Abstract: The present invention relates to a composition for improving skin conditions comprising matrine and oxymatrine as an active ingredient. Matrine and oxymatrine have lower cytotoxicity than retinol used as anti-wrinkle agents and exhibit the inhibition effect on collagenase activity and promotion effect on collagen biosynthesis at a molecular level, contributing to excellent efficacy in improvement of skin wrinkles. In addition, both matrine and oxymatrine exhibit the inhibition effect on melanin production by inhibiting intracellular tyrosinase activity, the improving effects of UV- induced skin damage and the skin growth promotion or hair loss prevention. Therefore, matrine and oxymatrine have the excellent improvement effects on skin conditions. Furthermore, matrine and oxymatrine have the excellent anti-obesity and anti-oxidation effects. The composition of this invention can be applied to cosmetic, pharmaceutical and food composition having no cytotoxicities and side effects.
COMPOSITIONS FOR IMPROVING SKIN CONDITIONS COMPRISING
MATRINE OR ITS OXIDIZED DERIVATIVES

FIELD OF THE INVENTION

The present invention relates to a composition comprising matrine and oxymatrine as active ingredients, exhibiting an excellent improvement effect on skin conditions with stability and safety.

DESCRIPTION OF THE RELATED ART

Wrinkles are caused from skin aging and aged skin is a result of natural changes related to aging process. Skin aging can be broadly divided into physiological aging and photo aging. The former represents changes of skin function and structure associated with aging in entire skin surface, and the latter is induced by ultraviolet radiation.

The changes in dermis become remarkable with aging and dermis atrophy is one of representative phenomena after the age of 70. The decrease in both the number of fibroblasts and their biosynthetic potential results in changes of biomolecules having large molecular weights in extracellular matrix, so that the dermis change comes to appear. The changes include segregation of collagen bundle, reduction of mucopolysaccharide synthesis, decrease in the number and diameter of collagen and elastin, decomposition of collagen and elastin, and expansion of blood vessel.

Generally, among several intricate causes such as moisture content of skin, collagen content and immune responsiveness to external environments, the main factor of wrinkle formation involves the expression and activity of collagenase, a collagen-degradating enzyme to reduce synthesis and content of collagen.

Meanwhile, the color of human skin is ascribed mainly to the concentration and distribution of melanin. Melanin is one of phenol-based high molecular weighted
biosubstances and plays an important role in preventing skin damage elicited by UV.

The tyrosinase activity present in melanocyte has reported as a pivotal factor for melanin biosynthesis, tyrosinase plays pivotal role in skin darkening process by converting tyrosine into DOPA and DOPA-quinone, which are intermediate product of melanin polymer generation.

Hormone imbalance has become increasingly worse due to environmental pollution and auto exhausts. Because of this, the incidence rate of hair loss has become higher, the incidence age is lowered and a variety of skin diseases such as atopy and psoriasis have occurred due to inducing disharmony of skin immune system.

In addition, number of young obesity patients has rapidly went on increasing because of the change of dietary life such as meat and instant food centered diets.

Accordingly, studies have been intensively made to develop substances capable of effectively resolving several phenomena (i.e., intrinsic aging and incidence of wrinkles by UV, melasma or freckles, obesity, immune imbalance or hair loss) which have became serious social problems.

Throughout this application, several patents and publications are referenced and citations are provided in parentheses. The disclosure of these patents and publications is incorporated into this application in order to more fully describe this invention and the state of the art to which this invention pertains.

**DETAILED DESCRIPTION OF THIS INVENTION**

The present inventors have made intensive researches to develop active substances having activities of improvement in wrinkle, skin whitening, UV-induced skin damage, and prevention in hair loss, oxidation and obesity with high stability and safety without side effects on skin. As a result, the present inventors have found that compositions comprising matrine or oxymatrine as active ingredients allowed to provide compositions for improving skin conditions, anti-oxidation and anti-obesity, having excellent effects and safety.
Accordingly, it is an object of this invention to provide a composition for improving skin conditions, wherein the skin conditions are wrinkles, whitening, UV-induced skin damage or hair growth.

It is another object of this invention to provide a composition for anti-oxidation.

It is still another object of this invention to provide a composition for anti-obesity.

It is another object of this invention to provide a method for improving skin conditions.

It is still another object of this invention to provide a method for preventing oxidation.

It is another object of this invention to provide a method for suppressing obesity.

Other objects and advantages of the present invention will become apparent from the detailed description to follow taken in conjunction with the appended claims and drawings.

In one aspect of the present invention, there is provided a composition for improving skin condition comprising matrine or oxymatrine as an active ingredient, wherein the skin condition is wrinkles, whitening, UV-induced skin damage or hair growth.

In another aspect of the present invention, there is provided a method for improving skin condition, which comprises administering to a subject a composition comprising matrine or oxymatrine as an active ingredient.

In still another aspect of the present invention, there is provided a use of matrine or oxymatrine for manufacturing a composition for improving skin condition.

In another aspect of the present invention, there is provided a composition for anti-oxidation comprising matrine or oxymatrine as an active ingredient.
In still another aspect of the present invention, there is provided a method for preventing oxidation, which comprises administering to a subject a composition comprising matrine or oxymatrine as an active ingredient.

In another aspect of the present invention, there is provided a use of matrine or oxymatrine for manufacturing a composition for anti-oxidation.

In still another aspect of the present invention, there is provided a composition for anti-obesity comprising matrine or oxymatrine as an active ingredient.

In another aspect of the present invention, there is provided a method for suppressing obesity, which comprises administering to a subject a composition comprising matrine or oxymatrine as an active ingredient.

In still another aspect of the present invention, there is provided a use of matrine or oxymatrine for manufacturing a composition for anti-obesity.

The present inventors have made intensive researches to develop active substances having activities of improvement in wrinkle, skin whitening, UV-induced skin damage, and prevention in hair loss, oxidation and obesity with high stability and safety without side effects on skin. As a result, the present inventors have found that compositions comprising matrine or oxymatrine as an active ingredient allowed to provide compositions for improving skin conditions, anti-oxidation and anti-obesity, having excellent effects and safety.

As representative alkaloid substances mainly extracted in sophora root, matrine and oxymatrine used as active ingredient in this invention are also observed in Euchresta japonica Benth. Tetracyclo-quinolizidine alkaloids, matrine and oxymatrine are compounds derived from plants, represented by the following formula I and II. It has been generally known that matrine and oxymatrine with a bitter taste, have the effects on cancer, hepatitis B, cirrhosis, bacteia, virus, heart diseases, or skin disease such as psoriasis or eczema.
Matrine and oxymatrine used as active ingredients in compositions of this invention may be extracted from natural source, *Sophora* such as *Sophora japonica*, *Sophora flavescens*, *Euchresta japonica* Benth. In detail, the matrine and oxymatrine may be obtained using conventional various extraction methods. Preferably, the matrine and oxymatrine may be obtained using various extraction solvents, e.g., in 0°C to 50°C of extraction temperature, (a) water, (b) absolute or hydrous lower alcohol containing 1-4 carbon atoms (methanol, ethanol, propanol, butanol, etc.), (c) mixture of lower alcohol and water, (d) acetone, (e) ethyl acetate, (f) chloroform or (g) 1,3-butyleneglycol.

If necessary, matrine and oxymatrine used in the present invention may be subjected to additional purification by the well-known methods in the art as well as those obtained by extraction. For instance, it could be appreciated that matrine and oxymatrine obtained using a variety of additional purification methods such as ultrafiltration with defined molecular weight cut-off value and various chromatography (designed for purification dependent upon size, charge, hydrophobicity and affinity) may be used in the present invention.

The compositions of the present invention have novel use to improve skin
wrinkles. The compositions of the present invention have lower cytotoxicity compared to retinol used as anti-wrinkle agents and exhibit the inhibition effect on collagenase activity and promotion effect on collagen biosynthesis at a molecular level, and as a result, excellent efficacy in improvement of skin wrinkles. Such effects and efficacies are demonstrated in Examples described hereunder. It would be appreciated that the improvement of skin wrinkles covers general uses of skin protection (e.g., prevention of skin wrinkles, removal of skin wrinkles and prevention of skin aging).

In addition, the compositions for improving skin conditions of the present invention have novel use of skin whitening. The term "whitening" used herein, refers to improvement of skin trouble preventing skin melanism induced by melanin pigmentation.

In the composition for skin whitening of this invention, matrine and oxymatrine exhibit the inhibition effect on melanin production by inhibiting intracellular tyrosinase activity and as a result, excellent efficacy in skin whitening. The matrine and oxymatrine show a high stability and no or a little side effects such as skin irritability induction. Furthermore, the content of the matrine and oxymatrine are constantly maintained in the composition. Such effects and efficacies are demonstrated in Examples described hereunder.

Moreover, the compositions for improving skin conditions of this invention have alleviating, improving, preventing or treating effects on UV-induced skin damage.

The compositions for improving skin conditions of this invention contribute very effectively to the prevention of hair loss or the promotion of hairs growth. The terms "hair loss prevention" and "hairs growth promotion" used herein have the same meaning.

The matrine and oxymatrine as active ingredients of the present compositions exhibit an anti-oxidation effect by eliminating free radicals.

The composition for anti-oxidation of the present invention may be applied to a variety of diseases, disorders or abnormal conditions capable of preventing or treating
by inhibiting or eliminating oxidation conditions. The diseases associated with anti-
oxidation to be treated by the present composition include neurodegenerative
disorders such as atherosclerosis, coronary heart disease, restenosis, reperfusion
injury, parkinson's disease or alzheimer's disease, stroke, cancer, aging, cardiovascular
disorder, osteoporosis, disorder of central nervous system, peripheral vascular disease
and dyspnea, but not limited to. Most preferably, the composition for anti-oxidation of
this invention is used to prevention or remedy of aging. The correlation between
various disorders and free radical damage has been reported generally and the
component of various cells comprising enzyme, ion channel, structural protein and
membrane lipid is a potential target against reactive free radical species (Rice-Evans C,
Mo/Aspects of Med 13(1):1-1U (1992)). The reaction of the potential target and free
radicals impairs cell function of specific ranges, induces pathological changes and
allows for cell death ultimately. A variety of diseases caused by physiological oxidation
are disclosed in U.S. Pat. No 5750351; 5773209; 5773231 and 5846959.

The composition for anti-oxidation of this invention protects DNA, protein
(including lipoprotein) and membrane lipid from oxidative damage by excellent effects
on elimination of free radicals.

Moreover, matrine and oxymatrine as active ingredients of this invention have
an excellent anti-obesity effect.

It is obvious to one skilled in the art that matrine and oxymatrine represented
by Formula I and II include derivatives obtained by chemical processes with
substituents performed conventionally in one skilled in the art. The derivatives show
the effects of wrinkles improvement by promotion of collagen synthesis and/or
inhibition of collagenase (MMP-I) activity, or skin whitening, improvement of UV-
induced skin damage, skin growth promotion or hair loss prevention, anti-oxidation
and anti-obesity effects. More particularly, matrine and oxymatrine used as active
ingredients of this invention include derivatives of Formula I and II as well as
compounds of Formula I and II. The derivatives with structural nucleus as Formula I
and II as nucleus may be obtained by chemical processes using various substituents well-known in the art. For example, it would be suggested that derivatives formed by linking hydroxy, halo, nitro or Cl₄ alkyl group to cyclic carbons of Formula I and II are likely to exert effects identical or similar to matrine and oxymatrine. These substituted derivatives may also fall into the scope of this invention.

According to a preferred embodiment, the active ingredient of matrine or oxymatrine in the composition is present in the amount of 0.00001-15.0 wt%, more preferably, 0.0001-10 wt%, most preferably, 0.0001-5 wt% based on the total weight of the composition. If the amount of the active ingredient of matrine or oxymatrine is lower than 0.00001 wt%, the effect of the composition may be negligible; in the case of exceeding 15.0 wt%, some adverse effects such as skin irritation and instability in formulation are very likely to occur.

According to the preferred embodiment, the composition of the present invention is a cosmetic composition.

The cosmetic compositions of the present invention may contain auxiliaries as well as carrier in addition to the matrine or oxymatrine as active ingredients. The non-limiting examples of auxiliaries include antioxidants, stabilizers, solubilizers, vitamins, colorants, odor improvers or mixtures of these ingredients. In addition, the cosmetic compositions may additionally comprise promoting materials of skin absorption to enhance the effects.

The cosmetic compositions of this invention may be formulated in a wide variety of form, for non-limited example, including a solution, a suspension, an emulsion, a paste, a gel, a cream, a lotion, a powder, a soap, a surfactant-containing cleanser, an oil, a powder foundation, an emulsion foundation, a wax foundation and a spray. In detail, the cosmetic composition of the present invention can be provided in a form of skin softener (skin lotion), nutrient emulsion (milk lotion), nutrient cream,
message cream, essence, eye cream, cleansing cream, cleansing foam, cleansing water, facial pack, spray or powder.

The cosmetically acceptable carrier contained in the present cosmetic composition, may be varied depending on the type of the formulation. For example, the formulation of pastes, creams or gels may comprise animal and vegetable fats, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silica, talc, zinc oxide or mixtures of these ingredients.

In the formulation of powder or spray, it may comprise lactose, talc, silica, aluminum hydroxide, calcium silicate, polyamide powder or mixtures of these ingredients. Spray may additionally comprise the customary propellants, for example, chlorofluorohydrocarbons, propane/butane or dimethyl ether.

The formulation of solution and emulsion may comprise solvent, solubilizer or emulsifier, for example water, ethanol, isopropanol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol oils, glycerol fatty esters, polyethylene glycol, fatty acid esters of sorbitan or mixtures of these ingredients.

The formulation of suspension may comprise liquid diluents, for example water, ethanol or propylene glycol, suspending agents, for example ethoxylated isostearyl alcohols, polyoxyethylene sorbitol esters and poly oxyethylene sorbitan esters, micocrystalline cellulose, aluminum metahydroxide, bentonite, agar and tragacanth or mixtures of these ingredients.

The formulation of cleansing compositions with surfactant may comprise aliphatic alcohol sulfate, aliphatic alcohol ether sulfate, sulfosuccinate monoester, isothionate, imidazolium derivatives, methyltaurate, sarcocinate, fatty acid amide ether sulfate, alkyl amido betain, aliphatic alcohol, fatty acid glyceride, fatty acid diethanolamide, vegetable oil, lanoline derivatives, ethoxylated glycerol fatty acid ester or mixtures of these ingredients.

The composition of this invention may be prepared as a pharmaceutical
composition, and the pharmaceutically acceptable carrier as well as the active ingredient contained in the pharmaceutical composition. The pharmaceutically acceptable carrier, which is commonly used in pharmaceutical formulations, but is not limited to, includes lactose, dextrose, sucrose, sorbitol, mannitol, starch, rubber arable, potassium phosphate, arginate, gelatin, potassium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrups, methyl cellulose, methylhydroxy benzoate, propylhydroxy benzoate, talc, magnesium stearate, and mineral oils. The pharmaceutical composition according to the present invention may further include a lubricant, a humectant, a sweetener, a flavoring agent, an emulsifier, a suspending agent, and a preservative. Details of suitable pharmaceutically acceptable carriers and formulations can be found in Remington's Pharmaceutical Sciences (19th ed., 1995).

The pharmaceutical composition of this invention may be administered to mammals such as rat, mouse, domestic animals and human via various routes, for example oral administration, rectal administration or intravenous injection, intramuscular injection, subcutaneous injection, intrauterine injection or intracerebroventricular injection, preferably subcutaneous injection, more preferably topical application.

A suitable dosage amount of the pharmaceutical composition of the present invention may vary depending on pharmaceutical formulation methods, administration methods, the patient's age, body weight, sex, pathogenic state, diet, administration time, administration route, an excretion rate and sensitivity for a used pharmaceutical composition, and physicians of ordinary skill in the art can determine an effective amount of the pharmaceutical composition for desired treatment. In case of oral formulation, a suitable dosage unit may be administered once to several times a day with 0.001-100 mg/kg on the basis of adult. In case of preparation for external use, a suitable dosage unit may be administered by applying once to five times a day in amounts of 1.0 to 3.0 ml on the basis of adult and it has better use for more than 1 month. However, the dosage unit does not limit the scope of this invention.
According to the conventional techniques known to those skilled in the art, the pharmaceutical composition of the present invention may be formulated with pharmaceutically acceptable carrier and/or vehicle as described above, finally providing several forms a unit dose form and a multi-dose form. Non-limiting examples of the formulations include, but not limited to, oral formulation such as a powder, a granule, a tablet, a capsule, a suspension, an emulsion, a syrup and an aerosol, preparation for external use such as an ointment and a cream, a suppository and sterile injection solution, and may further comprise a dispersion agent or a stabilizer.

The composition of this invention may be prepared as a food composition. The food composition of this invention may comprise conventional additives for preparing food compositions, e.g., protein, carbohydrates, lipids, nutritive substances and flavors.

Non-limiting examples of carbohydrates described above include, but not limited to, monosaccharide (e.g., glucose and fructose); disaccharide (e.g., maltose, sucrose and oligosaccharide); and polysaccharide (e.g., dextrin and cyclodextrin); and sugar alcohol (e.g., xylitol, sorbitol and erythritol). Non-limiting examples of Flavors include, but not limited to, natural flavors [thaumatin and extract of stevia (e.g., rebaudioside A and glycyrrhizin)] and synthetic flavors (e.g., saccharin and aspartame).

For example, where the food composition of this invention is provided as a drink, it may further comprise citric acid, liquid fructose, sugar, glucose, acetic acid, malic acid, fruit juice, extract of eucommia ulmoides oliv, jujube extract or extract of glycyrrhiza uralensis.

Meanwhile, experiment results of a cumulative skin irritation elucidated that matrine and oxymatrine as natural substances was harmless to human body in specific example of the present invention. Therefore, matrine and oxymatrine of the present invention may be used with confidence for long period due to not or a little having toxicities and side effects, particularly may be applied to cosmetic, pharmaceutical and food composition with safety as described above.
The summary of features and advantages of this invention is as follows:

(i) The composition of the present invention comprises matrine and oxymatrine as active ingredients.

(ii) Matrine and oxymatrine have lower cytotoxicity than retinol used as anti-wrinkle agents and exhibit the inhibition effect on collagenase activity and promotion effect on collagen biosynthesis at a molecular level, contributing to excellent efficacy in improvement of skin wrinkles. In addition, both matrine and oxymatrine exhibit the inhibition effect on melanin production by inhibiting intracellular tyrosinase activity, the improving effects of UV-induced skin damage and the skin growth promotion or hair loss prevention. Therefore, matrine and oxymatrine have the excellent improvement effects on skin conditions.

(iii) Furthermore, matrine and oxymatrine have the excellent anti-obesity and anti-oxidation effects.

(iv) The composition of this invention can be applied to cosmetic, pharmaceutical and food composition having no cytotoxicities and side effects.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 is a graph showing that matrine and oxymatrine as active ingredients have lower cytotoxicity against human fibroblasts compared to RA. RA represents retinoic acid. RA, matrine and oxymatrine were treated in amounts of 1 µM, 10 µM and 50 µM, respectively.

Fig. 2 is a graph showing the promotion effect on collagen (Type 1 collagen) biosynthesis by matrine and oxymatrine. RA was treated in amounts of 1 µM.

Fig. 3 represents the inhibition effect on collagenase (MMP-I) activity by matrine and oxymatrine as active ingredients of this invention. MMP-I and PMA show Type 1 collagenase and phorbol myristate acetate, respectively. PMA was treated in amounts of 100 nM.
The present invention will now be described in further detail by examples. It would be obvious to those skilled in the art that these examples are intended to be more concretely illustrative and the scope of the present invention as set forth in the appended claims is not limited to or by the examples.

EXAMPLES

Example 1: Measurement of effect of matrine and oxymatrine on wrinkles improvement

Test of wrinkle improvement effect can be generally measured through collagen biosynthesis ability, collagenase degradation inhibitory ability and clinical test to human.

Human fibroblasts (commercially available from pacific) were seeded into a 6-well plate (2 x 10^5 cells/well) and the well plate was incubated in a 5% CO₂ incubator for 24 hr at 37°C. After 24 hr, the medium in each well was removed and samples were treated with various concentrations, followed by incubating again for 24 hr. Following incubation, the cell medium was collected and was used as samples. To measure cytotoxicity of samples to fibroblasts, MTT reagent (1 mg/ml) was added to each well in 1/10 fold volume of the remaining medium, incubated for 3 hr and the medium was eliminated. The medium was dissolved in DMSO and its absorbance at 540 nm was measured.

The extent of collagen synthesis was determined by measuring the amount of procollagen type I C-peptide (PICP) in cell medium using Procollagen Type I C-peptide EIA kit (MKIOI, Takara, Kyoto, Japan). The method was performed in accordance with manufacturer's protocol.

As a method of measuring the activity of collagenase, an enzyme that decomposes collagen, an antibody against collagenase was used. As material inducing collagenase activity, PMA (phorbol myristate acetate, Sigma) was treated. For measuring collagenase activity, a Type 1 collagenase assay kit (Amersham Biosciences,
RPN2629) was used, and the absorbance was measured using an ELISA reader (Bio-
Tek ELx808™ Series Ultra Microplate Reader, U.K). The measured average values were represented as mean ± standard deviation. A T-test with SPSS/PC+ was conducted to determine significance, and the result is shown in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Test items</th>
<th>Matrine (1 μM)</th>
<th>Matrine (10 μM)</th>
<th>Matrine (50 μM)</th>
<th>Oxymatrine (1 μM)</th>
<th>Oxymatrine (10 μM)</th>
<th>Oxymatrine (50 μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Increasing rate of collagen synthesis (%)</td>
<td>20</td>
<td>23</td>
<td>40</td>
<td>11</td>
<td>15</td>
<td>22</td>
</tr>
<tr>
<td>Inhibition of collagenase (%)</td>
<td>19</td>
<td>20</td>
<td>32</td>
<td>6</td>
<td>13</td>
<td>17</td>
</tr>
</tbody>
</table>

As shown in Table 1, matrine and oxymatrine enhanced collagen synthesis and inhibited collagenase activity in concentration-dependent manner. Meanwhile, it was founded that effects of matrine on collagen synthesis and collagenase activity inhibition was more excellent than that of oxymatrine.

Therefore, these results demonstrate that matrine and oxymatrine have effect of wrinkles improvement.

**Preparation Example A and Comparative Preparation Example: Preparation of nutrient cream**

A nutrient cream (preparation example A) containing matrine and oxymatrine were prepared as indicated in Table 2. Aqueous phases including purified water, triethanolamine and propylene glycol, and oil phases including fatty acids, oil components, emulsifiers, and preservatives were heated to 70°C and mixed for emulsification. After completion of the emulsification, the emulsion was cooled to 45°C. Matrine, oxymatrine and perfumes were added and dispersed before cooling to 30°C. In contrast, Comparative Preparation Example was prepared in a manner similar to that of Preparation Example A, with the exception that purified water was used instead of matrine or oxymatrine.
Table 2

Components and contents of nutrient cream containing matrine or oxymatrine

<table>
<thead>
<tr>
<th>Components</th>
<th>Contents (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrine or oxymatrine</td>
<td>1.00 or 5.00</td>
</tr>
<tr>
<td>Jojoba oil</td>
<td>5.0</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>7.0</td>
</tr>
<tr>
<td>Cetearyl alcohol</td>
<td>2.0</td>
</tr>
<tr>
<td>Polyglycerol-3 methyl glucose distearate</td>
<td>2.0</td>
</tr>
<tr>
<td>Glyceryl stearate</td>
<td>0.5</td>
</tr>
<tr>
<td>Squalene</td>
<td>3.0</td>
</tr>
<tr>
<td>Propylene glycol</td>
<td>4.0</td>
</tr>
<tr>
<td>Glycerin</td>
<td>5.0</td>
</tr>
<tr>
<td>Triethanolamine</td>
<td>0.3</td>
</tr>
<tr>
<td>Carboxy vinyl polymer</td>
<td>0.3</td>
</tr>
<tr>
<td>Tocopherol acetate</td>
<td>0.2</td>
</tr>
<tr>
<td>Preservatives, Perfumes</td>
<td>Trace amounts</td>
</tr>
<tr>
<td>Purified water</td>
<td>Added to reach 100</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>

Example 2: Measurement of effects of cosmetics containing matrine and oxymatrine on the wrinkles improvement

The effects of cosmetics containing matrine and oxymatrine on the improvement of wrinkles were measured in a clinical demonstration. The nutrient creams prepared in Preparation Example A (nutrient creams containing matrine and oxymatrine in amounts of 1% and 5%, respectively) and Comparative Preparation Example (a nutrient cream containing purified water) were used.
The effects of wrinkle improvement were evaluated by measuring the changes in the elasticity of the skin. Measurements were conducted on 30 healthy female test subjects (aged 25 to 35) in a stable environment of temperature ranging from 24°C to 26°C and humidity ranging from 38% to 40%. After 4 types of nutrient creams of Preparation Example A and the nutrient cream of Comparative Preparation Example were applied to the facial skin of test subjects twice a day for 3 months, the elasticity was measured using a Cutometer SEM 474 (Courage+Khazaka, Cologne, Germany). Relative grades were set forth for skin elasticity within a range from zero for no elasticity to 5 for the highest elasticity measured, and the results are shown in Table 3.

**Table 3**

<table>
<thead>
<tr>
<th>Test Item</th>
<th>Preparation Ex. A (nutrient cream with 1% matrine)</th>
<th>Preparation Ex. A (nutrient cream with 5% matrine)</th>
<th>Preparation Ex. A (nutrient cream with 1% oxymatrine)</th>
<th>Preparation Ex. A (nutrient cream with 5% oxymatrine)</th>
<th>Comparative Preparation Ex. A (nutrient cream with 0% matrine and oxymatrine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin elasticity</td>
<td>4.1</td>
<td>4.8</td>
<td>3.6</td>
<td>4.5</td>
<td>1.45</td>
</tr>
</tbody>
</table>

As shown in Table 3, the preparation example A of the present invention showed significantly greater effects on the improvement of wrinkles compared to the comparative preparation example, and skin elasticity enhanced as the concentration of matrine and oxymatrine increased.

**Example 3: Measurement of effects of matrine and oxymatrine on inhibition of melanin production**

After measuring the inhibition of melanin production by matrine and oxymatrine using B16 mouse melanoma cells (Korean Cell Line Bank), the measurement was compared with that for the inhibition of melanin production by arbutin, known as a melanin production inhibitor.
B16 mouse melanoma cells FIO (Korean Cell Line Bank) were seeded into each well of a 6-well plate (1 x 10^5 cells/well) in DMEM (Dulbecco's modified Eagle's media) containing 10% FBS (fetal bovine serum) and the cells were cultured to about more than 80% confluence by incubating in a CO2 incubator under conditions of 37°C and 5.0% CO2. After cultivation, the medium was removed and samples were replaced in medium diluted at suitable concentration, followed by incubating for 3 days under conditions of 37°C and 5.0% CO2. The concentration of matrine and oxymatrine was determined with 10 µM, 100 µM and 500 µM, which did not show cytotoxicity. The cells removing medium were washed with PBS (phosphate buffer saline) and treated with trypsin to collect cells. Number of the collected cells was calculated using hematocytometer (Tiefe Depth Profondeur 0.100 mm, Paul Marienfeld GmbH & Co. KG, D.E), centrifuged at 5,000 to 10,000 rpm for 10 min and the supernatant was eliminated, thereby obtaining pellets. This cell pellets were dried at 60°C, 100 µl of 1M NaOH containing 10% DMSO was added to thereto, and intracellular melanin was obtained in incubator at 60°C. Then, the cell solution was measured for absorbance at 490 nm using microplate reader (Bio-Tek ELx8081U, U.S) and the amount of melanin per cell constant number was estimated. The experiment results were summarized in Table 4.

**Table 4**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatment concentration (µM)</th>
<th>Inhibitory rate of melanin production (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albutin</td>
<td>100</td>
<td>26</td>
</tr>
<tr>
<td>Matrine</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>31</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>39</td>
</tr>
<tr>
<td>Oxymatrine</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>31</td>
</tr>
</tbody>
</table>
As indicated in Table 4, matrine and oxymatrine inhibited melanin production in concentration-dependent manner. In addition, it was found that matrine and oxymatrine showed significantly greater effects on the inhibition of melanin production compared to albutin.

Example 4: Inhibitory effect on tyrosinase activity by matrine and oxymatrine

After measuring the inhibition of melanin production by matrine and oxymatrine using B16 mouse melanoma cells (Korean Cell Line Bank), the measurement was compared with that for the inhibition of intracellular tyrosinase activities by arbutin, known as a melanin production inhibitor.

Murine melanoma (B-16 Fl) cells were seeded into each well of a 6-well plate (1 x 10^5 cells/well) in DMEM containing 10% FBS (fetal bovine serum) and the cells were cultured to about more than 80% adherence by incubating in a CO_2 incubator under conditions of 37°C and 5.0% CO_2. After cultivation, the medium was removed and samples were replaced in medium diluted at suitable concentration, followed by incubating for 3 days under conditions of 37°C and 5.0% CO_2. The concentration of matrine and oxymatrine was determined with 10 µM, 100 µM and 500 µM, which did not show cytotoxicity. The cells removing medium were washed with PBS (phosphate buffer saline) and treated with trypsin to collect cells. Number of the collected cells was calculated using hematocytometer, centrifuged at 5,000 to 10,000 rpm for 10 min and the supernatant was eliminated, thereby obtaining pellets. This cell pellets were lysated using lysis buffer, centrifuged at 12,000 rpm for 10 min and the supernatant was collected. Then, the cell solution was measured for absorbance at 492 nm using microplate reader and the activity of tyrosinase per cell constant number was estimated. The results were summarized in Table 5.

Table 5

<table>
<thead>
<tr>
<th>Sample</th>
<th>Treatment concentration (µM)</th>
<th>Inhibitory rate of intracellular tyrosinase activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albutin</td>
<td>100</td>
<td>29</td>
</tr>
</tbody>
</table>
As shown in Table 5, the results demonstrate that matrine and oxymatrine inhibited significantly greater effects on the inhibition of intracellular tyrosinase activities than albutin.

Example 5: Evaluation of skin whitening effect in animal level

A whitening effect of matrine and oxymatrine was measured using brown guinea pigs (Charles River Laboratories, Inc.), known to increase its pigmentation upon exposure to ultraviolet light, like in humans.

To cause pigmentation in the brown guinea pig by ultraviolet (UV), aluminum foil with square windows of 3x3 cm² was adhered to hair-removed abdominal skin of brown guinea pig, and then UV light was irradiated thereon with a SE lamp (wavelength 290-320 nm, Toshiba) (total irradiation energy = 1350 mJ/cm²). After UV irradiation, the aluminum foil was removed and samples (matrine, oxymatrine or albutin) were applied as the following method. Increased pigmentation was observed at 2 or 3 days after UV irradiation and reached a maximum after about 2 weeks. From the maximum, samples were applied. Applications performed once or twice a day for 50 days. The samples were dissolved or diluted in a certain solvent (Propylene glycol : ethanol : water = 5 : 3 : 2) and applied by a swab. The control with only the solvent was applied to another site. Occurrence of cumulative irritation also was examined.

The degree of pigmentation of skin was determined using a chromameter (CR2002, MINOLTA, JP) to estimate the effects of applied samples. The results are shown in Table 6 below. L’-a’-b’ colorimetric system was used to classify color and L’ value was used as standard in the present invention. The L’ value was corrected using

<table>
<thead>
<tr>
<th></th>
<th>10</th>
<th>100</th>
<th>500</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrine</td>
<td>13</td>
<td>32</td>
<td>37</td>
</tr>
<tr>
<td>Oxymatrine</td>
<td>11</td>
<td>29</td>
<td>34</td>
</tr>
</tbody>
</table>
white board standard and was measured more than five times at one site, repeatedly. Pigmentation was evenly distributed. Skin color differences ($\Delta L^*$) between application initial point and application terminal point were obtained and then using these values, their effects of the applied samples were estimated.

**Equation 1**

$$\Delta L^* = L^* \text{ value at 00 days after application} - L^* \text{ value at application initial day}.$$  

$\Delta L^*$ values were obtained both at sample application site and control application site and compared, whereby the effects of the whitening substances can be estimated. These experiment results were summarized in the following Table 6.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatment concentration (%)</th>
<th>Whitening effects ($\Delta L$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrine</td>
<td>0.2</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.53</td>
</tr>
<tr>
<td>Oxymatrine</td>
<td>0.2</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.50</td>
</tr>
<tr>
<td>Albutin</td>
<td>1.0</td>
<td>0.49</td>
</tr>
<tr>
<td>Control</td>
<td>-</td>
<td>0.35</td>
</tr>
</tbody>
</table>

As described in Table 6, matrine and oxymatrine exhibited the whitening effects in concentration-dependant manner. Furthermore, matrine and oxymatrine showed more excellent whitening effects than albutin, and also had a safety due to not observing the occurrence of cumulative irritation.

**Example 6: Safety test of matrine and oxymatrine on human skin**

In order to find out whether matrine and oxymatrine are safe for use on human skin, a skin safety test was conducted. Suitable for this was a cumulative skin irritation test.

Squalene-based preparations containing matrine and oxymatrine in amounts of 1%, 5%, and 10% were applied in patches 9 times to the upper arms of 30 healthy adults once every other day for a total time period of 24 hours. This 24-hour
cumulative patch test was conducted to determine whether matrine and oxymatrine irritates the skin or not.

The Finn chamber (Epitest Ltd, Finland) was chosen as the patching method. The above preparation for external use on the skin was dropped into each chamber in an amount of 15 µl, and a patch was applied. The level of reaction on the skin for each test was scored using the following Equation 1, and the result was shown in Table 7.

**Equation 2**

Average reaction level = [((Reaction index x reaction level)/No. of test subjects x maximum points (4 points)) x 100] ÷ No. of test (9 times)

Points were marked in accordance with reaction level, for example, ± for 1 point, + for 2 points, and ++ for four points. The composition can be considered safe if the average reaction level is below 3.

**Table 7**

<table>
<thead>
<tr>
<th>Test materials</th>
<th>Week 1</th>
<th>Week 2</th>
<th>Week 3</th>
<th>Average reaction level</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
<td>4th</td>
</tr>
<tr>
<td>Control (squalene)</td>
<td>± +</td>
<td>± +</td>
<td>± +</td>
<td>± +</td>
</tr>
<tr>
<td>Matrine (1%) [Test 1]</td>
<td>- -</td>
<td>0 -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Matrine (5%) [Test 2]</td>
<td>1 -</td>
<td>0 -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Matrine (10%) [Test 3]</td>
<td>2 -</td>
<td>0 -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Oxymatrine (1%) [Test 4]</td>
<td>- -</td>
<td>0 -</td>
<td>- -</td>
<td>- -</td>
</tr>
<tr>
<td>Oxymatrine (5%)</td>
<td>2 -</td>
<td>0 -</td>
<td>- -</td>
<td>- -</td>
</tr>
</tbody>
</table>
In the Table 7, the number of people is zero, 1, 2, zero, 2 and 2, respectively, for ±, + and ++ all in Tests 1, 2, 3, 4, 5, and 6, the average reaction level was calculated to be not more than 3. As the average reaction level is below 3, matrine and oxymatrine was proven to be a safe substance for human skin, not showing any significant cumulative irritation.

**Example 7: Anti-oxidation effect**

Superoxide dismutase (SOD) has been known as an anti-oxidation-catalyzing enzyme which converts superoxide anion into \( \text{H}_2\text{O}_2 \) and \( \text{O}_2 \). This experiment evaluates anti-oxidation activity of sample by observing removal of superoxide anion generated by xanthine oxidase. The experiment was performed using the kit (SOD Assay Kit-WST) purchased from Dojindo (JP) in accordance with manufacture's protocol. The experiment results were summarized in Table 8.

**Table 8**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Treatment concentration (µM)</th>
<th>Removal rate of superoxide anion (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Matrine</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>24</td>
</tr>
<tr>
<td>Oxymatrine</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>32</td>
</tr>
</tbody>
</table>
As shown in Table 8, matrine and oxymatrine of the present invention exerted the anti-oxidation effects in concentration-dependant manner. Meanwhile, oxymatrine showed slightly more excellent anti-oxidation effects relative to matrine.

Example 8: Evaluation of anti-inflammatory effects

To determine whether matrine and oxymatrine had anti-inflammatory effects, the experiment of COX (cyclooxygenase) inhibitory ability was carried out using human monocytic cell, THP-I cell (Korean Cell Line Bank) according to conventional method. The COX activity was measured in accordance with Methods in Enzymology 43:9 (1994) published by F. J. Van de Ouderaa and Muytenhek. THP-I cell line was cultivated and aliquoted into 24-well plate. The incubation volume per well was adjusted to 500 µl, 2 µl of the sample compound, which dissolved in lipopolysaccharide (1 //g/ml, Sigma) and solvents described in the following Table 9 respectively, was added and cultivated for 24-48 hr under the same condition. After 24-48 hr, calcium ionophore (Sigma) and [1-14C] arachidonic acid 1 µl (in EtOH, 0.1 µCi/ml, Sigma) were added to each well, and cultivated for 10 min under the same condition. Following the cultivation, citric acid was added to each well for adjusting to pH 3.5, shaked, each 500 µl of cultivation solution taken from the plate was aliquoted into micro centrifuge tube, 700 µl of ethylacetate was added to thereto, and the solution was shaking extracted for 10 min. 500 µl of ethylacetate layer was concentrated using speed vacuum dryer for 20 min, 20 µl of the residual was dissolved in ethylacetate, and the resultant was employed with authentic standard in TLC plate. The radioactive band was identified by authentic eicosanoid standards, the radioactivity of the identified band was measured using BAS 2000 bio-imaging analyzer (Fuji, JP) (Table 9).

Table 9

<table>
<thead>
<tr>
<th>Samples</th>
<th>COX activities (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS (1 µg/ml)</td>
<td>100</td>
</tr>
</tbody>
</table>
As described in Table 9, the results address that matrine and oxymatrine effectively inhibited COX activities as treatment concentrations increased. From these results, it was founded that matrine and oxymatrine have the inflammatory inhibitory effects.

**Example 9: Evaluation of Immunosuppressive effects**

For examining whether the samples used in this example suppressed the immune response related to inflammatory, the experiment of interleukin-2 luciferase reporter activity was performed. Interleukin-2 promoter was reported to play an important role in the generation of cytokine related to inflammatory. The activity of interleukin-2 luciferase was measured using the following method: Human T lymphocytes cell line, 1 x 10^6 of Jurkat cell (Korean Cell Line Bank) were aliquoted into each well of 6-wells, IL-2 luciferase reporter plasmid DNA (Stratagene) was transfected using superfect transfection reagent (In vitrogen). 24 hr after transfection, PHA (Phytohemaglutinin, Sigma) (100 ng/ml) was treated to activate Jurkat cell and each samples was treated with varying concentration. Following 24 hr, the cell was collected and the luciferase activity was measured using luminometer (Berthold Technologies GmbH&Co.KG, Germany). The results were summarized in Table 10.

**Table 10**

<table>
<thead>
<tr>
<th>Samples</th>
<th>IL-2 luciferase activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHA (100 ng/ml)</td>
<td>100</td>
</tr>
</tbody>
</table>
As shown in Table 10, the results address that matrine and oxymatrine have the immuno-regulatory activities by inhibiting the IL-2 expression as treatment concentrations increased.

Example 10: Anti-obesity effect

An obesity inhibition test was performed by the following method using well-known animals. Table 11 shows the results.

Method for determining obesity-inhibitory activity was as follows:

Crj : ICR male mice (obtained from Charles River Japan Ltd) aged 7 weeks were preliminarily fed for 1 week, then classified into groups each having 7 animals and subjected to the test. The animals were fed in a thermo-hygrostat at a temperature of 23±1°C, and a humidity of 55±5% under illumination for 12 hours per day. They were fed with a feed Labo MR (manufactured by Nippon Nosan) and allowed to take water ad libitum. The matrine was in the form of liposome in 5% lecithin to become 0.1% and 1%. The concentration of each sample solution was regulated so that 0.1 ml of the solution was given per 10 g body weight of mice. The doses employed were 1.5 g/kg and 1 g/kg. To a control group, 5% lecithin emulsion were administered. After fasting the mice, the sample was administered once by force on the next day. During the test period over 2 weeks, the body weight and general conditions were monitored.

Table 11
As shown in Table 11, the body weight gain was inhibited in matrine and oxymatrine. It was observed to the excellence of the effects, as administered concentration was high.

Example 11: Effect of hair loss prevention and hairs growth promotion

For measuring the effect of hair loss prevention and hairs growth promotion, the samples were prepared in the form of hydrogel base containing only viscosity-increasing agent and preservative and test was performed. Each 3 cc of liquids for external use for promoting hairs growth prepared were applied to the hair loss sites of 10 baldness patients twice a day for 3 months. As a result, the liquids containing the samples showed an excellent effect such as the generation of the root of hair from 8 baldness patients. The experiment results were as follows. The control group used the moxidil commercially available from Hanmi pharmaceutical Co. Ltd.

<table>
<thead>
<tr>
<th>Solvents</th>
<th>Days</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Untreatment group</td>
</tr>
<tr>
<td>Matrine liposome</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>37.1</td>
</tr>
<tr>
<td>Oxymatrine</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>liposome</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>37.1</td>
</tr>
</tbody>
</table>

As indicated in Table 12, the results demonstrate that matrine and oxymatrine of this invention exerted a similar hairs growth effect to commercially available moxidil as hairs growth formulations.
As described hereinabove, the present invention provides a composition for improving skin conditions comprising matrine and oxymatrine as an active ingredient. The matrine and oxymatrine have lower cytotoxicity compared to retinol used as anti-wrinkle agents and exhibit the inhibition effect on collagenase activity and promotion effect on collagen biosynthesis at a molecular level, and as a result, excellent efficacy in improvement of skin wrinkles. In addition, the matrine and oxymatrine exhibit the inhibition effect on melanin production by inhibiting intracellular tyrosinase activity, the improving effects of UV-induced skin damage and the skin growth promotion or hair loss prevention. Therefore, the matrine and oxymatrine have the excellent improvement effects on skin conditions. Furthermore, the matrine and oxymatrine have the excellent anti-obesity and anti-oxidation effects. The composition of this invention can be applied to cosmetic, pharmaceutical and food composition due to having no cytotoxicities and side effects.

Having described a preferred embodiment of the present invention, it is to be understood that variants and modifications thereof falling within the spirit of the invention may become apparent to those skilled in this art, and the scope of this invention is to be determined by appended claims and their equivalents.
What is claimed is:

1. A composition for improving a skin condition comprising matrine or oxmatrine as an active ingredient, wherein the skin condition is wrinkles, whitening, UV-induced skin damage or hair growth.

2. A composition for anti-oxidation comprising matrine or oxmatrine as an active ingredient.

3. A composition for anti-obesity comprising matrine or oxmatrine as an active ingredient.

4. The composition according to any one of claims 1 to 3, wherein the matrine or oxmatrine is present in the amount of 0.0001-10 wt% based on the total weight of the composition.

5. The composition according to any one of claims 1 to 4, wherein the composition is a cosmetic composition.

6. The composition according to claim 5, wherein the composition is in the form of one selected from the group consisting of a solution, a suspension, an emulsion, a paste, a gel, a cream, a lotion, a powder, a soap, a surfactant-containing cleanser, an oil, a powder foundation, an emulsion foundation, a wax foundation and a spray.

7. The composition according to any one of claims 1 to 4, wherein the composition is a pharmaceutical composition.

8. The composition according to any one of claims 1 to 4, wherein the composition is a food composition.
9. A method for improving skin condition, which comprises administering to a subject a composition comprising matrine or oxymatrine as an active ingredient.

10. The method according to claim 9, wherein the skin condition is wrinkles, whitening, UV-induced skin damage or hair growth.

11. A method for preventing oxidation, which comprises administering to a subject a composition comprising matrine or oxymatrine as an active ingredient.

12. A method for suppressing obesity, which comprises administering to a subject a composition comprising matrine or oxymatrine as an active ingredient.

13. The method according to any one of claims 9 to 12, wherein the matrine or oxymatrine is present in the amount of 0.0001-10 wt% based on the total weight of the composition.

14. The method according to any one of claims 9 to 13, wherein the composition is a cosmetic composition.

15. The method according to claim 14, wherein the composition is in the form of one selected from the group consisting of a solution, a suspension, an emulsion, a paste, a gel, a cream, a lotion, a powder, a soap, a surfactant-containing cleanser, an oil, a powder foundation, an emulsion foundation, a wax foundation and a spray.

16. The method according to any one of claims 9 to 13, wherein the composition is a pharmaceutical composition.
17. The method according to any one of claims 9 to 13, wherein the composition is a food composition.

18. A use of matrine or oxymatrine for manufacturing a composition for improving skin condition.

19. The use of the matrine or oxymatrine according to claim 18, wherein the skin condition is wrinkles, whitening, UV-induced skin damage or hair growth.

20. A use of matrine or oxymatrine for manufacturing a composition for anti-oxidation.


22. The use of the matrine or oxymatrine according to any one of claims 18 to 21, wherein the matrine or oxymatrine is present in the amount of 0.0001-10 wt% based on the total weight of the composition.

23. The use of the matrine or oxymatrine according to any one of claims 18 to 22, wherein the composition is a cosmetic composition.

24. The use of the matrine or oxymatrine according to claim 23, wherein the composition is in the form of one selected from the group consisting of a solution, a suspension, an emulsion, a paste, a gel, a cream, a lotion, a powder, a soap, a surfactant-containing cleanser, an oil, a powder foundation, an emulsion foundation, a wax foundation and a spray.
25. The use of the matrine or oxymatrine according to any one of claims 18 to 22, wherein the composition is a pharmaceutical composition.

26. The use of the matrine or oxymatrine according to any one of claims 18 to 22, wherein the composition is a food composition.
**Fig 1**

![Cell Viability Graph](image_url)
Fig 2
Fig 3

[Bar chart showing MMP-1 activity (% of control) for different treatments: PMA, Martine 1μM, Martine 10μM, Martine 50μM, oxymartine 1μM, oxymartine 10μM, oxymartine 50μM. The chart compares the activity levels before and after PMA 100 nM treatment.]
INTERNATIONAL SEARCH REPORT

PCT/ISA/210 (second sheet) (April 2007)

INTERNATIONAL SEARCH REPORT

PCTYKR2008/001021

A. CLASSIFICATION OF SUBJECT MATTER

A61K 8/49(2006.01)i, A61Q 19/08(2006.01)i, A61Q 19/02(2006.01)i, A61Q 17/00(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)

IPC 8 A61K 8/49, A61Q 19/08, 19/02, 17/00

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

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

eKIPASS(KIPO internal), PubMed, JPO, USPTO, Google

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
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<tr>
<td>A</td>
<td>KR 100311 199 B1 (LG CHEM INVESTMENT, LTD ) 24 SEPTEMBER 2001&lt;br&gt;See abstract, 2 page, claim 1</td>
<td>1-4, 9-13, 18-22</td>
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<tr>
<td>A</td>
<td>JP 2003355677 A2 (Japan Science and Technology Agency) 25 NOVEMBER 2003&lt;br&gt;See abstract, paragraphs 0007-0012 and 0027</td>
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<td>A</td>
<td>JP 01128934 A (JAISEITOU, LTD) 22 MAY 1989&lt;br&gt;See abstract, example 1</td>
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* Special categories of cited documents
  "A" documentation defining the general state of the art which is not considered to be of particular relevance
  "E" earlier application or patent but published on or after the international filing date
  "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)
  "O" document referring to an oral disclosure, use, exhibition or other means
  "P" document published prior to the international filing date but later than the priority date claimed

See patent family annex

Further documents are listed in the continuation of Box C

Date of the actual completion of the international search

09 JUNE 2008 (09.06.2008)

Date of mailing of the international search report

09 JUNE 2008 (09.06.2008)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
Government Complex-Daejeon, 139 Seomsa-ro, Seo-gu, Daejeon 302-761, Republic of Korea

Facsimile No 82-42-472-7140

Authorized officer

PARK, Yeong Gwan

Telephone No 82-42-481-8407

Form PCT/ISA/210 (second sheet) (April 2007)
**INTERNATIONAL SEARCH REPORT**

**International application No**
PCT/KR2008/001021

**Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos because they relate to subject matter not required to be searched by this Authority, namely.

2. [x] Claims Nos 6, 15 and 24
   - because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically.
   - Claims 6, 15 and 24 refer to claims which are not searchable due to not being drafted in accordance with the third sentence of Rule 6.4(a).

3. [x] Claims Nos 5, 7, 8, 14, 16, 17, 23, 25 and 26
   - because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims, it is covered by claims Nos.

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- [ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- [ ] No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2007)
<table>
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<td></td>
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