Abstract: The present invention provides a pharmaceutical composition for preventing or treating a metabolic disease, comprising an extract from Smilax China Linne as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver. The present invention results in decreases of gene expressions of major transcription factors LXRα and SREBP1-C, which play an important role in decrease of body fats, decrease of visceral fats and adipogenesis, and their target genes CD36 and aP2, whereby the present invention exhibits effects for preventing or treating obesity. The present composition improves fat accumulation in liver tissue by decreasing lipid levels in liver tissue such that it has effects for preventing or treating fatty liver. The present composition provides a food composition for improving or alleviating a metabolic disease, comprising an extract from Smilax China Linne as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver. In addition, the present composition provides a method for preventing or treating a metabolic disease, comprising administering to a subject in need thereof a therapeutically effective amount of a composition comprising an extract from Smilax China Linne as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.
PHARMACEUTICAL COMPOSITIONS COMPRISING AN EXTRACT FROM SMILAX
CHINA LINNE FOR PREVENTING OR TREATING OBESITY, HYPERLIPIDEMIA OR
FATTY LIVER

BACKGROUND OF THE INVENTION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority from Korean Patent Application No. 2012-0033316, filed on March 30, 2012, in the Korean Intellectual Property Office, the disclosure of which is incorporated herein by reference.

FIELD OF THE INVENTION

The present invention relates to a pharmaceutical composition for preventing or treating obesity, hyperlipidemia or fatty liver, containing an extract from Smilax china Linne as an active ingredient.

DESCRIPTION OF THE RELATED ART

The World Health Organization has warned that overweight and the present obese population (BMI > 30) amounting to 1.0 billion people will increase by 50% to reach 1.5 billion people in 2015, thereby serious health problems may be spawned (September 22, 2005). Obesity is considered as the cause of a variety of diseases. It is the cause of 80% in diabetes patients and 21% in heart disease patients. The socioeconomic loss caused by obesity is enormous [the loss in the United States increased approximately 1,170 billion dollars each year; the loss in Korea was 1 trillion 17 billion won in 2001, however, it increased to 1.8 trillion won in 2005 (direct cost 1,087 billion and indirect cost 7,152 billion won)]. Therefore, developments of safe and effective therapeutic drugs for obesity can reduce the enormous socioeconomic loss. However, therapeutic agents which can resolve the market situation are limited to appetite suppressants and fat absorption inhibitors such that it falls short of expectation and has various side effects as follows. Xenical (Roche), which reduces absorption
of fat by inhibiting lipase which secreted in pancreas and digestive system, has side effects such as 2-3% of low weight-loss effect, frequent diarrhea and fatty stools. In addition, Reductil (Abbott), which suppresses appetite by inhibiting reabsorption of serotonin and noradrenaline, shows 5-10% weight-loss effect. However, Reductil has adverse effects for cardiovascular diseases such as stroke and myocardial infarction. For this reason, it was subject to ban in prescription and administration and voluntary recall by European Medicines Agency (EMA) and USA Food and Drug Administration (FDA) in 2010, finally excluded from the market. Meanwhile, it was stopped in sales by Korea Food and Drug Administration in October 2010. Besides, a large number of anti-obesity drugs have been withdrawn from the market due to severe side effects. For example, aminophylline is reported to have various side effects in the nervous, circulatory and digestive systems despite its excellent effect of reducing body fat. Also, fenfluramine, dexfenfluramine, topiramate, ephedrine, etc. have been banned from being marketed as obesity drugs. As the synthetic drugs show limitations in side effects and in overcoming chronic diseases, foods and drugs derived from natural sources are drawing attentions.

NAFLD (non-alcoholic fatty liver disease) refers to a liver disease associated with triglyceride accumulation in the liver regardless of drinking alcohols, including steatosis and NASH (non-alcoholic steatohepatitis). Steatosis is considered benign diseases with good prognosis in clinic, but NASH accompanied with fatty liver, inflammation or fibrosis is recognized as progressive liver diseases inducing cirrhosis or liver cancer.

The obesity and the insulin resistance are representative of risk factors for NAFLD. The risk factors of hepatic fibrosis progression are, for example, obesity (BMI> 30), ratio of liver function parameters detected in serum (AST/ALT> 1) and diabetes. Hepatitis C carriers having the non-alcoholic fatty liver would be progressed to liver cancer; and therefore there are emerging needs for prevention and treatment of non-alcoholic fatty liver. 69-100% of patients with non-alcoholic fatty liver have obesity and 20-40% of patients in obesity are accompanied with fatty liver. Especially, prevalence of liver disease in the male obesity patients is higher than obesity women patients. There has been reported that 3-30% of adults
with normal weight as well as obesity patients have fatty liver diseases in the Western society. In Japan having similar diet patterns to Korea, the prevalence of non-alcoholic fatty liver is estimated to be approximately 20% and 1% of them is estimated to be NASH. Non-alcoholic fatty liver became problems in obese children as well as adults. 10-77% of obese children (inhabited in Europe, USA and Asia) show non-alcoholic fatty liver lesions, because the most important risk factor for non-alcoholic liver disease is obesity.

The pathogenesis of non-alcoholic fatty liver may be explained by two mechanisms. The first mechanism is that the increase in free fatty acids inhibits fatty acid oxidation in hepatocytes, thereby accumulating fatty acids in hepatocytes to cause non-alcoholic fatty liver. The second mechanism involves a variety of physiological factors associated with inflammation and fibrosis progression. More specifically, the increase in levels of fatty acid induces to elevate the expression of cytochrome peroxidase 2E1 and CYP2E1 and to generate reactive oxygen species resulting in lipid peroxidation of liver cell membrane and the increase in LPS and oxidative stress, increasing the level of TNF-a being responsible for apoptosis of hepatocytes, finally inducing liver damage. The insulin resistance and the accumulation of fatty acids contribute to mitochondrial dysfunction, and the latter increases reactive oxygen species and nitric oxide synthase (NOS), thereby inducing cell death.

The best way to treat NAFDL may be considered a weight loss through lifestyle changes (e.g., the exercise). However, when it is difficult to treat NAFDL with only exercise, chemotherapeutics may be combined with exercise. Chemotherapeutics for non-alcoholic fatty liver are classified: First, there are drugs with remediation of risk factors for treating and improving fatty liver, including obesity drugs (e.g., orlistat), insulin resistance drugs (e.g., metformin, pioglitazone and rosiglitazone), and hyperlipidemia drugs (e.g., clofibrate, gemfibrozil, bezafibrate, atorvastatin and simvastatin). Metformin increases oxidation of fatty acids, decreases lipogenic enzymes and improves hyperinsulinism and insulin resistance. Meanwhile, thiazolidinedione, rosiglitazone and pioglitazone are capable of activating PPARy as nuclear hormone receptors to promote the glucose uptake in muscles. Secondly, there are drugs with potentials to recover liver cell damage being independent on remediation of risk.
factors of non-alcoholic fatty liver, including hepatocyte protectors (e.g., ursodeoxycholoc acid and taurine), antioxidants (e.g., Vitamins E and C), and nutritional supplements (e.g., lecithin, betaine, and N-acetylcysteine). Unfortunately, there are no more plausible drugs with excellent therapeutic effects without adverse effects.

According to the Korean Herbal Pharmacopoeia, *Smilax china Linne* is one of rhizome plants belonging to Liliaceae. *Smilax china Linne* is also called by various synonyms such as Berchemia berchemiaefolia, Myeonggamnamu, Chamyolmaenamu, JongGasidunggul, Maebaltopgasi, Sangwirae, Myeonggam, Donggorinang, Maenggenang, Belnaegi, Belrangjinang, Maenggamnamu, Kkambagwi, Cheongyeolmaedeombul and Bangye.

*Smilax china Linne* grows in Japan, China and Korea. A root of *Smilax china Linne* is used as herb medicine. It is 5-15 cm in length, 2-5 cm in diameter, uneven, and sometimes cylindrical shape with cracked knot-shape. Externally, it is grayish yellow brown. Rhizome of *Smilax china Linne* is odorless and tasteless.

*Smilax china Linne*s known to be effective in removing wind and dampness, diuretic, detoxification and healing abscess. It has been used as a medicine in joint pain, paresthesia, dropsy, enteritis, dysentery, lymphadenitis, bloody leucorrhea, syphilis and cancer. Pharmacological actions of *Smilax china Linne*, which has been scientifically proved until now, are the anti-diabetic, anti-inflammatory, antioxidant, and anti-cancer effects. It was reported that where albino rat induced diabetes by alloxan injection was administered *Smilax china Linne* extract, the extract decreased fasting blood glucose (Rajesh Bhatii., Et al, *Asian Journal of Traditional Medicines*, 6:218-223 (2011)). In addition, it was determined that *Smilax china Linne* extract has an anti-inflammatory effect by decreasing activity of cyclooxygenase 2 in macrophage cell, which is induced by LPS, and by inhibiting accumulation of PGE2 (Xiao-Shun Shu., et al. *Journal of Ethnopharmacology* 103:327-332(2006)). It was reported that *Smilax china Linne* extract has an effect of antioxidant removing reactive oxygen species (Lee SE., et al. *Exp Mol Med.* 33:263-268(2001)). In addition, it was determined that *Smilax china Linne* extract has an anti-cancer effect (Li-Sheng Wu., et al. *Journal of Ethnopharmacology*.
It is known that active substances contained in *Smilax china Linne* are smilax saponin, prosapogenin A, dioscin, gracillin, pseudoprotodioscin, methylgracillin and methylprotodioscin (Kim, S.W. et al. 20:145-146(1989), Sashida, Y. et al, *Phytochemistry* 31: 2439-2443(1992)).

Throughout this application, various patents and publications are referenced and citations are provided in parentheses. The disclosure of these patents and publications in their entities are hereby incorporated by references into this application in order to more fully describe this invention and the state of the art to which this invention pertains.

**SUMMARY OF THE INVENTION**

The present inventors have made intensive studies to develop safe substances to human, particularly plant-derived substances having therapeutic efficacies for preventing or treating obesity, hyperlipidemia or fatty liver. As a result, they have found out that *Smilax china Linne* used as conventional medicinal herb is significantly effective in prevention or treatment of obesity, hyperlipidemia or fatty liver.

Accordingly, it is an object of this invention to provide a pharmaceutical composition for preventing or treating a metabolic disease, comprising an extract from *Smilax china Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

It is another object of this invention to provide a food composition for improving or alleviating a metabolic disease, comprising an extract from *Smilax china Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

It is still another object of this invention to provide a method for preventing or treating a metabolic disease, comprising an extract from *Smilax china Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.
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.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 represents images of body weight gain (g) in mice fed with test diets over time, total accumulated body weight gain for 8 weeks (g/56 days) and daily food intake (g/day).

Fig. 2 represents image of visceral fat-pad weight (g) of mice fed with test diets.

Fig. 3 represents images of lipids and glucose levels in blood of mice fed with test diets.

Fig. 4 represents images of measurement results on biochemical indicators for non-alcoholic fatty liver in mice fed with test diets.

Fig. 5 represents images of changes of adiponectin level in blood, and gene and protein expressions related adipogenesis in liver tissue.

DETAILED DESCRIPTION OF THIS INVENTION

In one aspect of the present invention, there is provided a pharmaceutical composition for preventing or treating a metabolic disease, comprising an extract from Smilax china Linne ets an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

The present inventors have made intensive studies to develop safe substances to human, particularly plant-derived substances having therapeutic efficacies for preventing or treating obesity, hyperlipidemia or fatty liver. As a result, they have found out that Smilax china Linne used as conventional medicinal herb is significantly effective in prevention or treatment of obesity, hyperlipidemia or fatty liver.

The present invention is significantly effective in prevention or treatment of obesity, hyperlipidemia or fatty liver.

According to the present invention, where the Similac china Linne
extract-supplemented high-fat diet is fed, visceral fat-pad weights and levels of plasma lipids such as triglyceride, total cholesterol and free fatty acids decrease. In addition, where lipid levels of liver tissues and changes of liver function are measured, the *Smiiax china Linne* exhibit significant effects.

According to the present invention, the *Smiiax china Linne* contributes to decrease in body weights and visceral fat-pad weights, levels of plasma lipids such as triglyceride, total cholesterol and free fatty acids and triglyceride level of liver tissues, thereby considerably alleviating obesity induced by HFD (high-fat diet). In addition, the gene expressions of both nuclear transcription factors and their target gene, which are increased by obesity induced with HFD in liver tissue, are decreased to inhibit adipogenesis in liver tissue, thereby considerably attenuating fatty liver.

The *Smiiax china Linne* used as an active ingredient in the present invention means to a rhizome plant belong to Liliaceae.

When the *Smiiax china Linne* extract used in the present composition is obtained by treating an extraction solvent to the *Smiiax china Linne*, the extract may be prepared using various extraction solvents. Preferably, the extraction solvent includes polar and non-polar solvents. The suitable polar solvent includes (i) water, (ii) alcohols (preferably, methanol, ethanol, propanol, butanol, n-propanol, iso-propanol, n-butanol, 1-pentanol, 2-butoxyethanol or ethylene glycol), (iii) acetic acid, (iv) DMFO (dimethyl formamide) and (v) DMSO (dimethyl sulfoxide). The suitable non-polar solvent includes acetone, acetonitrile, ethyl acetate, methyl acetate, fluoroalkane, pentane, hexane, 2,2,4-trimethylpentane, decane, cyclohexane, cyclopentane, diisobutylene, 1-pentene, 1-chlorobutane, 1-chloropentane, o-xylene, diisopropyl ether, 2-chloropropane, toluene, 1-chloropropane, chlorobenzene, benzene, diethyl ether, diethyl sulfide, chloroform, dichloromethane, 1,2-dichloroethane, aniline, diethylamine, ether, carbon tetrachloride and THF.

More preferably, the extraction solvent used in this invention includes (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, (g) butyl acetate, (h) 1,3-butyleneglycol, (i) hexane and 0) diethylether. Most
preferably, the extraction solvent used in this invention includes water, methanol, ethanol and
their combination.

The term used herein "extract" encompasses not only a crude extract but also a
fraction obtained by fractionation of the crude extract. In other words, the extract from the
*Smilax china Linne* includes not only the resultant of extraction using the extraction solvent
described above but also the resultant of additional purifications. For instance, it could be
appreciated that active fractions obtained using a variety of additional purification methods
such as an ultrafiltration with defined molecular weight cut-off value and various
chromatography (designed for purification dependent upon size, charge, hydrophobicity and
affinity) are included in the present extracts.

The *Smilax china Linne* extract used in present invention may be powdered through
additional processes such as lyophilization and spray drying.

Preferably, the extract from *Smilax china Linne* used in present invention is an extract
of *Smilax china Linne* stalk or an extract of *Smilax china Linne* leaf, and more preferably an
extract of *Smilax china Linne* stalk.

The term used herein "obesity" refers to disorders characterized by excessive
accumulation of body fats in body. As used herein, the term "fatty liver" refers to a condition
where fat accumulates excessively in hepatocytes due to the disorder of lipid metabolism. It
may cause various diseases such as angina, myocardial infarction, stroke, arteriosclerosis and
pancreatitis.

As used herein the term "hyperlipidemia" refers to a disease caused by higher level of
blood lipids due to poor metabolism of lipids such as triglyceride and cholesterol. More
specifically, hyperlipidemia is characterized by increased levels of lipids such as triglyceride,
LDL cholesterol, phospholipids and free fatty acids in blood, including hypercholesterolemia
and hypertriglyceridemia.

As used herein the term non-alcoholic fatty liver refers to liver function dysfunctions
or tissue damages, which are similar with alcoholic liver disease, in patient regardless of
drinking alcohols. More detailed, it means a case that fat accumulation in the liver of patient regardless of drinking alcohols accounts for more than about 5-10% of liver weight.

As used herein the term "metabolic diseases" refer to a group of a wide variety of diseases caused by risk factors for various cardiovascular diseases and type 2 diabetes, including insulin resistance and its related diverse and complicated metabolic and clinical abnormalities. In 1988, Reaven suggested that a common cause of these symptoms is insulin resistance and named insulin resistance syndrome; however, in 1998, WHO newly introduced the term "metabolic syndrome or metabolic diseases", because insulin resistance may not explain all the elements of these symptoms.

According to an embodiment, the present composition decreases liver fat or visceral fat.

As used herein the term "liver" or "visceral" is used to encompass organ, tissue and cell.

According to the present invention, the groups fed with the composition of the present invention showed significantly reduced lipid level in blood and liver tissues, and the total visceral fat weight was significantly reduced by 38% as compared with HFD.

According to an embodiment, the fat comprises triglyceride, cholesterol and free fatty acid.

According to an embodiment, the visceral fat comprises epididymal fat, perirenal fat, mesenteric fat and/or retroperitoneal fat.

According to an embodiment, the composition of the present invention decreases the level of ALT (alanine aminotransferase) and AST (aspartate aminotransferase) in blood.

As used herein the term "ALT (alanine aminotransferase)" and "AST (aspartate aminotransferase)" as indicators for liver function are enzymes exhibiting increased levels in blood upon damage of liver.

As used herein the term "decrease (of ALT and AST levels)" means that the levels of ALT and AST are significantly reduced to the extent being measurable, as compared with non-treated subjects (control group), preferably decrease by more than 30%, more preferably
by more than 20%, and still more preferably by more than 10%.

According to the present invention, the composition of the present invention significantly reduced both ALT (by 22%) and AST (by 38%) in blood as compared with the HFD group, addressing that the present composition has the excellent efficacies of improving fatty liver, preferably non-alcoholic fatty liver. Therefore, the present composition has the excellent efficacies of improving fatty liver in obesity induced by HFD.

According to an embodiment, the composition increases the level of adiponectin and phosphorylation of AMPK (AMP-activated protein kinase) in blood. Adiponectin in blood is adipokine secreted from adipocyte. Where adiponectin level in blood increases, it increases phosphorylation of AMPK (AMP-activated protein kinase) and decreases expressions of major transcription factors LXRα and SREBP1-C, which promote adipogenesis, and their target genes CD36 and aP2.

As used herein the term "increase (of adiponectin level in blood)" means that the levels of ALT and AST are significantly enhanced to the extent being measurable, as compared with the non-treated subjects (control group), preferably increase by more than 40%, and more preferably by more than 10%.

As used herein the term "increase (of phosphorylation of AMPK)" means that the levels of ALT and AST are significantly enhanced to the extent being measurable, as compared with the non-treated subjects (control group), preferably increase by more than 40%, and more preferably by more than 10%.

According to an embodiment, the composition decreases the expression of LXRα (Liver X receptor a), SREBP1-C (Sterol response element binding protein), CD36 (Fatty acid translocase) or aP2 (fatty acid binding protein). The present composition decreases the expression of LXRα (Liver X receptor a) and SREBP1-C (Sterol response element binding protein) and it results in decreases of expressions of their target genes CD36 and aP2, whereby adipogenesis in liver tissue is improved.

When the composition of the present disclosure is prepared as a pharmaceutical composition, the pharmaceutical composition of the present disclosure may comprise a
pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier included in the pharmaceutical composition of the present disclosure is one commonly used in the preparation of formulations and includes lactose, dextrose, sucrose, sorbitol, mannitol, starch, gum acacia, calcium phosphate, alginate, gelatin, calcium silicate, microcrystalline cellulose, polyvinyl pyrrolidone, cellulose, water, syrup, methyl cellulose, methyl hydroxybenzoate, propyl hydroxybenzoate, talc, magnesium stearate, mineral oil, etc., but is not limited thereto. The pharmaceutical composition of the present disclosure may further include, in addition to the above-described components, a lubricant, a wetting agent, a sweetener, a fragrance, an emulsifier, a suspending agent, a preservative, or the like. Suitable pharmaceutically acceptable excipients and formulations are described in detail in Remington's Pharmaceutical Sciences (19th ed., 1995).

The pharmaceutical composition of the present disclosure may be administered orally or parenterally. Preferably, it may be administered orally.

An appropriate administration dosage of the pharmaceutical composition of the present disclosure may be determined variously depending on such factors as preparation method, administration method, age, body weight and sex of a patient, pathological condition, diet, administration time, administration route, excretion rate or response sensitivity. Specifically, a daily dosage of the pharmaceutical composition of the present disclosure may be 0.001-100 mg/kg.

The pharmaceutical composition of the present disclosure may be prepared into a unit dosage form or multiple dosage form along with a pharmaceutically acceptable carrier and/or excipient according to a method that can be easily employed by those skilled in the art. The formulation may be in the form of solution in oily or aqueous medium, suspension, syrup, emulsion, extract, dust, powder, granule, tablet or capsule, and may further include a dispersant or stabilizer.

In another aspect of the present invention, there is provided a food composition for improving or alleviating a metabolic disease, comprising an extract from *Smilax china Linneas*
an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

When the composition of the present disclosure is prepared as a food composition, the food composition of the present disclosure may comprise, in addition to the extract of *Smilax chinensis* var. *Linne* of the present disclosure as the active ingredient, ingredients commonly added for preparation of food. For example, proteins, carbohydrates, fats, nutrients, seasoning or flavors may be added. The carbohydrate may be, for example, a sugar such as a monosaccharide, e.g. glucose, fructose, etc., a disaccharide, e.g. maltose, sucrose, oligosaccharide, etc. or a polysaccharide, e.g. dextrin, cyclodextrin, etc. or a sugar alcohol such as xylitol, sorbitol, erythritol, etc. The flavor may be a natural flavor [thumatin, stevia extract (e.g. rebaudioside A, glycyrrhizin, etc.)] or a synthetic flavor (saccharin, aspartame, etc.).

For example, when the food composition of the present disclosure is prepared as a drink, it may further comprise, in addition to the extract of *Smilax chinensis* var. *Linne* of the present disclosure as the active ingredient, citric acid, high-fructose corn syrup, sugar, glucose, acetic acid, malic acid, fruit juice, eucommia extract, jujube extract, licorice extract, or the like.

In still another aspect of the present invention, there is provided a method for preventing or treating a metabolic disease, comprising administering to a subject in need thereof a therapeutically effective amount of a composition comprising an extract from *Smilax chinensis* var. *Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

The features and advantages of the present invention may be summarized as follows:

(i) The present invention provides a pharmaceutical composition for preventing or treating a metabolic disease, comprising an extract from *Smilax chinensis* var. *Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.
(ii) The present invention results in decreases of gene expressions of major transcription factors LXRα and SREBP1-C, which play an important role in decrease of body fats, decrease of visceral fats and adipogenesis, and their target genes CD36 and aP2, whereby the present invention exhibits effects for preventing or treating obesity.

(iii) The present composition improves fat accumulation in liver tissue by decreasing lipid levels in liver tissue such that it has effects for preventing or treating fatty liver.

(iv) The present composition provides a food composition for improving or alleviating a metabolic disease, comprising an extract from *Smilax china Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

(v) In addition, the present composition provides a method for preventing or treating a metabolic disease, comprising administering to a subject in need thereof a therapeutically effective amount of a composition comprising an extract from *Smilax china Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

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: Preparation of extract of *Similac china Linne***

1 kg of stalks of *Similac china Linne* were ground into powder and extracted three times using 6 L of ethanol (90%) in a reflux extractor. The extract was filtered through a filter paper and cold-concentrated under vacuum, followed by drying using a cold dryer to give 80.8 g of an extract concentrate (yield 8.08%). 1 kg of leaves of *Similac china Linne* were ground into powder and extracted three times using 6 L of ethanol (90%) in a reflux extractor. The
extract was filtered through a filter paper and cold-concentrated under vacuum, followed by drying using a cold dryer to give 18.3 g of an extract concentrate (yield 18.3%).

**Example 2: Reduction of body and visceral fat-pad weights by extract of Similac china Linne**

*Preparation of test diets and maintenance of test animals*

The obesity-inducing control diet used in the test was high-fat diet (HFD: 40% fat calorie, 17 g lard + 3% corn oil/100 g diet). Diets supplemented with stem extract of *Similac china Linne* (*Similac china Linne* stalk extract-supplemented diet, T1D) had the same composition as HFD, except that the extract of *Similac china Linne* was contained in 0.4% concentration. Diets supplemented with stem extract of *Similac china Linne* (*Similac china Linne* leaf extract-supplemented diet, T2D) had the same composition as HFD, except that the extract of *Similac china Linne* was contained in 0.4% concentration. The normal diet (Chow) group was fed commercial rodent diet composition (Table1).

5-week-old male C57BL/6J mice (Orient, Korea) were accustomed to the laboratory environment for 1 week while feeding solid feed. Then, they were randomly divided into a high-fat diet group and a test group according to randomized block design and bred for a total of 8 weeks. The diet was given between 10 and 11 A.M. every day together with water. Food intake was measured every day and body weight was measured once a week. In order to avoid transient body weight increase after feed intake, body weight was measured 2 hours after removing the feed. After fasting the test animal for at least 12 hours and anesthetizing with diethyl ether, blood, liver and visceral fat (epididymal fat, perirenal fat, mesenteric fat and retroperitoneal fat) were taken and weighed after washing with 0.1 M PBS (pH 7.4). Blood taken from the abdominal aorta was centrifuged at 1000 x g for 15 minutes for the separation of plasma.
### Table 1

#### Compositions of test diets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>High-fat diet (HFD) (g/kg diet)</th>
<th>Similar china Linne stalk extract-supplemented diet (T1D) (g/kg diet)</th>
<th>Similar china Linne leaf extract-supplemented diet (T2D) (g/kg diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>D/L-Methionine</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Corn starch</td>
<td>111</td>
<td>107</td>
<td>107</td>
</tr>
<tr>
<td>Sucrose</td>
<td>370</td>
<td>370</td>
<td>370</td>
</tr>
<tr>
<td>Cellulose</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Corn oil</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Lard</td>
<td>170</td>
<td>170</td>
<td>170</td>
</tr>
<tr>
<td>Vitamin complex</td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>Mineral complex</td>
<td>42</td>
<td>42</td>
<td>42</td>
</tr>
<tr>
<td>Choline bitartrate</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Tert-butylhydroquinone</td>
<td>0.04</td>
<td>0.04</td>
<td>0.04</td>
</tr>
</tbody>
</table>

#### Changes of body and visceral fat-pad weights

After feeding the test diet for 8 weeks, the *Similac china Linne* stalk extract-supplemented group (T1D) showed decrease in the final body weight by 22% and the cumulative body weight gain by 40% as compared with HFD. The *Similac China Linne* leaf extract-supplemented group (T2D) showed decrease in the cumulative body weight gain by 25%. The dietary supplementations with the *Similac china Linne* stalk extract and the *Similac china Linne* leaf extract induced no significant change in daily food intake. The food efficiency ratio for the T1D and T2D group calculated by dividing the total body weight gain by the amount of the diet intake was analyzed to be significantly lower than that for the HFD group (Fig. 1). Therefore, it would be understood that the body weight-decreasing effect is exhibited...
by the *Similac china Linne* stalk extract and the *Similac china Linne* leaf extract and is not due to the suppression of appetite.

After feeding the test diet for 8 weeks, the epididymal, perirenal, mesenteric, and retroperitoneal fat-pads contained in the visceral fat were removed and weighed as shown in Fig. 2. The total visceral fat weight was significantly reduced by 32% in the *Similac china Linne* stalk extract-supplemented group (T1D) group than in the HFD group. In addition, the total visceral fat weight was significantly reduced by 16% in the *Similac china Linne* leaf extract-supplemented group (T2D) group than in the HFD group (*p* < 0.001, Fig. 2). Accordingly, it would be appreciated that the *Similac china Linne* stalk extract has excellent effects to reduce body weights and visceral fat-pad weights.

**Example 3: Prevention and treatment of hyperlipidemia and non-alcoholic fatty liver by *Similac china Linne* extracts**

*Biochemical analysis in blood and liver tissue*

The mice maintained for 8 weeks were analyzed for total cholesterol, triglyceride and glucose levels in the plasma and lipid levels in the liver tissue. Total cholesterol, triglyceride, free fatty acid and glucose levels in the plasma were measured twice for each using a commercially available kit (Bio Clinical System). ALT (alanine aminotransferase) and AST (aspartate aminotransferase), which are used as indicators for liver function, levels in blood were measured using a commercially analysis kit (Bio Clinical System, Korea).

Lipids were extracted from the liver tissue according to Folch et al.’s method Folch J et al., *J Biol Chem.* 226: 497-509 (1957). After adding 1 mL of distilled water to 0.25 g of the liver tissue, the liver tissue was homogenized using a Polytron homogenizer (IKA-Werke GmbH & Co., Ultra-Turrax, Staufen, Germany). After adding 5 mL of chloroform:methanol solution (2:1, v/v) to the homogenate and mixing well, the mixture was centrifuged at 1000 x g for 10 minutes. After adding 2 mL of chloroform:methanol solution (2:1, v/v) again to the supernatant, the same procedure was repeated to completely separate the lipid components of the liver. After adding 3 mL of chloroform:methanol:0.05% CaCl₂ (3:48:47, v/v/v) solution
to the remaining pellets and mixing well for 1 minute, followed by centrifugation at 1000 x g for 10 minutes, the resulting pellets were completely dried with nitrogen gas. The dried lipids were dissolved in 1 mL of methanol and then analyzed. The commercially available kits (Bio Clinical System, Korea) were used to measure the levels of triglyceride, cholesterol and free fatty acids of the liver tissue.

Changes of plasma lipid and glucose levels

After feeding the test diet for 8 weeks, the *Similac china Linne* stalk extract-supplemented group (T1D) showed significantly lower plasma levels of total cholesterol (by 36%), triglyceride (by 41%) and free fatty acid (by 45%) as compared with the HFD group (Fig. 3). Moreover, *Similac china Linne* stalk extract supplemented to the HFD resulted in a significant reduction of the fasting blood sugar level by 40%, as compared with HFD. Accordingly, it could be recognized that the *Similac china Linne* extract has the excellent effects of improving hyperlipidemia and fasting blood sugar level in obesity induced by the HFD.

Changes of hepatic lipid levels

After feeding the test diet for 8 weeks, liver absolute weight (g) in the *Similac china Linne* stalk extract-supplemented group (T1D) was significantly reduced by 22%, as compared with the HFD group and liver absolute weight (g) in the *Similac china Linne* leaf extract-supplemented group (T2D) was significantly reduced by 20%, as compared with the HFD group. The *Similac china Linne* stalk extract-supplemented group (T1D) showed significantly decreased levels of triglyceride (by 47%), cholesterol (by 71%) and free fatty acid (by 69%) in liver tissue as compared with HFD (Fig. 4). The *Similac china Linne* stalk extract-supplemented group (T1D) showed significantly decreased plasma activities of ALT (by 22%) and AST (by 39%), which are indicators for hepatic function, as compared with HFD group (Fig. 4). Accordingly, it would be understood that the *Similac china Linne* stalk extract has the excellent effect of significantly improving fatty liver which is induced by HFD.
Example 4: Regulation of protein expressions in liver tissues by *Similac china Linne* stalk extract

**Measurement of adiponectin level in blood**

Adiponectin levels in blood were measured using a commercial kit (Millipore, MA, USA). 1X assay buffer and plasma sample were added to 96-well, treated antibodies for detection and then incubated for 2 hours at room temperature. After removing the solution of each well, it was washed 5 times using washing buffer. The resultant was added with the substrate solution, incubated for 20 min at room temperature and then added with the stop solution to stop the reaction. The final reaction product was measured using a spectrometer (450 nm).

**RNA extraction and verification**

After adding 1 mL of Trizol agent (Invitrogen, USA) per 0.1 g of epididymal fat tissues, the mixture was homogenized and centrifuged at 12,000 x g for 10 min at 4°C. The supernatant was transferred to a new tube and 200 µL of chloroform was added to the tube, followed by vortexing. The same procedure was repeated twice and then the supernatant was transferred to a new tube, followed by addition of isopropanol and the supernatant at 1:1 ratio. The mixture was vigorously shaken 10 times and then incubated for 10 min at room temperature, followed by centrifugation at 12,000 x g for 10 min at 4°C to remove the supernatant. After adding 1 mL of 70% ethanol to the remaining pellet, it was centrifuged at 7,500 x g for 5 min at 4°C. After removing the ethanol, the RNA pellet contained in the tube was dried for 5 min at 4°C and dissolved in nuclease-free water. The RNA sample concentration was measured at a wavelength of 260 nm and 280 nm using a UV/VIS spectrophotometer (Beckman Coulter, DU730) and the integrity of RNA sample was verified by agarose gel electrophoresis.

**RT-PCR (reverse transcription-polymerase chain reaction) analysis results**

The RNA sample obtained from the epididymal fat tissues was transcribed using oligo
dT primer and reverse transcriptase (GIBCO BRL, Gaithersburg, MD, USA) to synthesize cDNA. The PCR amplification was performed using the cDNA as templates and primers complementary to cDNA 5' and 3' flanking sequence. The sequences of the primers used are presented in Table 2. 1 μl of the amplified products were resolved on agarose gel electrophoresis to verify DNA band.

### Table 2

<table>
<thead>
<tr>
<th>Gene</th>
<th>Direction</th>
<th>Sequence(5'→3')</th>
<th>Annealing Temp (°C)</th>
<th>Size of PCR product (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>aP2</td>
<td>F</td>
<td>AGCATCATAACCCTAGATGG</td>
<td>55</td>
<td>128</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GAAGTCACG CCTTTT CATAAC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SREBP1-c</td>
<td>F</td>
<td>ATCGCAAACA AAGCTGACCTG</td>
<td>55</td>
<td>115</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>AGATCCAGGTTT GAGGTGGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD36</td>
<td>F</td>
<td>ATGACGTGGCAAAGA AAGGC</td>
<td>55</td>
<td>160</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>GAAGGCTCAAAAGATGCCCTCC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LXR-α</td>
<td>F</td>
<td>TCCTACACGAGGATCAAGCG</td>
<td>55</td>
<td>119</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>AGTCGCAATGCAAAGACCTG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GAPDH</td>
<td>F</td>
<td>AGAACATCAT CCGCTGCATCC</td>
<td>55</td>
<td>321</td>
</tr>
<tr>
<td></td>
<td>R</td>
<td>TCCACCACCCCTGGTGCTGTA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Western blot analysis method

A certain amount of liver tissue in mortar was homogenized with liquid nitrogen and cell lysis buffer. The mixture was transferred to a new tube, followed by vortexing. After centrifugation at 12,000 x g for 10 min at 4°C, the middle layer of the tube was obtained and proteins were quantified by Bradford method. 50 μg of protein was electrophorized to SDS polyacrylamide gel, followed by immunoblotting to PVDF hyper film. The proteins were reacted using appropriate antibodies for the proteins [AMPK (AMP-activated protein kinase) and phospho AMPK (Thr172), Cell Signaling Technology, MA, USA] and detected by ECL to quantify the specific proteins.
Changes of adiponectin level in blood

After feeding the normal diet (Chow), the high-fat diet (HFD) and 0.4% of the Similac china Linne stalk extract-supplemented high fat diet (T1D) for 8 weeks, the mice were analyzed for the plasma using ELISA. As a result, it was observed that whereas adiponectin level of the plasma in HFD group was significantly reduced as compared with the Chow group, adiponectin level of the plasma in the Similac china Linne stalk extract-supplemented group (T1D) was significantly increased as compared with the HFD group (Fig. 5).

Changes of adipogenesis-related gene expression and AMPK phosphorylation in liver tissues

Adiponectin in blood is adipokine secreted from adipocyte. Where adiponectin level in blood increases, it binds to adiponectin receptors in liver tissue such that phosphorylation of AMPK (AMP-activated protein kinase) is increased. It has been known that activated AMPK inhibits phosphorylation of S6 kinase 1 (S6K1) through mTOR (mammalian target of rapamycin), it results in decreases of mRNA expressions of major transcription factors LXRα, SREBP1-C, which promote adipogenesis, and their target genes CD36 and aP2, whereby adipogenesis in liver tissue is improved.

Under the hypothesis that the Similac china Linne stalk extract is involved in adiponectin receptor-AMPK signaling of mechanism for improving fatty liver in high fat diet-supplemented mice, protein phosphorylation levels and gene expression levels for molecules related to adiponectin receptor-AMPK signaling were measured in the present invention. As a result of western blot in liver tissue, it was observed that whereas phosphorylation of AMPK in the HFD group was significantly reduced as compared with the Chow group, phosphorylation of AMPK in the T1D group was significantly increased as compared with the HFD group (Fig. 5). mRNA expressions for LXRα and SREBP1-C, which are signal transduction molecules for AMPK as well as major nuclear transcription factors, and their target genes (CD36 and aP2) in the HFD group were significantly increased as compared with the Chow group. In contrast, mRNA expressions for LXRα, SREBP1-C, CD36 and aP2 in the T1D group were significantly reduced as compared with the HFD group (Fig. 5).
Accordingly, it would be understood that the *Similac china Linne* stalk extract inhibits adipogenesis through signal pathway system mediated by adiponectin receptor-AMPK in liver tissue of high fat diet-supplemented mice.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present disclosure. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the disclosure as set forth in the appended claims.
What is claimed is:

1. A pharmaceutical composition for preventing or treating a metabolic disease, comprising an extract from *Smilax china Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

2. The composition according to claim 1, wherein the extract from *Smilax china Linne* is an extract of *Smilax china Linne* stalk or an extract of *Smilax china Linne* leaf.

3. The composition according to claim 1, wherein the composition decreases liver fat or visceral fat.

4. The composition according to claim 3, wherein the fat comprises triglyceride, cholesterol or free fatty acid.

5. The composition according to claim 3, wherein the visceral fat comprises one or more selected from the group consisting of epididymal fat, perirenal fat, mesenteric fat and retroperitoneal fat.

6. The composition according to claim 1, wherein the fatty liver is non-alcoholic fatty liver.

7. The composition according to claim 1, wherein the composition decreases the level of ALT (alanine aminotransferase) or AST (aspartate aminotransferase) in blood.

8. The composition according to claim 1, wherein the composition increases the level of adiponectin in blood.

9. The composition according to claim 1, wherein the composition increases phosphorylation of AMPK (AMP-activated protein kinase).
10. The composition according to claim 1, wherein the composition decreases the expression of LXRα (Liver X receptor α), SREBP-C (Sterol response element binding protein), CD36 (Fatty acid translocase) or aP2 (fatty acid binding protein).

11. A food composition for improving or alleviating a metabolic disease, comprising an extract from *Smilax china Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

12. A method for preventing or treating a metabolic disease, comprising administering to a subject in need thereof a therapeutically effective amount of a composition comprising an extract from *Smilax china Linne* as an active ingredient; wherein the metabolic disease is selected from the group consisting of obesity, hyperlipidemia and fatty liver.

13. The method according to claim 12, wherein the extract from *Smilax china Linne* is an extract of *Smilax china Linne* stalk or an extract of *Smilax china Linne* leaf.

14. The method according to claim 12, wherein the composition decreases liver fat or visceral fat.

15. The method according to claim 14, wherein the fat comprises triglyceride, cholesterol or free fatty acid.

16. The method according to claim 14, wherein the visceral fat comprises one or more selected from the group consisting of epididymal fat, perirenal fat, mesenteric fat and retroperitoneal fat.

17. The method according to claim 12, wherein the fatty liver is non-alcoholic fatty liver.
18. The method according to claim 12, wherein the composition decreases the level of ALT (alanine aminotransferase) or AST (aspartate aminotransferase) in blood.

19. The method according to claim 12, wherein the composition increases the level of adiponectin in blood.

20. The method according to claim 12, wherein the composition increases phosphorylation of AMPK (AMP-activated protein kinase).

21. The method according to claim 12, wherein the composition decreases the expression of LXRα (Liver X receptor α), SREBP1-C (Sterol response element binding protein), CD36 (Fatty acid translocase) or aP2 (fatty acid binding protein).
Fig. 1
Fig. 3

- FFA
- Glucose
- Total Cholesterol
- Triglyceride
Fig. 4

Liver weight (g)

Cholesterol

Triglyceride

Free fatty acid

ALT

AST

(a, b, c, d)
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
A61K 36/90(2006.01)i, A61E 3/04(2006.01)i, A61P 3/06(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)
A61K 36/90; A61P 7/12; A61K 31/70; A61K 36/896; A61K 45/00; A61K 31/7048; A61K 36/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic database consulted during the international search (name of data base and, where practical, search terms used)
eKOMPASS (KIPO internal) & Keywords: .

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>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>US 2010-0173022 A1 (RAMAUTARSING DEVINDRA) 08 July 2010</td>
<td>1-11</td>
</tr>
<tr>
<td></td>
<td>See claims, Table 8</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>KR 10-1093730 B1 (KOREA BIO POLYTECHNIC INDUSTRIAL &amp; ACADEMIC, SHINDO INDUST</td>
<td>1-11</td>
</tr>
<tr>
<td></td>
<td>KY Co., LTD.) 19 December 2011</td>
<td></td>
</tr>
<tr>
<td></td>
<td>See abstract</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>JP 2000-154151 A (JO KYO) 06 June 2000</td>
<td>1-11</td>
</tr>
<tr>
<td></td>
<td>See abstract</td>
<td></td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search 24 June 2013 (24.06.2013)

Date of mailing of the international search report 24 June 2013 (24.06.2013)

Name and mailing address of the ISA/KR

Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-70, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer
JEONG, Sei Joon

Telephone No. 82-42-481-5592

Form PCT/ISA/210 (second sheet) (July 2009)
<table>
<thead>
<tr>
<th>Box No. II</th>
<th>Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:</td>
<td></td>
</tr>
<tr>
<td>1. ☒ Claims Nos.: 12-21 because they relate to subject matter not required to be searched by this Authority, namely:</td>
<td></td>
</tr>
<tr>
<td>Claims 12-21 pertain to methods for treatment of the human body by therapy, as well as diagnostic methods, and thus relate to a subject matter which this International Searching Authority is not required, under Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.</td>
<td></td>
</tr>
<tr>
<td>2. ☐ Claims Nos.: 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:</td>
<td></td>
</tr>
<tr>
<td>3. ☐ Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Box No. III</th>
<th>Observations where unity of invention is lacking (Continuation of item 3 of first sheet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>This International Searching Authority found multiple inventions in this international application, as follows:</td>
<td></td>
</tr>
<tr>
<td>1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</td>
<td></td>
</tr>
<tr>
<td>2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.</td>
<td></td>
</tr>
<tr>
<td>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.:</td>
<td></td>
</tr>
<tr>
<td>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.:</td>
<td></td>
</tr>
</tbody>
</table>

**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.
<table>
<thead>
<tr>
<th>Patent document cited in search report</th>
<th>Publication date</th>
<th>Patent family members</th>
<th>Publication date</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>EP 2008-118010 Al</td>
<td>02.10.2008</td>
</tr>
<tr>
<td>JP 2000-154151 A</td>
<td>06.06.2000</td>
<td>AU 1999-47395 Al</td>
<td>23.03.2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>AU 1999-47395 B2</td>
<td>06.11.2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CA 2282147 Al</td>
<td>14.03.2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1154488 CO</td>
<td>23.06.2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CN 1260173 A0</td>
<td>19.07.2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0987024 A2</td>
<td>22.03.2000</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EP 0987024 A3</td>
<td>30.05.2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>KR 10-0663688 Bl</td>
<td>02.01.2007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 2002-0040050 Al</td>
<td>04.04.2002</td>
</tr>
<tr>
<td></td>
<td></td>
<td>US 6531505 B2</td>
<td>11.03.2003</td>
</tr>
</tbody>
</table>