutmeg for preparing diabetes treating reagent. The method of extraction and isolation for preparing extracts of the aril of nut is well described above, and it may be used
5 to those skilled in the art according method which is well known in the art for preparing diabetes treating reagent.

In addition, the present invention provides a
10 method comprising administering C1-C6 organic solvent extracts of the aril of nutmeg for preventing or treating PPAR γ mediated diseases.

The method of extraction and isolation for
15 preparing extracts of the aril of nut is the same as described above. In addition, administration method of the said organic solvent extract is the same as described and it is not limited thereto, as far as it sustain effect on preventing or treating diabetes.

20

In another aspect, the present invention provides a use of one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by
25 Formula 3, or their pharmaceutically acceptable salts for preparing a treating reagent of diabetes.

Because a compound represented by Formula 1, a

compound represented by Formula 2, and a compound represented by Formula 3 have excellent effect on treating diabetes, it may be used for preparing diabetes treating reagent by those skilled in the art with well known skills thereof.

In addition, the present invention provides a method comprising administering an effective amount of one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or their pharmaceutically acceptable salts for preventing or treating diabetes.

The administration method of the said a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3 is the same as described and it is not limited thereto, as far as it sustain effect on preventing or treating diabetes.

In another aspect, the present invention provides a use of one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or their pharmaceutically acceptable salts for preparing a treating reagent of PPAR γ -mediated diseases.

Because a compound represented by Formula 1, a compound represented by Formula 2, and a compound

represented by Formula 3 have excellent effect on PPAR γ -mediated diseases, it may be used for preparing PPAR γ -mediated diseases treating reagent by those skilled in the art with well known skills thereof. The PPAR γ -
5 mediated disease means the disease which is prevented, cured, reduced, or moderated by PPAR γ activation.

In addition, the present invention provides a method comprising administering an effective amount of
10 one which is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or their pharmaceutically acceptable salts for preventing or treating PPAR γ -mediated disease.

15 The administration method of the said a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3 is the same as described and it is not limited thereto, as far as it sustain effect on preventing or treating PPAR γ -
20 mediated disease.

As used herein, the term "subject in need" means mammals, which need prevention and treatment of PPAR γ -mediated disease, but not limited thereto, diabetes, and
25 preferably it means human beings.

The inventive compositions may be administered to patients with the amount of single dose and may be

administered with the amount of multiple doses in fractionated treatment protocol and the dose level may vary according to disease condition. Preferably, the effective amount of the inventive composition is about 5 0.01 μ g to 1,000mg/kg body weight/day, and most Preferably it may be 0.1 μ g to 100mg/kg body weight/day. However, because it may be suitably determined by considering various factors, such as age, body weight, health condition, sex, disease severity, diet and excretion of a 10 subject in need of treatment, as well as administration time and administration route, if he is skilled in the art, he could determine proper effective amount for administration for preventing or treating diabetes or PPAR γ -mediated disease. However, the pharmaceutical 15 composition, administration route, and administration method is not limited as far as it shows effect of the present invention.

Advantageous Effects

20 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 and licarin E of the present invention are compounds which are isolated from the aril of nutmeg extract, and the said compound and the aril of nutmeg extract act as ligand of PPAR γ and 25 activated PPAR γ . Therefore, they may be used for preventing and treating PPAR γ -mediated diseases, preferably, but not limited thereto, such as diabetes and

diabetic complication. Accordingly, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7, licarin E, and the aril of nutmeg extract may be used as preventing and treating reagent for PPAR γ -mediated diseases, preferably, but not limited thereto, such as diabetes and diabetic complication.

Description of Drawings

FIG.1 shows isolation scheme of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan-7 from the aril of nutmeg.

FIG.2 shows ^{13}C -NMR spectrum of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane.

FIG.3 shows ^1H -NMR spectrum of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane.

FIG.4 shows ^1H - ^1H -COSY spectrum of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane.

FIG.5 shows ^1H - ^{13}C -HMBC spectrum of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane.

FIG.6 shows FAB/Mass spectrum of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane.

FIG.7 shows ^{13}C -NMR spectrum of austrobailignan-7.

FIG.8 shows ^1H -NMR spectrum of austrobailignan-7.

FIG.9 shows ^1H - ^1H -COSY spectrum of austrobailignan-7.

FIG.10 shows ^1H - ^{13}C -HMBC spectrum of austrobailignan-7.

FIG.11 shows FAB/Mass spectrum of austrobailignan-7.

FIG.12 is a graph showing PPAR γ activating effect of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 in dose-dependant manner.

FIG. 13 shows effect of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 on activating target gene expression.

A : aP2

B : LPL

C : PEPCK

D : GLUT4

FIG.14 shows effect of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 on activating fat cell differentiation in 3T3-L1 mouse fibroblast cells.

FIG.15 shows isolation scheme of licarin E 7 from the aril of nutmeg.

FIG.16 shows FAB/Mass spectrum of licarin E 7.

FIG.17 shows ^{13}C -NMR spectrum of licarin E 7.

FIG.18 shows ^1H -NMR spectrum of licarin E 7.

FIG.19 shows ^{13}C -DEPT spectrum of licarin E 7.

FIG.20 is a graph showing PPAR γ activating effect of licarin E 7 in dose-dependant manner.

FIG.21 shows effect of licarin E 7 on activating

target gene expression.

A : LPL

B : aP2

5 Mode for Invention

Hereinafter, the present invention will be described in detail by examples. It is to be understood, however, that these examples are for illustrative purpose only and are not constructed to limit the scope of the present invention. In addition, every activity analysis were performed 3 times and the results are shown as Means $\pm$ SD.

Example 1: Separation and purification of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 from aril of nutmeg and determination of structures thereof

<1-1> Separation and purification of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7

100 g of the dried and ground aril of nutmeg was added to 400 ml of 75% methanol and left to stand at room temperature for 2 days. The extracted solution was filtered and concentrated in a vacuum, thus preparing 13.26 g of a methanol extract of the aril of nutmeg. The extracted solution was fractionated with ethylacetate, butanol and water. The ethylacetate fraction (10.29 g)

was eluted by silica gel column chromatography with a mixed solution of chloroform and ethylacetate (9:1 (v/v)) to obtain fraction V (0.52 g) and fraction VI (0.19 g). The fraction V was subjected to column chromatography with a mixed solvent of chloroform, ethylacetate and acetone (24:0.3:1 (v/v/v)) to obtain fraction V-C (0.05 g). Then, the fraction V-C was eluted by recycling high-performance liquid chromatography with 100% methanol to obtain single material fraction V-C-2 (0.03 g; found to be 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane in Example 1-2 below). Also, the fraction V was subjected to column chromatography with a mixed solvent of chloroform, ethylacetate and acetone (30:1:2 (v/v/v)) to obtain fraction VI-B (0.52 g). Then, the fraction VI-B was eluted by recycling high-performance liquid chromatography with 100% methanol to obtain single material fraction VI-B-5 (0.011 g; found to be austrobailignan-7 in Example 1-3 below). This separation process is shown in FIG. 1.

<1-2> Structural analysis of single material V-C-2

To determine the structure of the separated single material V-C-2, the ^1H -NMR spectrum and ^{13}C -NMR spectrum of the material were measured at 600 MHz and 150 MHz (solvent: CDCl_3), respectively. The measurement results are shown in FIGS. 2 and 3. The results of comprehensive analysis of ^1H -NMR and ^{13}C -NMR are shown in Table 1 below.

[Table 1]

25

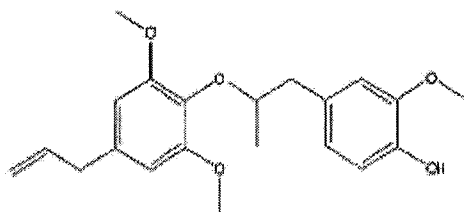
Position	^{13}C -NMR	^1H -NMR
1	134.5	
2	112.4	6.77 (1H, d)
3	146.3	3.82 (3H, s, 3-OMe)
4	144.0	5.48 (1H, s, -OH)
5	114.1	6.82 (1H, d)
6	122.3	6.70 (1H, dd)
α	43.0	2.72 (1H, dd) 3.12 (1H, dd)
β	79.8	4.33 (1H, dd)
γ	19.6	1.20 (3H, d)
1'	135.6	
2'	153.8	3.80 (6H, s)
3'	105.7	6.40 (2H, d', 5'-H)
4'	131.2	
5'	105.7	6.40 (2H, 3', 5'-H)
6'	153.8	3.80 (6H, s, 2'-OMe, 6'-OMe)
α	40.5	3.34 (2H, d)
β	137.4	5.92-6.02 (1H, m)
γ	115.8	5.07-5.15 (2H, m)
-OMe	56.0	
	56.2	

In order to measure ^1H - ^1H correlation and ^1H - ^{13}C correlation on the basis of the results of the ^{13}C -NMR spectrum and the ^1H -NMR spectrum, the ^1H - ^1H COSY spectrum and the ^1H - ^{13}C HMBC spectrum were measured. The measurement results are shown in FIGS. 4 and 5,

respectively. The results of FAB/MS measured for the mass spectrometry of the separated single material are shown in FIG. 6. The $[M]^+$ of the single material was observed at m/z 358 in FAB/MS, suggesting that the material had a molecular weight of 358 and a molecular formula of $C_{21}H_{26}O_5$.

Through the above results of 1H -NMR, ^{13}C -NMR, 1H - 1H COSY, 1H - ^{13}C HMBC and FAB/MS and the prior reference (*Chem. Pharm. Bull.*, 35: 668, 1987), the separated single material was found to be 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane represented by the following formula 1:

<Formula 1>



15

<1-3> Structural analysis of single material VI-B-5

To determine the structure of the separated single material VI-B-5, the 1H -NMR spectrum and ^{13}C -NMR spectrum of the single material were measured at 600 MHz and 150 MHz (solvent: $CDCl_3$). The measurement results are shown in FIGS. 7 and 8, respectively. The results of comprehensive analysis of 1H -NMR and ^{13}C -NMR are shown in Table 2 below.

[Table 2]

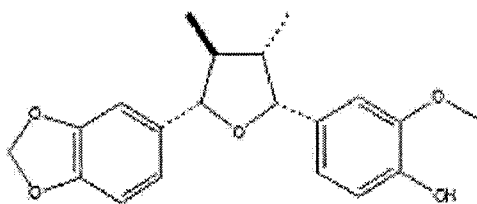
Position	^{13}C -NMR	^1H -NMR
1	137.4	
2	108.9	6.92 (1H, d)
3	148.1	
4	147.1	
5	106.7	6.78 (1H, d)
6	119.8	6.82 (1H, dd)
α	85.9	4.61 (1H, d)
β	43.7	2.39-2.43 (2H, m)
γ	12.0	1.00 (3H, d)
1'	132.7	
2'	119.0	6.91 (1H, d)
3'	114.2	
4'	144.5	5.54 (1H, s, -OH)
5'	146.5	6.88 (1H, d)
6'	108.2	6.77 (1H, dd)
α'	85.0	5.42 (1H, d)
β'	47.9	2.39-2.43 (2H, m)
γ'	9.6	0.61 (3H, d)
-OMe	56.2	3.89 (3H, s)
-OCH ₂ O-	101.1	5.94 (1H, d)
		5.95 (1H, d)

To measure ^1H - ^1H correlation and ^1H - ^{13}C correlation on the basis of the results of the ^{13}C -NMR spectrum and the ^1H -NMR spectrum, the ^1H - ^1H COSY spectrum and the ^1H - ^{13}C HMBC spectrum were measured. The measurement results are shown in FIGS. 9 and 10, respectively. The results of

FAB/MS measured for the mass spectrometry of the separated single material are shown in FIG. 11. The $[M]^+$ of the separated single material was observed at m/z 342 in FAB/MS, suggesting that the material had a molecular weight of 342 and a molecular formula of $C_{20}H_{22}O_5$.

Through the above results of 1H -NMR, ^{13}C -NMR, 1H - 1H COSY, 1H - ^{13}C HMBC and FAB/MS and the prior reference (Aust. *J. Chem.*, 28: 81, 1975), the separated single material was found to be austrobailignan-7 represented by the following formula 2:

<Formula 2>



<1-4> Separation and purification of licarin E

100 g of the dried and ground aril of nutmeg was added to 400 ml of 100% ethanol and left to stand at room temperature for 5 days. The extracted solution was filtered and concentrated in a vacuum, thus preparing 26.61 g of an ethanol-extract of the aril of nutmeg. The extract was fractionated with ethylacetate, butanol and water. The ethylacetate fraction (18.44 g) was eluted by silica gel column chromatography with a mixed solvent of chloroform and ethylacetate (9:1 (v/v)) to obtain

fraction II (3.04 g). The fraction II was subjected to column chromatography with a mixed solvent of chloroform, hexane, ethylacetate and acetone (15:10:0.1:0.1 (v/v/v/v)) to obtain fraction II-B (0.4 g). Then, the
 5 fraction II-B was eluted by recycling high-performance liquid chromatography with 100% methanol to obtain protein fraction II-B-2 (0.016 g; found to be licarin E in Example 1-5 below). This separation process is shown in FIG. 15.

10 <1-5> Structural analysis of single material II-B-2

To determine the structure of the separated single material II-B-2, the ^1H -NMR spectrum and ^{13}C -NMR spectrum of the separated material were measured at 600 MHz (solvent: CDCl_3). The measurement results are shown in
 15 FIGS. 17 and 18, respectively. The results of comprehensive analysis of ^1H -NMR and ^{13}C -NMR are shown in Table 3 below.

[Table 3]

Carbon No. ^	δ_{C}	δ_{H}
1		
2	93.0	
3	45.5	1.35 (3H, d, 7-OMe)
3a	132.7	5.48 (1H, s, -OH)
4	113.0	6.82 (1H, d)
5	131.8	6.70 (1H, dd)
6	109.2	5.15 (d, 9)
7	143.7	3.2-3.7 (m)
7a	146.2	1.35 (3H, d, 7)

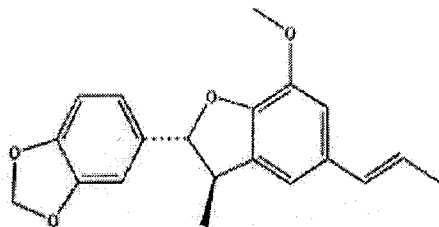
3-Me	17.6	5.92
OMe	55.7	
1'	134.0	
2'	106.3	3.80 (6H, s)
3'	147.5	3.88 (s)
4'	147.2	
5'	107.7	6.40 (2H, 3', 5'-H)
6'	119.7	3.80 (6H, s, 2'-OMe, 6'-OMe)
α	130.6	3.34 (2H, d)
β	122.9	5.92-6.02 (1H, m)
γ	18.1	1.84 (3H, d, 5)

Based on the ^{13}C -NMR spectrum and the ^1H -NMR spectrum, the ^{13}C -DEPT spectrum of the separated material was measured. The measurement results are shown in FIG.

5 19. The results of FAB/MS measured for the mass spectrometry of the separated single material are shown in FIG. 16. The $[\text{M}]^+$ of the separated material was observed at m/z 324 in FAB/MS, suggesting that the separated material had a molecular weight of 324 and a
10 molecular formula of $\text{C}_{20}\text{H}_{20}\text{O}_4$.

Through the above results of ^1H -NMR, ^{13}C -NMR, ^{13}C -DEPT and FAB/MS and the prior reference (*J.Chromatogr.* 110, 91-102), the separated material was found to be licarin E represented by the following formula 3:

15 <Formula 3>



Example 2: Activation of peroxisome proliferator
activated receptor γ (PPAR γ) by 2-(4-allyl-2,6-
5 dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane
and austrobailignan-7

In order to confirm whether 2-(4-allyl-2,6-
dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane
and austrobailignan-7 act as ligands for PPAR γ , a test
10 was carried out according to a known method using a
vector, having a PPAR γ expression plasmid and a
luciferase gene under the control of PPRE (peroxisome
proliferator responsive element) (*Cell*, 68: 879-887,
1992; *J. Biol. Chem.*, 272: 25252-25259, 1997).

15 The activation of luciferase expression was
measured by transfecting COS-7 monkey kidney cells (ATCC
CRL-1651) with a PPAR γ plasmid and a pFR-luciferase
vector (Stratagene, USA), and then treating the cells
with 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-
20 methoxyphenyl)-propane and austrobailignan-7, separated
in Example 1, for 24 hours. The PPAR γ plasmid was
prepared by performing RT-PCR with primers of SEQ ID NO:
1 (TCGGTTTAAGATTCATCTTTATT) and SEQ ID NO: 2

(GTCTCCGGTACCTTGATCACCTGC) to obtain a PCR product encoding amino acids 176-477 (PPAR γ ligand binding domain) of the entire amino acid sequence of human PPAR γ (Genbank Accession No. NM_138712), and then by cloning
5 the PCR product into the XbaI and KpnI restriction enzyme sites of a pFA vector (Stratagene, USA). RT-PCR was performed by synthesizing cDNA at 42 °C for 60 minutes using reverse transcriptase, and then by performing PCR using Taq polymerase in the following conditions: 30
10 cycles of 1 min at 95 °C, 30 sec at 54 °C and 2 min at 72 °C. Herein, test groups treated with each of the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 at varying concentrations of 3 μ M, 5 μ M and 10 μ M were compared with
15 a control group treated with 0.01% DMSO (dimethyl sulfoxide) and a group treated with 5 μ M troglitazone (Sigma, USA), a compound known as a PPAR γ ligand.

As a result, as shown in FIG. 12, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane
20 and austrobailignan-7 increased PPAR γ activity in a concentration-dependent manner and showed a significant difference (**, $p < 0.01$) from the control group at all the treatment concentrations. For example, at a treatment concentration of 5 μ M, the activity of the
25 comparative compound troglitazone was about 10-fold higher than that of the control group, and luciferase activities induced by the inventive 2-(4-allyl-2,6-

dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 were higher than that of the control group by about 6.6 times and about 3.1 times, respectively. This suggests that the inventive natural materials, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7, acted as PPAR γ ligands to activate PPAR γ , and additionally showed activity similar to that of the synthetic material troglitazone that is a known PPAR γ ligand, thus effectively activating PPAR γ .

Example 3: Examination of expression of target gene resulting from PPAR γ activation caused by 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7

3T3-L1 mouse preadipocytes (ATCC CL-173), cultured in 10% FBS (fetal bovine serum)-containing DMEM (Dulbeccos Modified Eagles Medium) medium, were plated at a concentration of 1×10^6 cells/well and were additionally cultured for 5 hours. To the medium of the cultured cells, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 were added at concentrations of 5 μ M and 10 μ M, and the cells were left to stand for 24 hours. The medium of the cultured cells was replaced with MDI (0.5 mM 3-isobutyl-1-methylxanthine, 0.5 μ M dexamethasone, 10 μ g/ml insulin) medium to induce the differentiation of the adipocyte cells (*Am. J. Physiol. Cell Physiol.*, 280: C807-C813,

2001). After 2 days from the time of replacement with the MDI medium, the cells were collected, and the total RNA of the cells was isolated using TRIZOL (Invitrogen, USA). The isolated total RNA was quantified, and then

5 the RNA in the same amount for each sample was synthesized into cDNA at 42 °C for 20 minutes using reverse transcriptase. The synthesized cDNA was subjected to RT-PCR using Taq polymerase with each of an aP2 amplification primer pair (SEQ ID NO: 3

10 (ACAGCTCCTCCTCGAAGG) and SEQ ID NO: 4 (GCGTAAATGGGGATTGGTCA)), an LPL amplification primer pair (SEQ ID NO: 5 (TATCCGCGTGATTGCAGAGA) and SEQ ID NO: 6 (AGAGAGTCGATGAAGAGATGAATGG)), a PEPCK amplification primer pair (SEQ ID NO: 7 (CAGGCGGCTGAAGAAGTATGA) and SEQ

15 ID NO: 8 (AACCGTCTTGCTTTCGATCCT)) and a GLUT4 amplification primer pair (SEQ ID NO: 9 (ATCCATGGATGGACGGTAACG) and SEQ ID NO: 10 (CTGGATCCCAATACTTCGACCA)) in the following conditions: 30 cycles of 1 min at 95 °C, 30 sec at 56 °C and 2 min at

20 72 °C.

As a result, as shown in FIG. 13, the mRNA expressions of the target genes aP2, LPL, PEPCK and GLUT4, the expressions of which are increased due to PPAR γ , were significantly (**, $p < 0.01$; *, $p < 0.05$) increased in

25 all the test groups compared to the control group in proportion to the concentrations of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane

and austrobailignan-7. This suggests that the inventive
2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-
methoxyphenyl)-propane and austrobailignan-7 can activate
PPAR γ to regulate the expression of the target genes of
5 PPAR γ .

Example 4: Examination of stimulation of adipocyte
differentiation resulting from PPAR γ activation caused by
2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-
methoxyphenyl)-propane and austrobailignan-7

10 3T3-L1 mouse preadipocytes, cultured in 10% FBS-
containing DMEM medium, were plated at a concentration of
1 x 10⁶ cells and cultured for 5 hours. To the cultured
cells, each of the inventive 2-(4-allyl-2,6-
dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane
15 and austrobailignan-7 was added at a concentration of 10
 μ M, and the cells were left to stand for 24 hours. The
medium of the cultured cells was replaced with an MDI
(0.5 mM 3-isobutyl-1-methylxanthine, 0.5 μ M dexamethasone
and 10 μ g/ml insulin) medium to induce adipocyte
20 differentiation (*Am. J Physiol. Cell Physiol.*, 280: C807-
C813, 2001). After 2 days from the time of replacement
with the MDI medium, the morphology of the cells was
observed with an optical microscope.

As a result, as can be seen in FIG. 14, adipocyte
25 differentiation in the groups treated with each of 10 μ M
2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-
methoxyphenyl)-propane and 10 μ M austrobailignan-7,

respectively, was stimulated compared to the control group treated with 0.01% DMSO and the group treated with 10 μ M troglitazone. This suggests that the inventive 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane and austrobailignan-7 activated PPAR γ to maximize the sugar absorption of adipocytes, thus stimulating adipocyte differentiation.

Example 5: Examination of anti-diabetic effect of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane in ob/ob mouse model

Ob/ob mice used as diabetic model animals continually ingest excessive food because their appetite is not regulated due to the deletion of a leptin gene. As a result, fat is excessively accumulated in the body, and the ob/ob mice become typical type 2 diabetic models, in which fat is excessively accumulated in the body and which have blood glucose levels higher than those of wild-type mice. Thus, in order to examine the effect of preventing and treating diabetes, four 10-week-old ob/ob mice were used in each of a test group and a control group. In the case of the test group, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane was diluted in corn oil and administered to the mice at a dosage of 20 mg/kg one time a day for 10 days, and in the case of the control group, only the same amount of corn oil was administered. At 10 days after the start of

administration, the blood glucose levels of the test group and the control group were measured and analyzed.

As a result, the control group was maintained at a blood glucose level of 456.23 ± 15.27 mg/dl, which was higher than a blood glucose level of 226.68 ± 13.26 mg/dl for normal mice, whereas the group treated with 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane showed a blood glucose level of 316.95 ± 22.76 mg/dl, which was significantly (*, $p < 0.01$) lower than that of the control group by 30.53%, suggesting that 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane had the effect of preventing and treating diabetes.

Example 6: Examination of anti-diabetic effect of austrobailignan-7 in ob/ob mouse model

In order to examine the effect of preventing and treating diabetes, four 10-week-old ob/ob mice were used in each of a test group and a control group. In the case of the test group, austrobailignan-7 was diluted in corn oil and administered to the mice at a dosage of 20 mg/kg one time a day for 10 days, and in the case of the control group, only the same amount of corn oil was administered to the mice. At 10 days after the start of administration, the blood glucose levels of the test group and the control group were measured and analyzed.

As a result, the control group was maintained at a blood glucose level of 456.23 ± 15.27 mg/dl, which was

higher than a blood glucose level of 226.68 ± 13.26 mg/dl for normal mice, whereas the group treated with austrobailignan-7 showed a blood glucose level of 352.95 ± 19.54 mg/dl, which was significantly (*, $p < 0.01$) lower than that of the control group by 22.64%, suggesting that austrobailignan-7 had the effect of preventing and treating diabetes.

Example 7: Activation of PPAR γ by licarin E

In order to examine whether licarin E acts as a PPAR γ ligand, a test was carried out according to the method of Example 2 and a known method using a vector, having a PPAR γ expression plasmid and a luciferase gene under the control of PPRE (*Cell*, 68: 879-887, 1992; *J. Biol. Chem.*, 272: 25252-25259, 1997).

The activation of luciferase expression was measured by COS-7 monkey kidney cells (ATCC CRL-1651) transfected with a PPAR γ plasmid and a pFR-luciferase vector (Stratagene, USA), and then treating the cells with licarin E, separated in Example 1, for 24 hours. RT-PCR was performed by synthesizing cDNA using reverse transcriptase at 42 °C for 60 minutes, and then performing PCR in the following conditions using Taq polymerase: 30 cycles of 1 min at 95 °C, 30 sec at 54 °C and 2 min at 72 °C. Herein, test groups treated with licarin E of the present invention at varying concentrations of 5 μ M, 10 μ M and 25 μ M were compared with a control group treated with 0.01% DMSO and a group

treated with 10 μ M troglitazone (Sigma, USA), a compound known as a PPAR γ ligand.

As a result, as shown in FIG. 20, licarin E increased PPAR γ activity in a concentration-dependent manner and showed a significant difference (**, $p < 0.05$) from the control group at all the treatment concentrations. For example, at a treatment concentration of 5 μ M, the activity of the comparative compound troglitazone was about 10 times higher than that of the control group, and luciferase activities induced by licarin E of the present invention were higher than that of the control group by about 5 times and about 8 times. This suggests that the natural material licarin E of the present invention acted as a PPAR γ ligand to activate PPAR γ , and additionally showed activity similar to that of the synthetic material troglitazone that is a known PPAR γ ligand, thus effectively activating PPAR γ .

Example 8: Examination of expression of target genes resulting from PPAR γ activation induced by licarin E

3T3-L1 mouse preadipocytes (ATCC CL-173), cultured in 10% FBS-containing DMEM medium, were plated at a concentration of 1×10^6 cells and additionally cultured for 5 hours. To the medium of the cultured cells, 10 μ M licarin E was added, and the cells were left to stand for 24 hours. The medium of the cultured cells was replaced with MDI (0.5 mM 3-isobutyl-1-methylxanthine, 0.5 μ M

dexamethasone and 10 µg/ml insulin) medium to induce adipocyte differentiation (*Am. J. Physiol. Cell Physiol.*, 280: C807-C813, 2001). After 2 days from the time of replacement with the MDI medium, the cells were collected, and the total RNA of the cells was isolated using TRIZOL (Invitrogen, USA). The isolated total RNA was quantified, and then the RNA in the same amount for each sample was synthesized into cDNA at 42 °C for 20 minutes using reverse transcriptase. The synthesized cDNA was subjected to RT-PCR using Taq polymerase with each of an aP2 amplification primer pair of SEQ ID NO: 3 and SEQ ID NO: 4 and an LPL amplification primer pair of SEQ ID NO: 5 and SEQ ID NO: 6 in the following conditions: 30 cycles of 1 min at 95 °C 30 sec at 56 °C and 2 min at 72 °C

As a result, as shown in FIG. 21, the mRNA expressions of the target genes aP2 and LPL, the expressions of which are increased due to PPAR γ , were significantly (**, $p < 0.01$) increased in all the test groups compared to the control group in proportion to the treatment concentration of II-B-2. This suggests that licarin E of the present invention can activate PPAR γ to regulate the expression of the target genes of PPAR γ .

Example 9: Examination of anti-diabetic effect of licarin E in ob/ob mouse model

In order to examine the effect of preventing and treating diabetes, four 10-week-old ob/ob mice were used in each test group. In the case of the test group,

licarin E was diluted in corn oil and administered to the mice at a dosage of 20 mg/kg one time a day for 10 days, and in the case of the control group, only the same amount of corn oil was administered to the mice. At 10
5 days after the start of administration, the blood glucose levels of the test group and the control group were measured and analyzed.

As a result, the control group was maintained at a blood glucose level of 456.23 ± 15.27 mg/dl, which was
10 higher than 226.68 ± 13.26 mg/dl for normal mice, whereas the group treated with licarin E showed a blood glucose level of 316.95 ± 22.76 mg/dl, which was significantly (*, $p < 0.01$) lower than that of the control group by 30.53%, suggesting that licarin had the effect of preventing and
15 treating diabetes.

Example 10: Examination of anti-diabetic effect of ethanol extract of aril of nutmeg in ob/ob mouse model

100 g of the dried and ground aril of nutmeg was added to 400 ml of ethanol and left to stand at room
20 temperature for 2 days. The extracted solution was filtered and concentrated in a vacuum, thus preparing 14.29 g of an ethanol extract of the aril of nutmeg.

The anti-diabetic effect of the prepared ethanol extract was examined in an ob/ob mouse model according to
25 the same method as in Example 5, and as a result, the blood glucose level of the group treated with the ethanol

extract was reduced by 37.6% compared to that of the control group.

Example 11: Examination of anti-diabetic effect of hexane extract of aril of nutmeg in ob/ob mouse model

5 100 g of the dried and ground aril of nutmeg was added to 400 ml of hexane and left to stand at room temperature for 2 days. The extracted solution was filtered and concentrated in a vacuum, thus preparing 16.78 g of a hexane extract of the aril of nutmeg.

10 The anti-diabetic effect of the prepared hexane extract was examined in an ob/ob mouse model according to the same method as in Example 5, and as a result, the blood glucose level of the group treated with the ethanol extract was reduced by 32.9% compared to that of the
15 control group.

Preparative example 1

Preparation of medicine comprising pharmaceutical composition for preventing and treating diabetes
20 according to the present invention

<1-1> Preparation of Tablets

First, 25mg of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan 7, licarin E or an extract of the aril of nutmeg of the
25 present invention was introduced into a U-shaped mixer together with 26mg of lactose and 3.5mg of Avicel (amorphous cellulose) as excipients for direct tablet

making, 1.5mg of sodium starch glyconate as a supplementary disintegrating agent, and 8mg of L-HPC (low-hydroxypropylcellulose) as a binder, and then the mixture were mixed for 20 minutes. After the mixing, 1mg
5 of magnesium stearate as a lubricant was further added thereto, followed by mixing for additional 3 minutes. The mixture was subjected to a weight determination test and an anti-humidity test. Then, tablets were made from the mixture and coated with a film to provide finished
10 tablets.

<1-2> Preparation of Syrup

Syrup comprising 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan 7,
15 licarin E or a pharmaceutically acceptable salt thereof in an amount of 2% (W/V) as an active component was prepared by the following process: 2g of an acid addition salt of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan 7 of the present
20 invention, 0.8g of saccharin and 25.4 g of sugar were dissolved into 80g of hot water. After cooling the solution, 8.0g of glycerin, 0.04g of a perfume, 4.0g of ethanol, 0.4g of sorbic acid and an adequate amount of distilled water were mixed with the solution. Water was
25 added to the resultant mixture to the final volume of 100ml.

<1-3> Preparation of Capsule

50mg of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan 7, licarin E or an extract of the aril of nutmeg of the present invention, 50mg of lactose, 46.5mg of starch, 1mg of talc and an adequate amount of magnesium stearate were mixed. Then, the mixture was filled into hard gelatin capsules to provide finished capsules.

10 <1-4> Preparation of Injection Solution

An injection solution comprising 10mg of the active component was prepared by the following process: 1g of hydrochloride salt of 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan 7, or licarin E of the present invention, 0.6g of sodium chloride and 0.1g of ascorbic acid were dissolved in distilled water to the final volume of 100ml. The solution was introduced into ampoules, and the ampoules were heated and sterilized at 120°C for 30 minutes.

20

Industrial Applicability

As can be seen from the foregoing, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)-propane, austrobailignan 7 or licarin E are the compounds isolated from an extract of the aril of nutmeg. The said compound and extract of the aril of nutmeg acts as ligands for

PPAR γ , and they may be used for preventing or treating PPAR γ -mediated diseases, not limited thereto, such as diabetes and diabetic complication. Accordingly, 2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-

5 methoxyphenyl)-propane, austrobailignan 7 or licarin E and extract of the aril of nutmeg may be useful as preventing or treating reagent for PPAR γ -mediated diseases, not limited thereto, such as diabetes and diabetic complication.

CLAIMS**Claim 1**

A pharmaceutical composition for preventing or treating diabetes comprising a C1-C6 organic solvent
5 extract of an aril of nutmeg as an active ingredient.

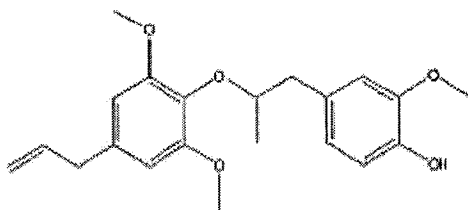
Claim 2

The composition according to claim 1, wherein the C1-C6 organic solvent is selected from the group
10 consisting of methanol, ethanol, propanol, isopropanol, butanol, acetone, ether, benzene, chloroform, ethyl acetate, methylene chloride, hexane, cyclohexane and petroleum ether.

Claim 3

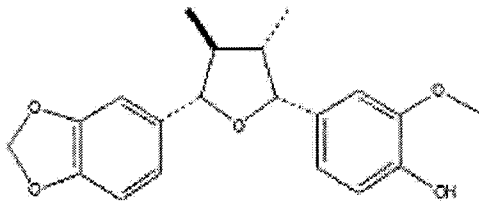
A pharmaceutical composition for preventing or treating diabetes comprising a compound selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound
20 represented by Formula 3, or pharmaceutically acceptable salts thereof as an active ingredient.

<Formula 1>

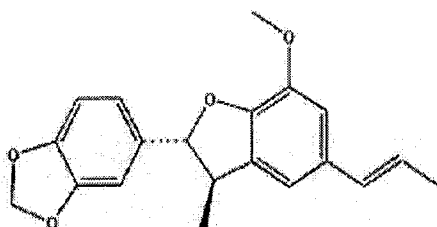


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<Formula 2>



<Formula 3>



5

Claim 4

The composition according to claim 3, wherein the diabetes is 2 type 2 diabetes.

10

Claim 5

A pharmaceutical composition for preventing a treating PPAR γ (peroxisome proliferator activated receptor γ)-mediated diseases comprising a compound
15 selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or pharmaceutically acceptable salts thereof as an active ingredient.

20

Claim 6

The composition according to claim 5, wherein the PPAR γ -mediated diseases are selected from the group
5 consisting of type 2 diabetes(NIDDM; non-insulin-dependent diabetes mellitus), hyperinsulinemia, obesity, hyperglycemia, hyperlipidemia, syndrome X, hypercholesterolemia, hyperlipoproteinemia, atherosclerosis, hypertension, insulin resistance,
10 dysmetabolic syndrome, diabetic complications, impaired glucose homeostasis, impaired glucose tolerance, hypertriglyceridemia, osteoporosis, glomerulonephritis and glycosurian nephropathy.

15 Claim 7

Use of a C1-C6 organic solvent extract of a aril of nutmeg for the manufacture of an agent for treating diabetes.

20 Claim 8

A method for preventing or treating diabetes comprising administering a C1-C6 organic solvent extract of an aril of nutmeg with an effective amount to subject in need thereof.

25

Claim 9

Use of a compound selected from the group

consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or pharmaceutically acceptable salts thereof for the manufacture of an agent for
5 treating diabetes.

Claim 10

A method for preventing or treating diabetes comprising administering a compound selected from the
10 group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or pharmaceutically acceptable salts thereof with an effective amount to subject in need thereof.

15

Claim 11

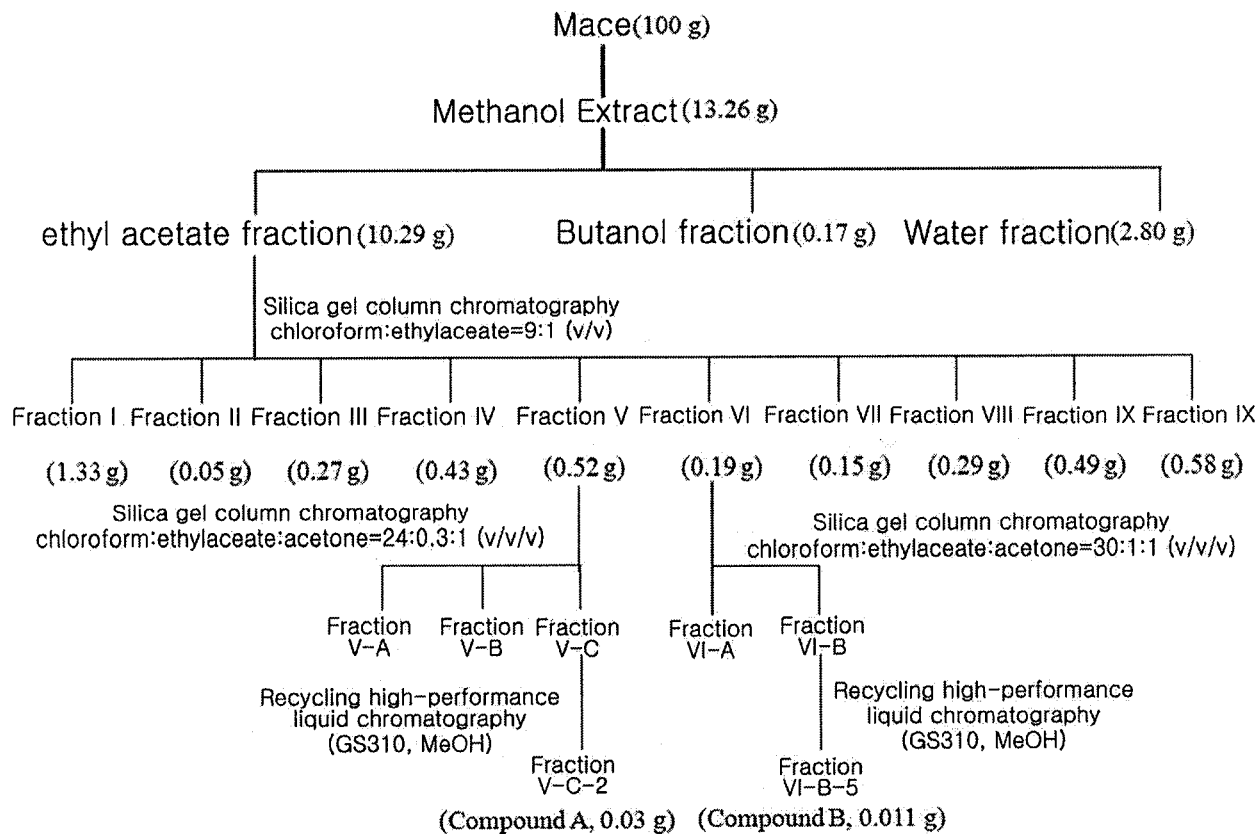
Use of a compound is selected from the group consisting of a compound represented by Formula 1, a compound represented by Formula 2, and a compound
20 represented by Formula 3, or pharmaceutically acceptable salts thereof for the manufacture of an agent for treating PPAR γ -mediated diseases.

Claim 12

25 A method for preventing or treating PPAR γ -mediated diseases comprising administering a compound selected from the group consisting of a compound represented by

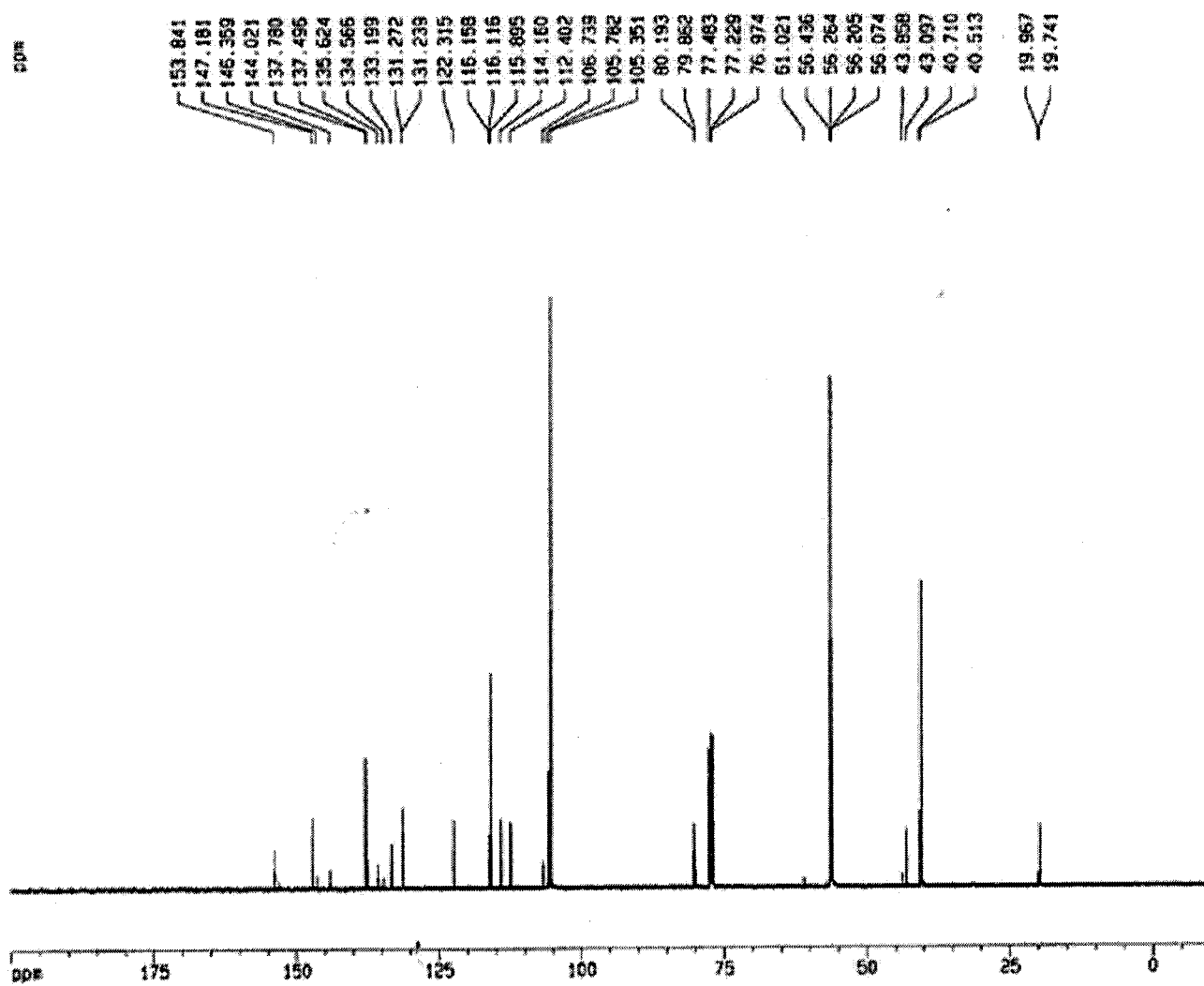
Formula 1, a compound represented by Formula 2, and a compound represented by Formula 3, or pharmaceutically acceptable salts thereof with an effective amount to subject in need thereof.

1/21

FIG. 1

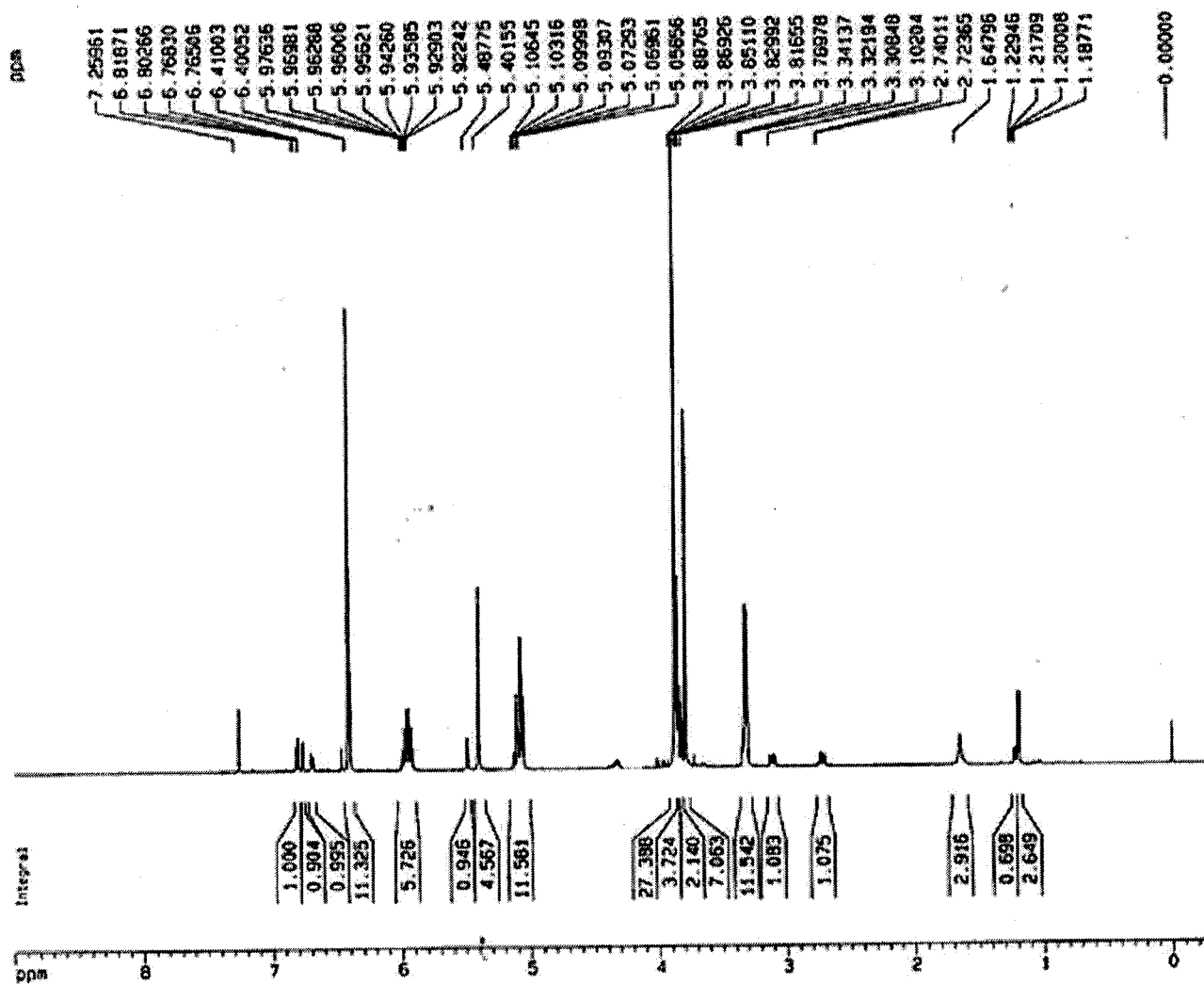
2/21

FIG. 2



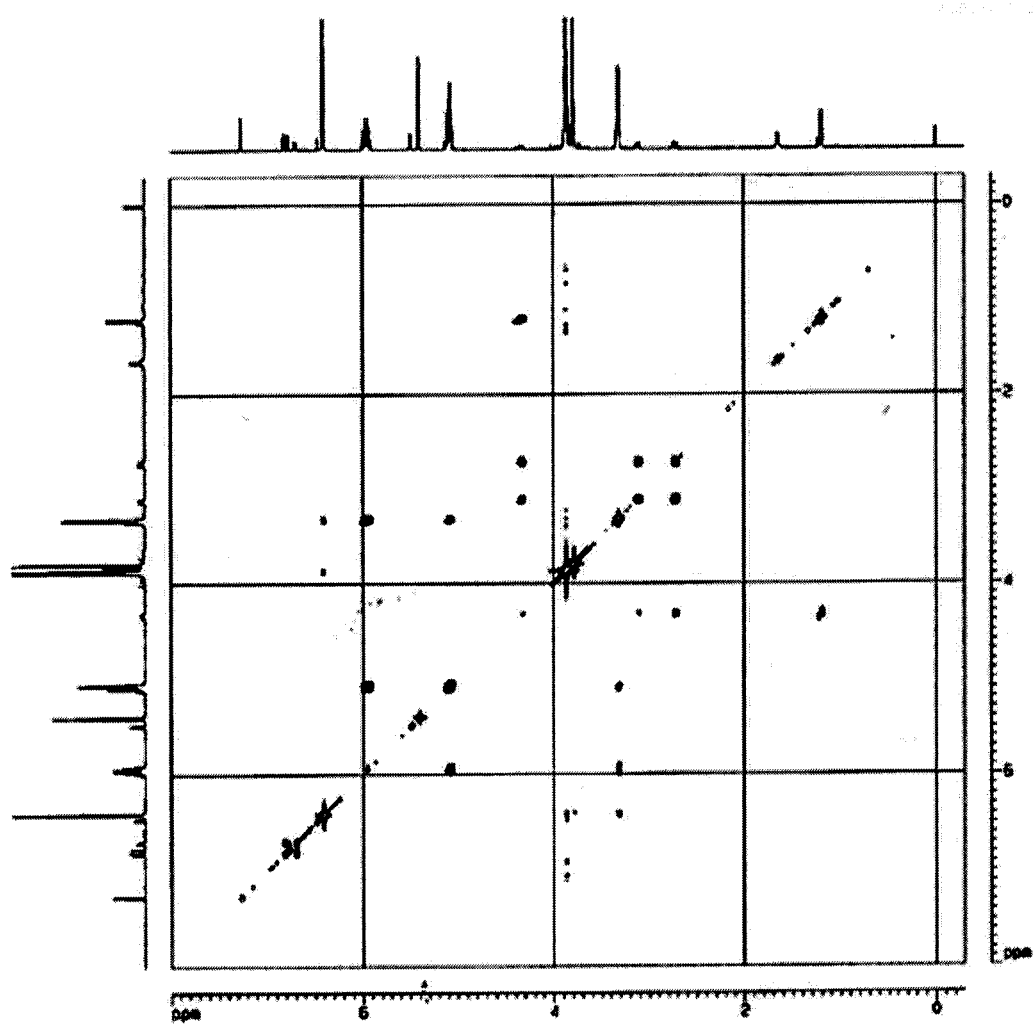
3/21

FIG. 3



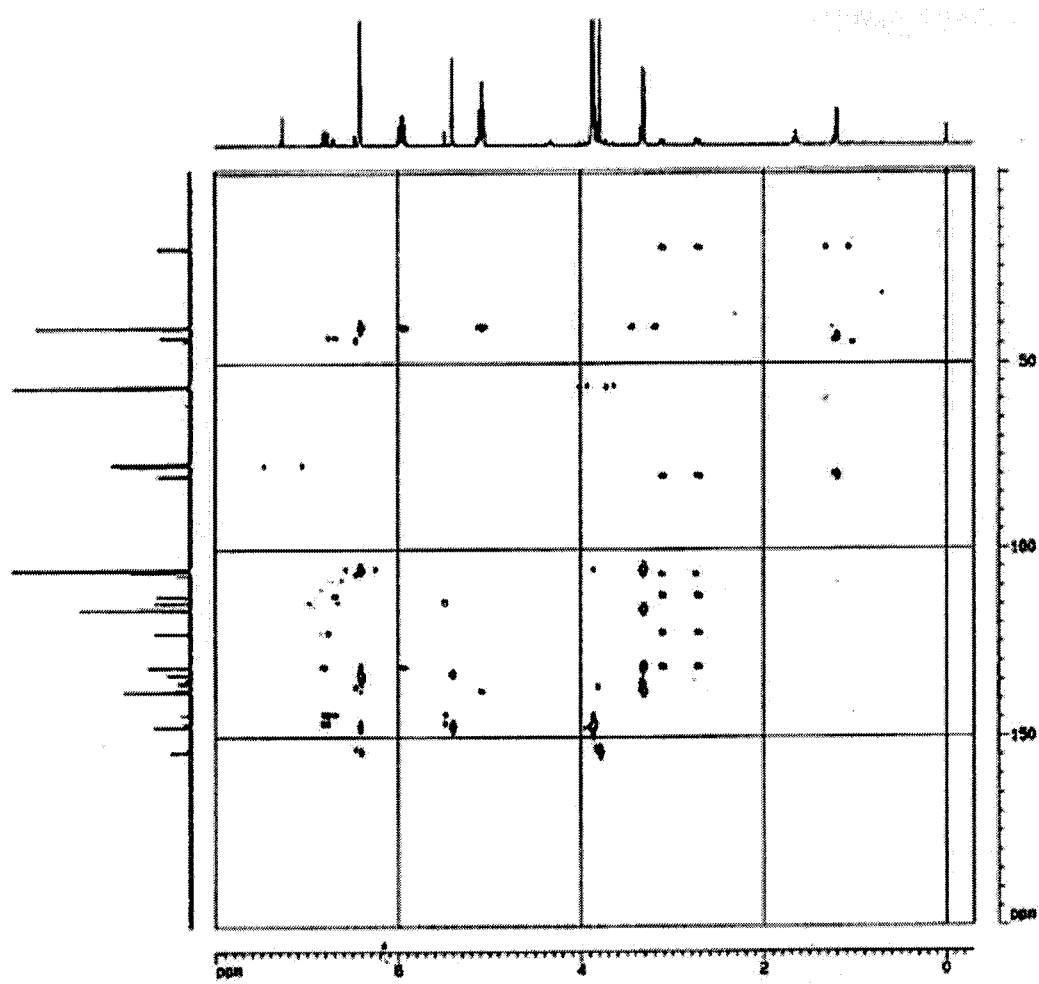
4/21

FIG. 4



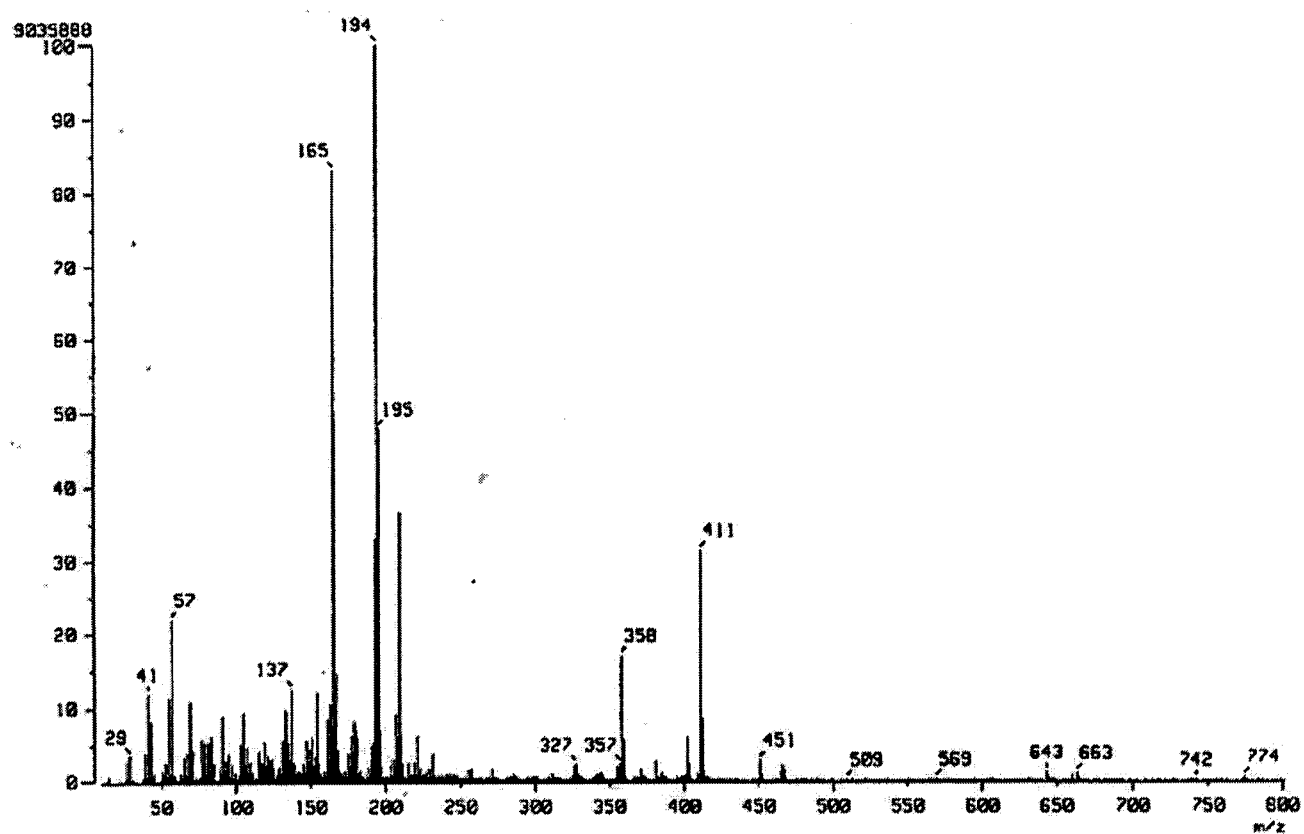
5/21

FIG. 5



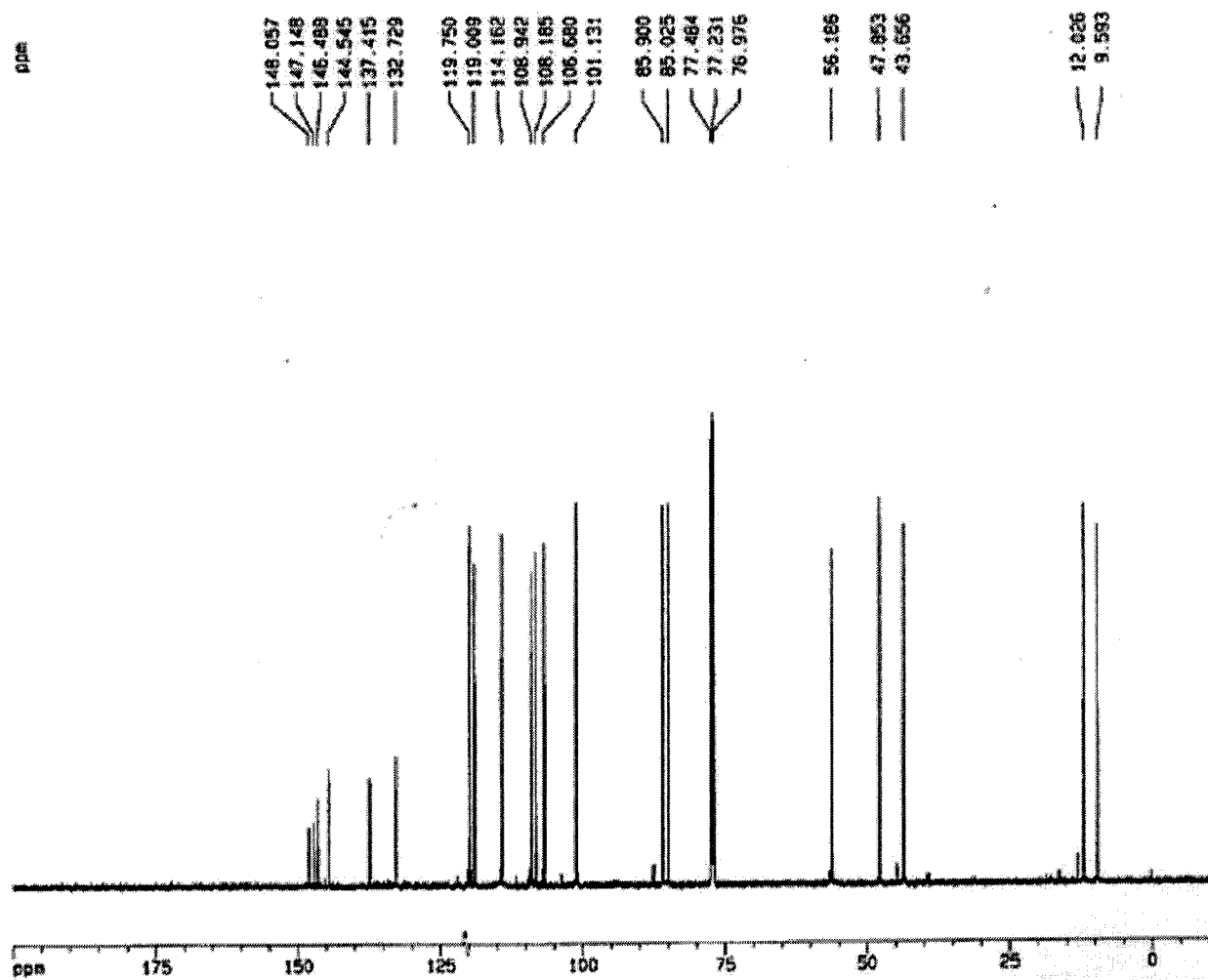
6/21

FIG. 6



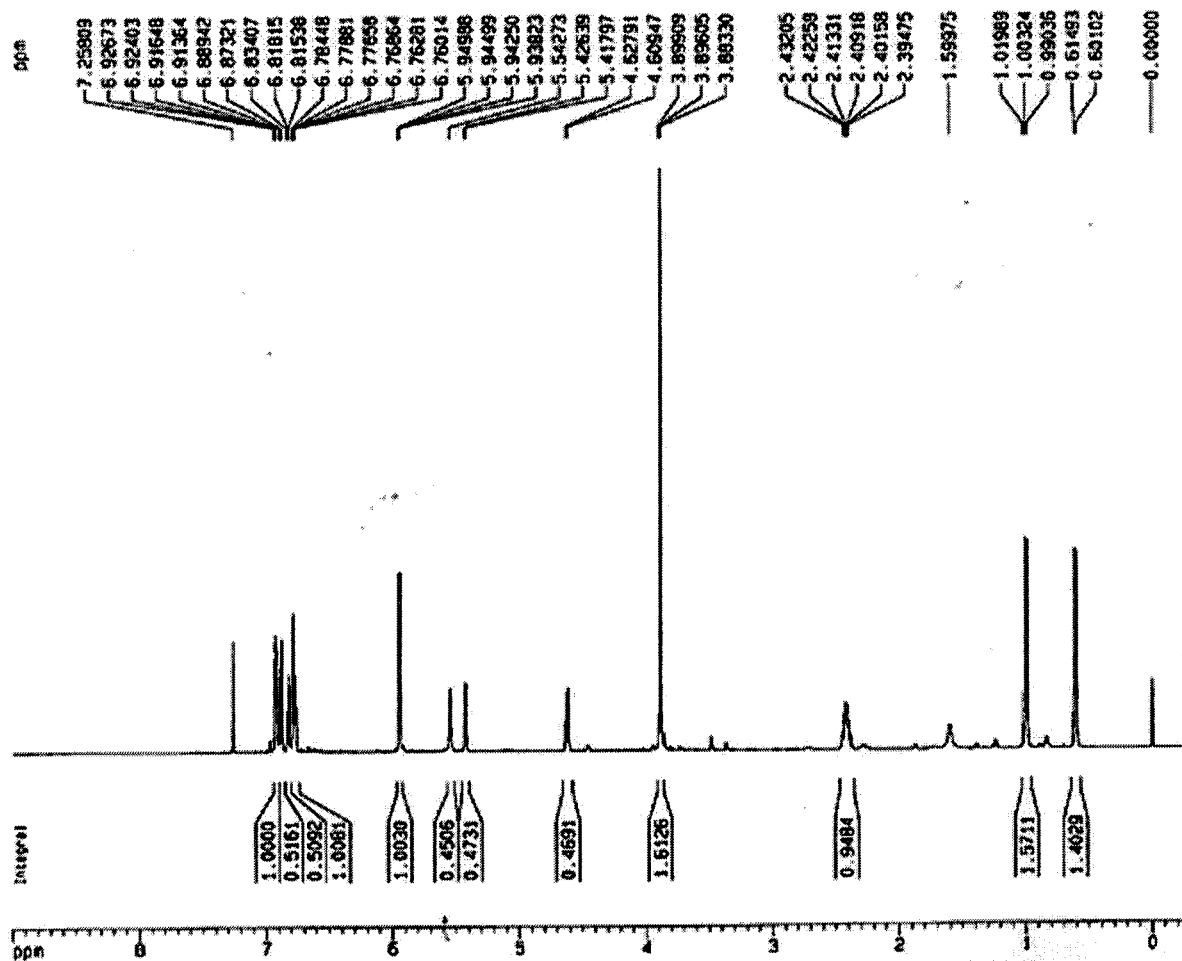
7/21

FIG. 7



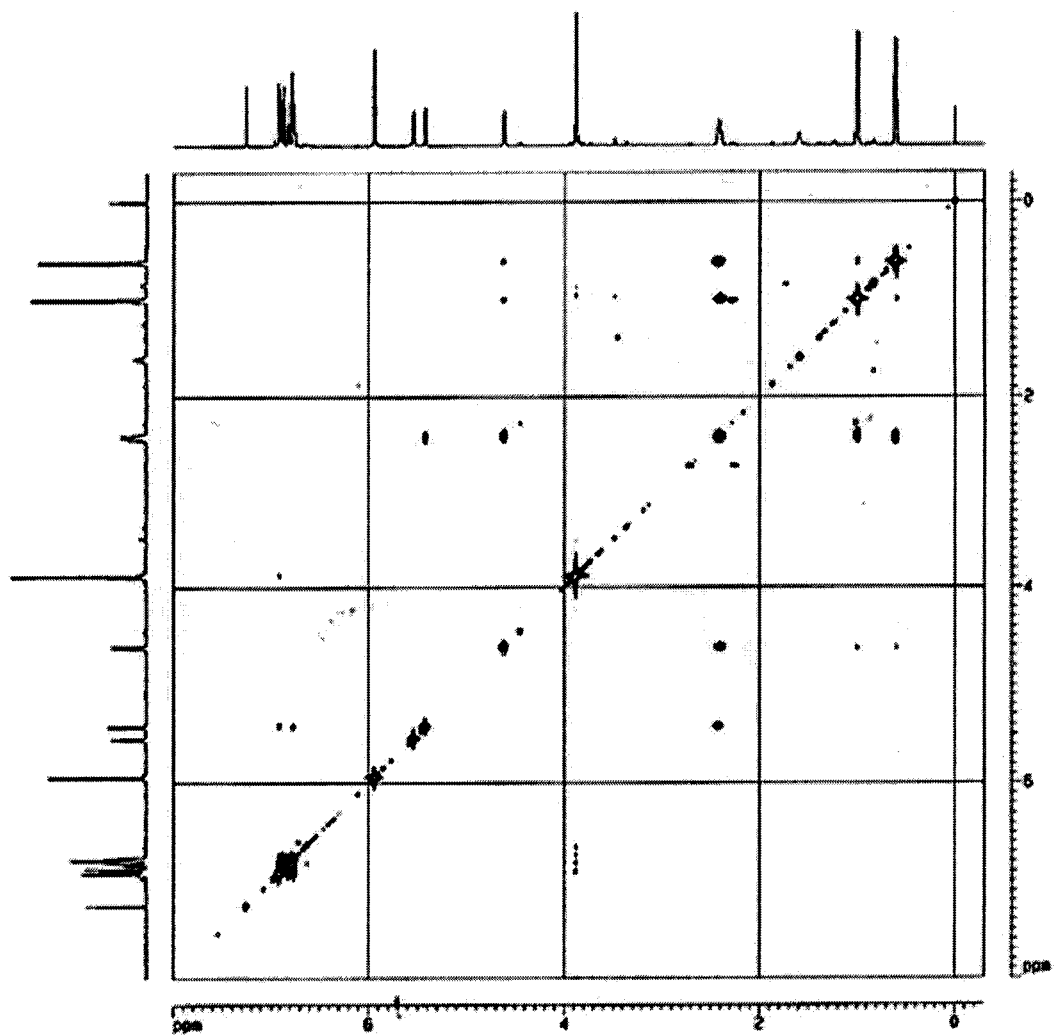
8/21

FIG. 8



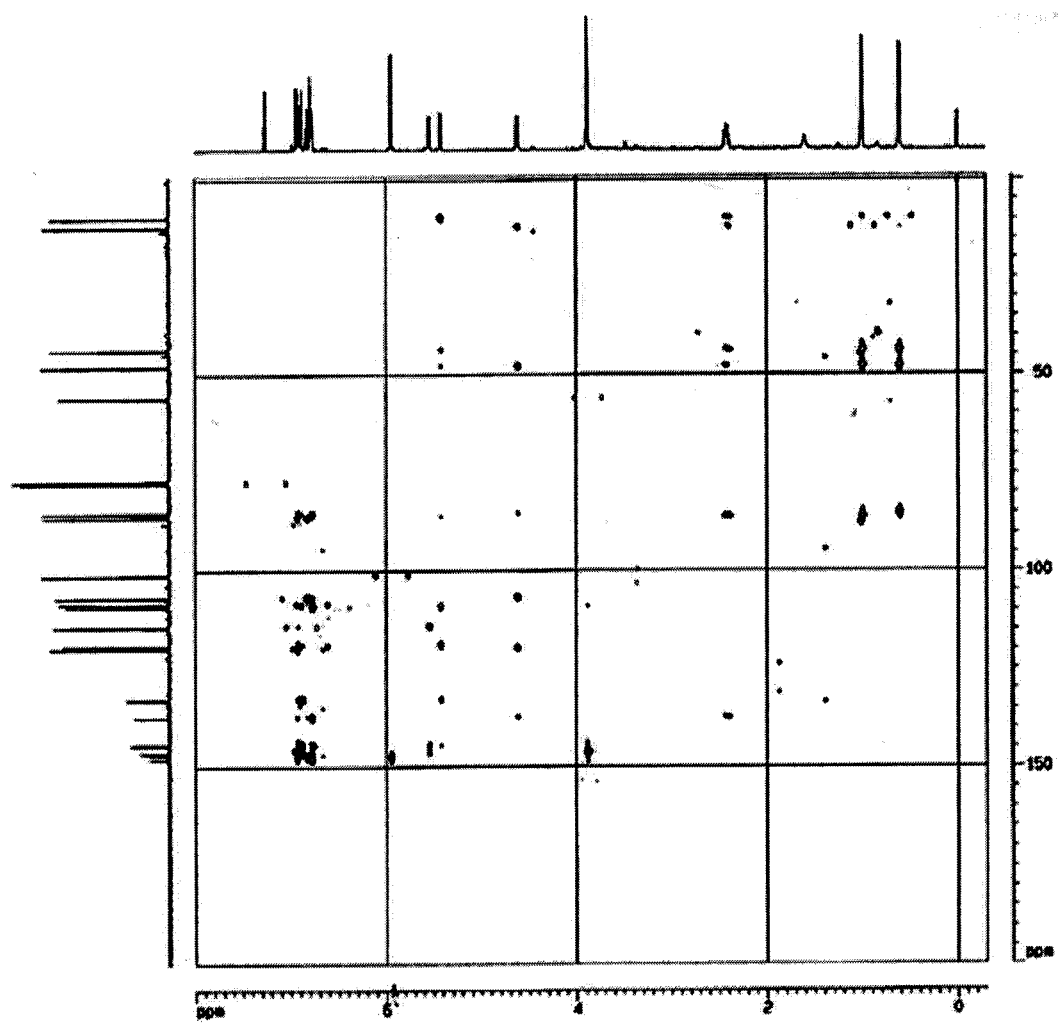
9/21

FIG. 9



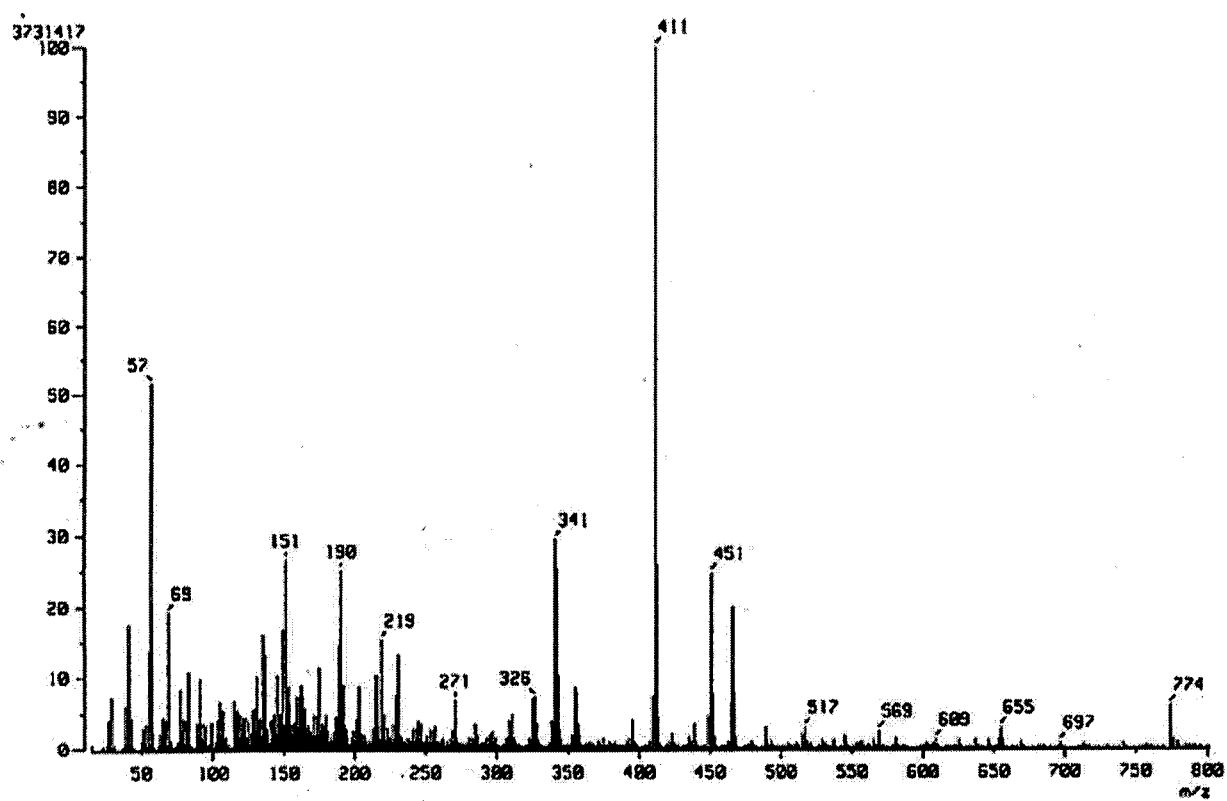
10/21

FIG. 10



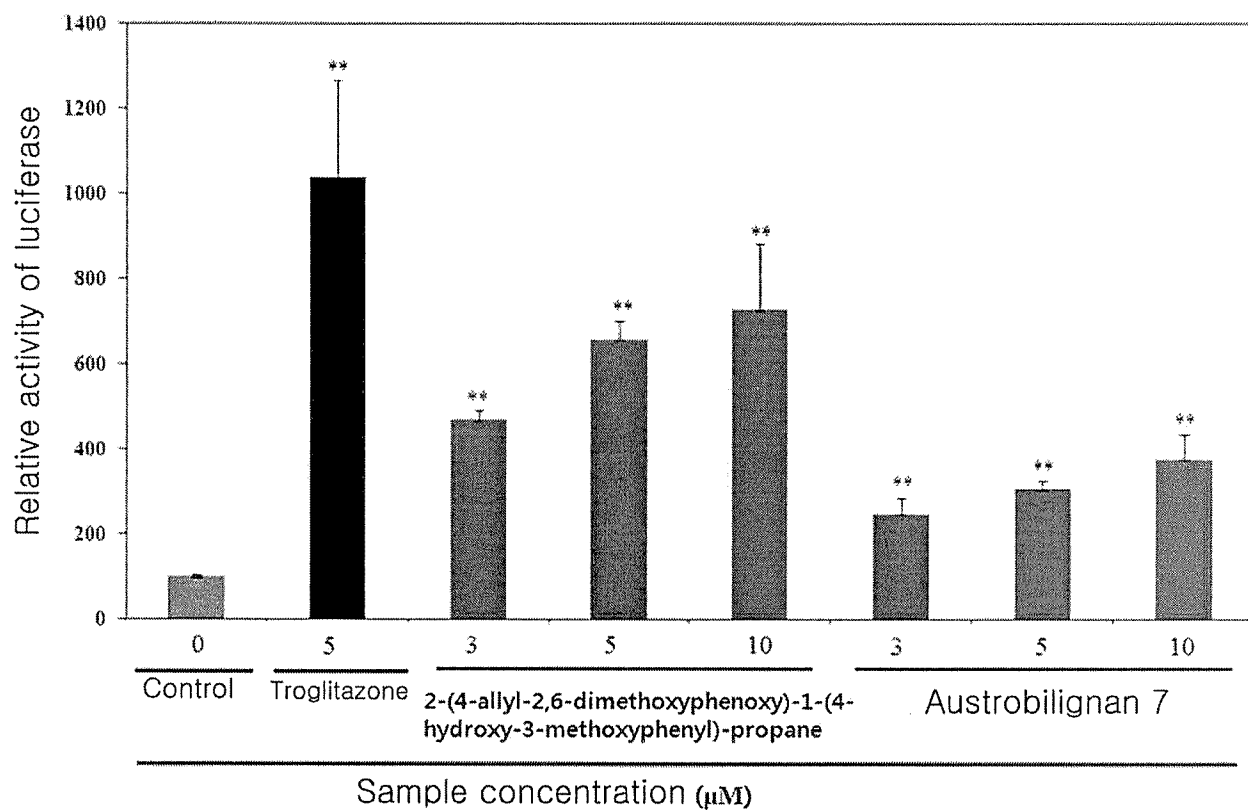
11/21

FIG. 11



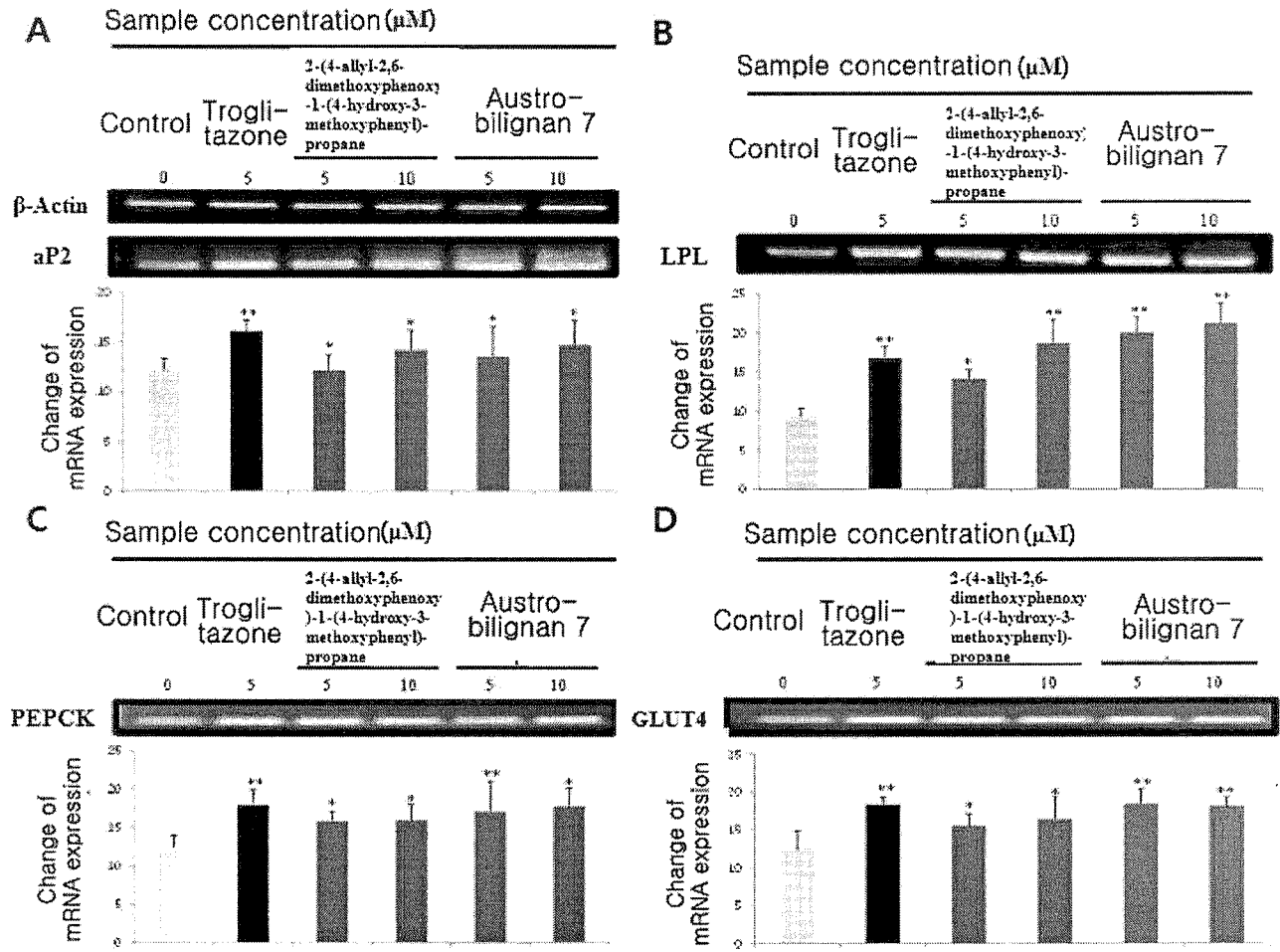
12/21

FIG. 12



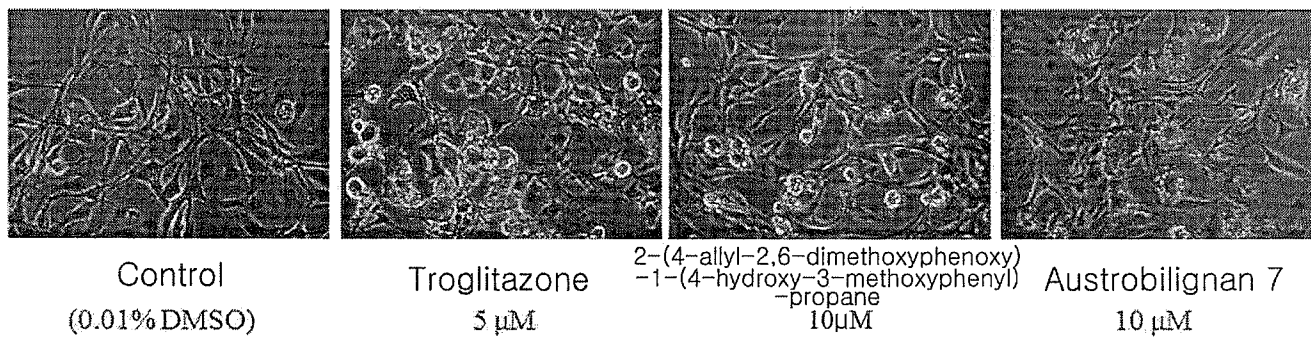
13/21

FIG. 13

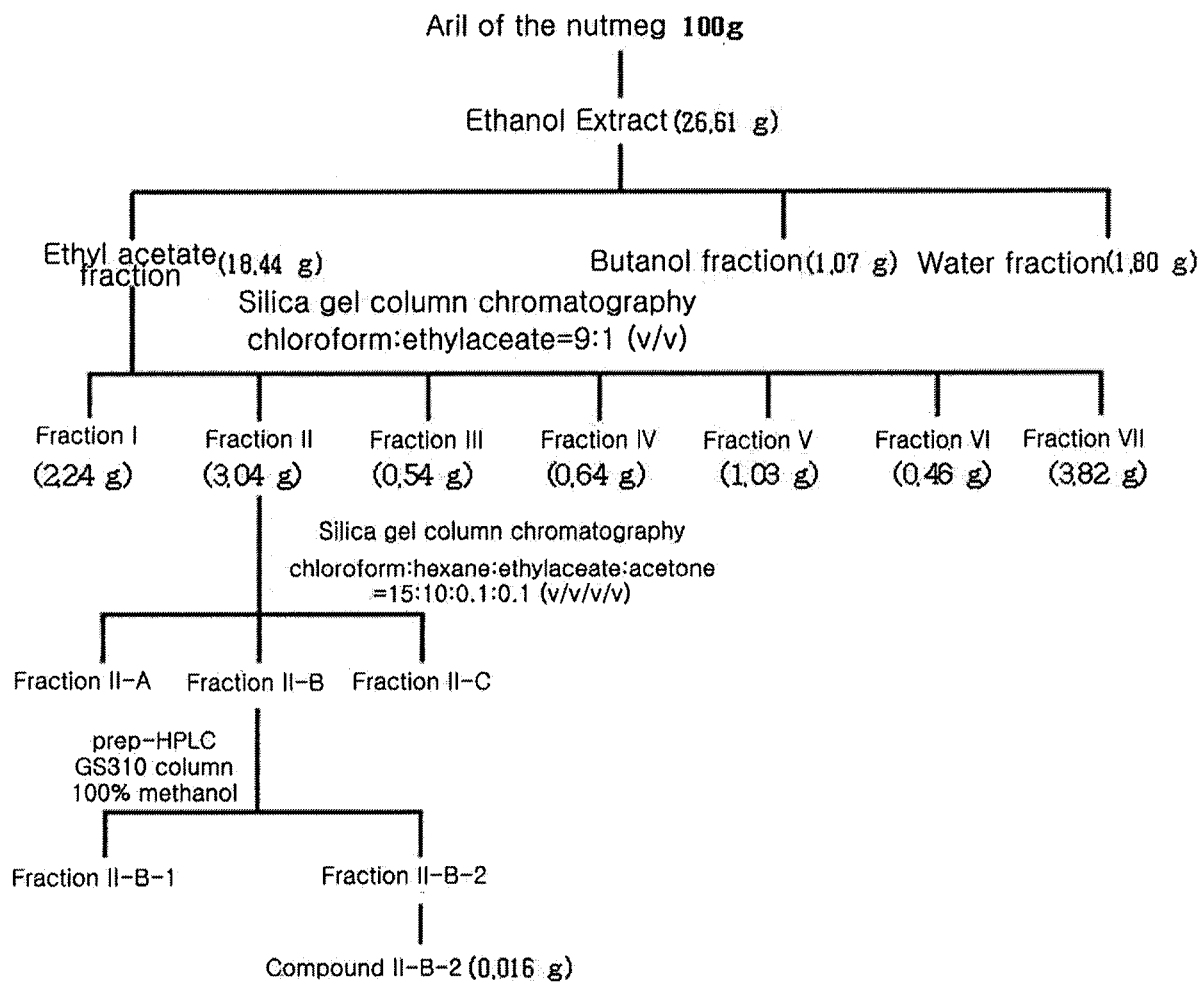


14/21

FIG. 14

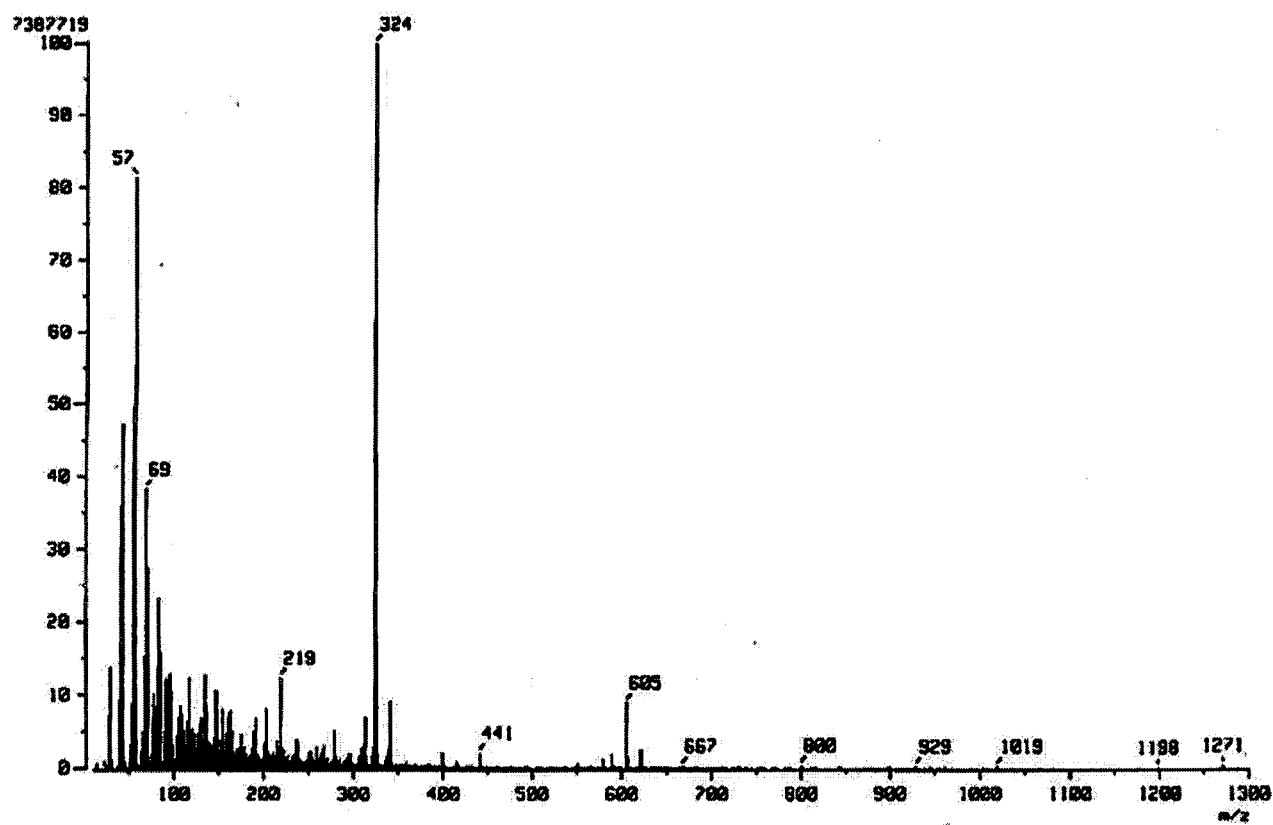


15/21

FIG. 15

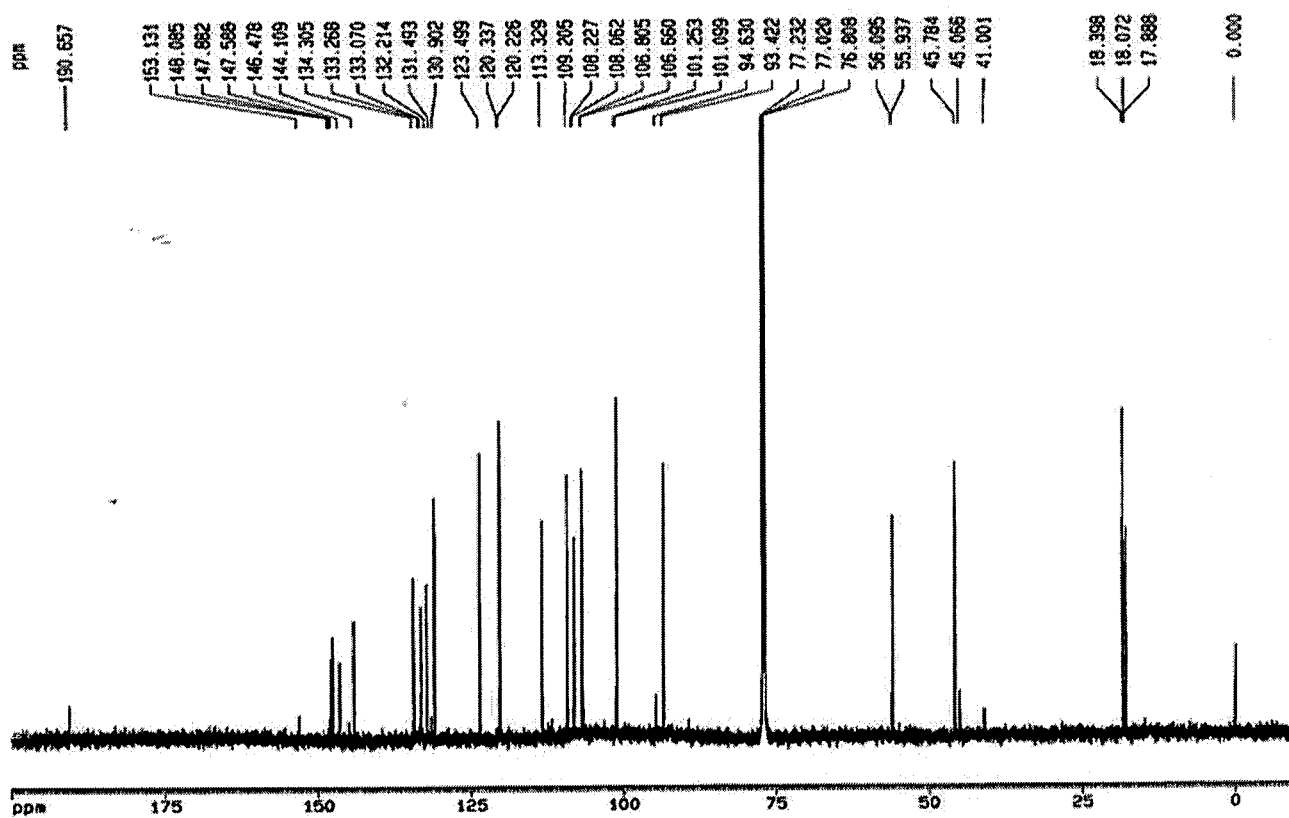
16/21

FIG. 16



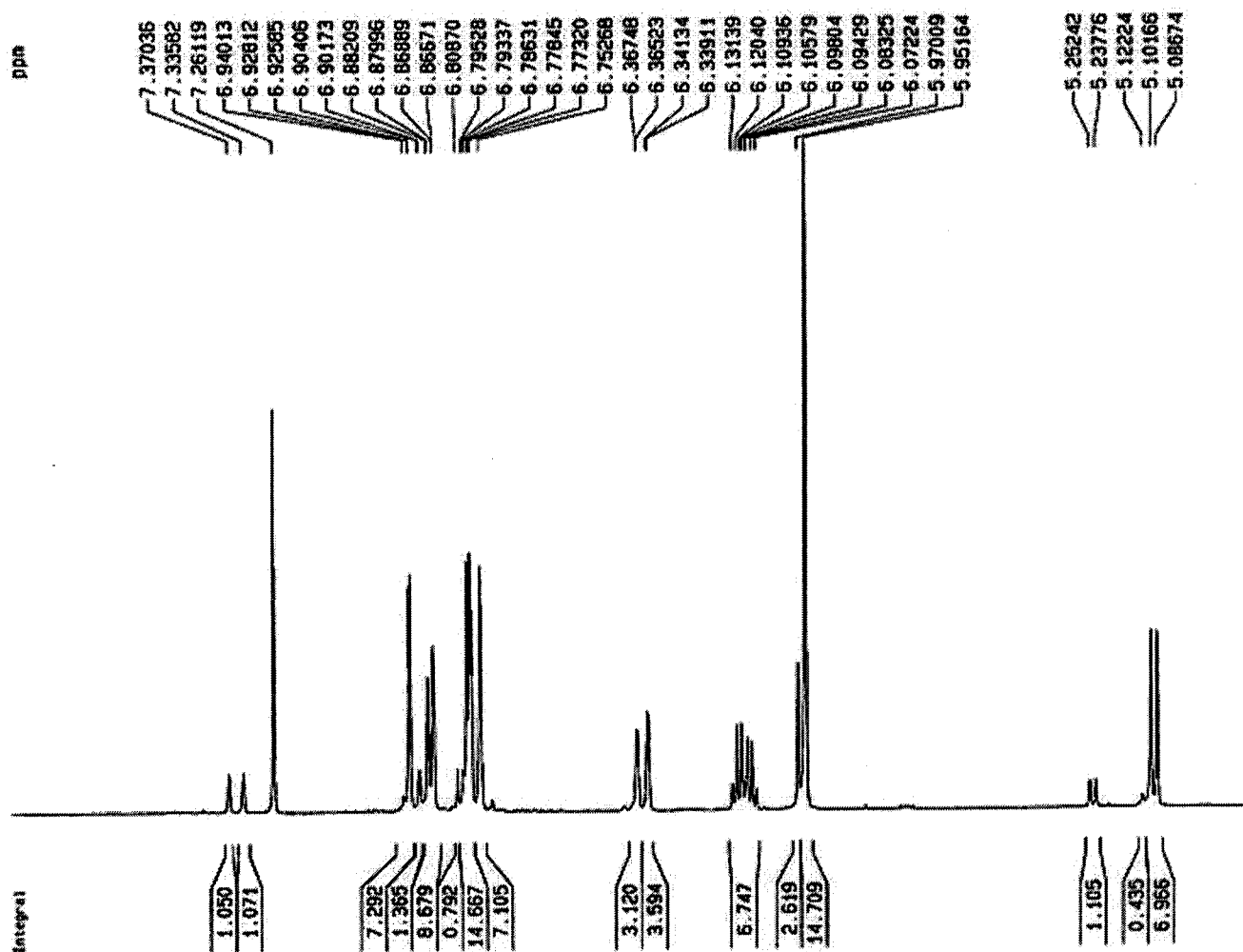
17/21

FIG. 17



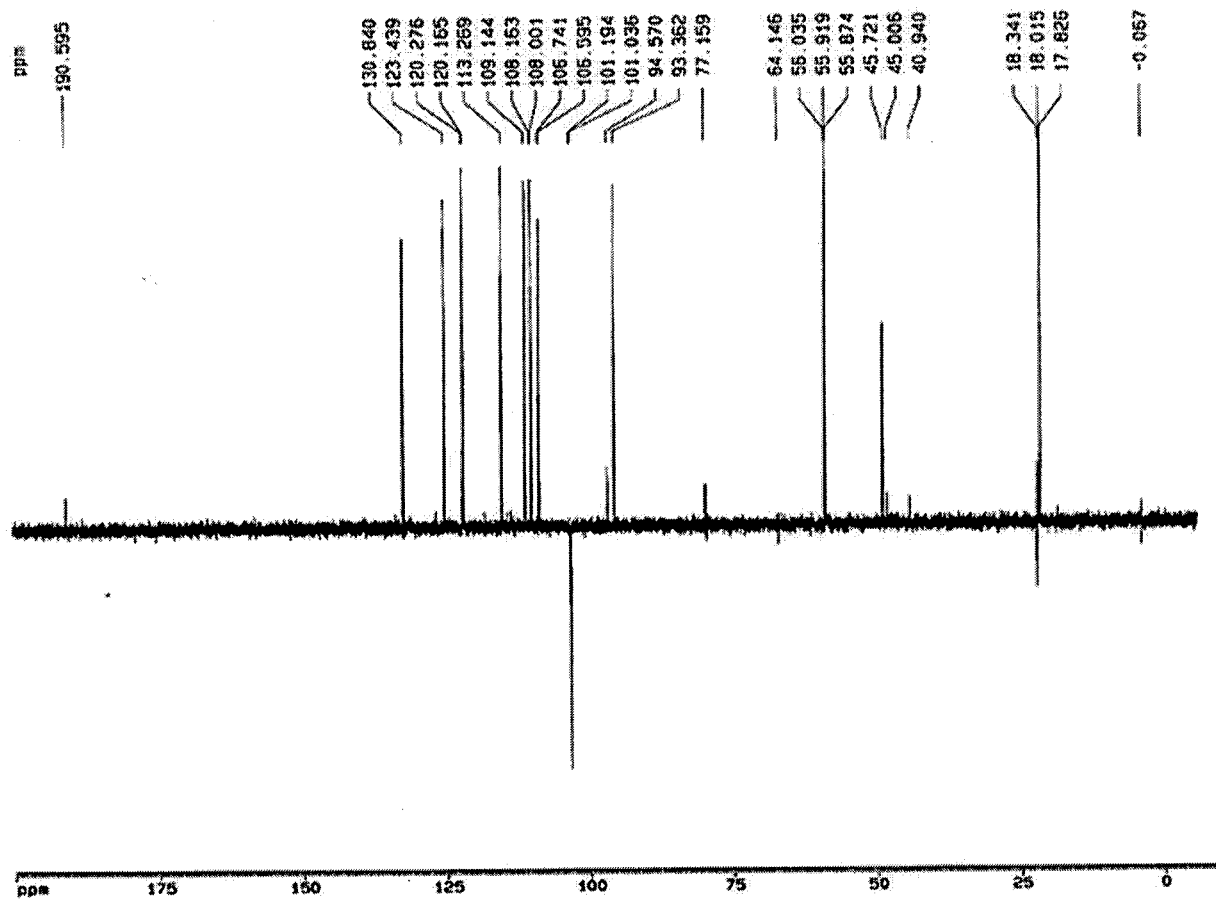
18/21

FIG. 18



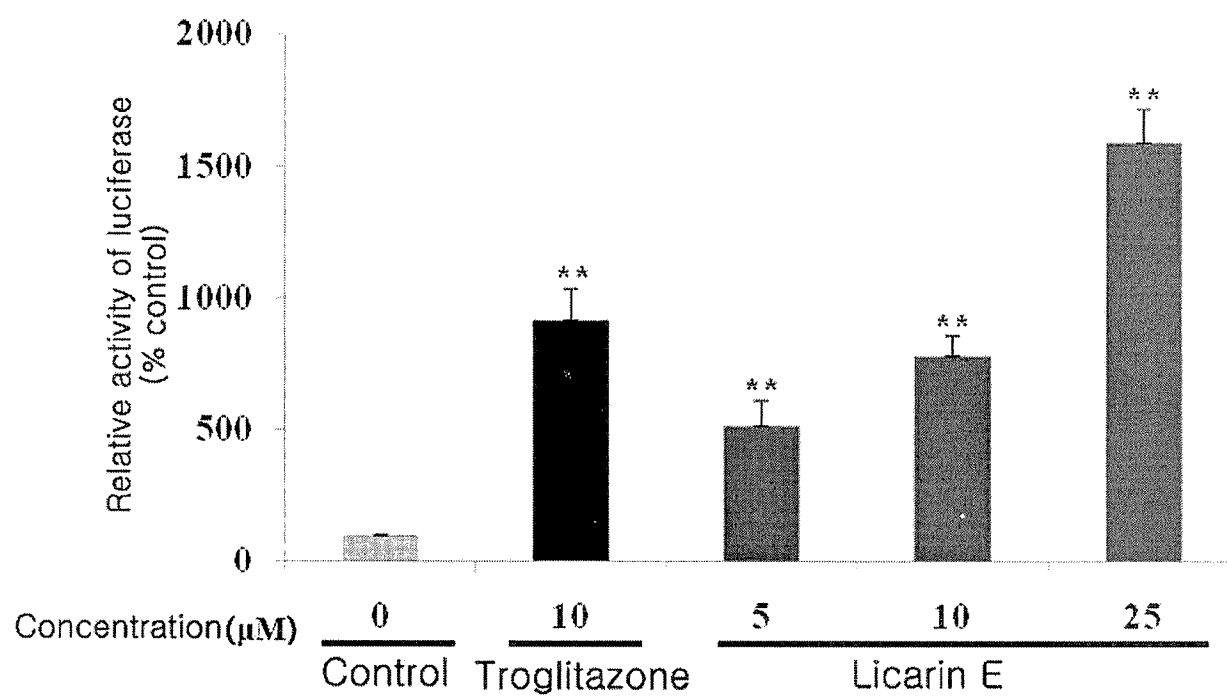
19/21

FIG. 19



20/21

FIG. 20



21/21

FIG. 21

