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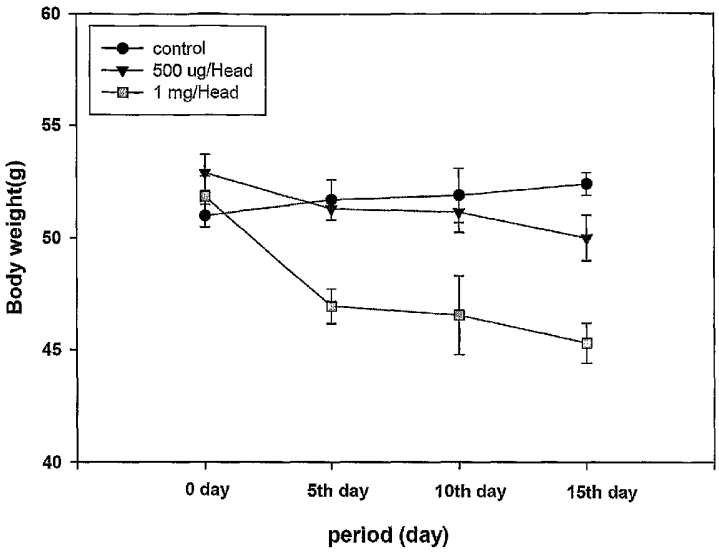
(54) Title: POLYSACCHARIDES OF PLANTS BELONGING TO PANAX HAVING EFFECT ON TREATMENT AND PREVENTION OF OBESITY



(57) Abstract: The present invention relates to a polysaccharide of plants belonging to the *Panax* family, a method for extracting the same and a pharmaceutical composition containing the same as an active ingredient. The polysaccharide in accordance with the present invention can be easily extracted from plants belonging to the*Panax* family and exhibits excellent effects for treatments and prevention of obesity-causing diseases by lowering of body fat. In addition, when extracting a polysaccharide from leaves or stems of plants belonging to the *Panax* family, it is possible to prepare a polysaccharide at very low production cost, thus being advantageous from economical point of view. Further, since plants belonging to the *Panax* family have been used as the raw material of medicines for a long period of time, general problems associated with use thereof as medicines, including toxicity can be naturally solved.

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POLYSACCHARIDES OF PLANTS BELONGING TO PANAX HAVING EFFECT ON TREATMENT AND PREVENTION OF OBESITY

[Technical Field]

The present invention relates to a polysaccharide of plants belonging to the *Panax* family producing effective treatment and prevention of obesity, a method for extracting the same and a pharmaceutical composition containing the same as an active ingredient, and more specifically to a polysaccharide extracted from plants belonging to the *P*anax family such as *Panax ginseng C.A. Mayer* and producing effective treatment and prevention of obesity by lowering of body fat.

[Background Art]

Obesity is broadly divided into primary obesity (simple obesity) and secondary obesity (symptomatic obesity: morbid obesity). Causes of the primary obesity include excessive calorie intake, insufficient energy consumption (lack of physical exercise) and reduction of calorie expenditure (basal metabolism). Most cases that are judged as obesity from clinical point of view are primary obesity. Expression and then progression of primary obesity causes various health hazards. Meanwhile, secondary obesity develops due to any underlying diseases. As examples of such obesity, mention may be made of endocrine obesity, hypothalamic obesity, genetic obesity and obesities due to drugs.

Obesity is a risk factor to health, and is responsible for diseases due to increased load of circulation system (for example, hypertension and coronary disease), diseases due to metabolism disorder (for example, diabetes, hypertriglyceridemia), disorder of the liver or biliary system (for example, cholelithiasis), a lowering of respiratory function, or diseases caused by excessive loading to bones or articular system (for example, degenerative articular diseases), or causes problems such as depression of activity.

A large number of methods for preventing and treating obesity have been studied from diverse directions. For example, there have been proposed calorie limiting diet therapy, exercise therapy, surgical therapy and pharmacotheraphy such as use of an anorexigenic agent or energy metabolism promoter, but these methods are not effectively employed due to various reasons.

At present, red ginseng, prepared by steaming ginseng, is developed and commercially available as a product for relieving obesity, and such product exerts indirect obesity prevention efficacy by taking advantage of functions that saponin, one of the constituents of ginseng, inhibits absorption of saccharides and cholesterol in the gastrointestinal tract and at the same time, promotes release of absorbed energy. However, methods of relieving obesity using ginseng polysaccharides have yet to be proposed.

As another approach to prevent or treat obesity, dietary control includes control of diet through fasting, and control of diet by use of drugs such as morphine or hormone preparations inducing satiety.

Recently, it was confirmed that administration of cytokines also induces loss of body weight, in such a manner that induction of changes in metabolism, similar to

immune reactions against infections, leads to reduction of dietary intake. Hormones such as corticotrophin releasing hormone, cholecystokinin, prostaglandins, glucagons, insulin and corticosteroids, known to inhibit appetite, were known as being induced by pyrogenic materials such as lipopolysaccharide and cytokines.

However, the fact that polysaccharides of plants belonging to the *Panax* family exhibit excellent effects on prevention and treatment of obesity has remained unknown. Further, if it is confirmed that some ingredients from plants belonging to the *Panax* family which have been used as the raw material of drugs for a long period of time have such obesity prevention and treatment effects, problems associated with toxicity on empirical basis may be naturally solved and thus it can be said that such effects will be significant.

The present inventors carried out extensive studies on polysaccharides of plants belonging to the *Panax* family and consequently filed PCT/KR03/01042 relating to anticancer effects of such polysaccharides. Subsequently, the present inventors have found that repetitive administration of such polysaccharides has significant effects on control of body weight and created the present invention based on this finding.

[Disclosure]

[Technical Problem]

Therefore, the present invention has been made in view of the above problems, and it is an object of the present invention to provide a polysaccharide (or polysaccharide fraction) extracted from plants belonging to the *Panax* family, and

capable of being used in treating and preventing obesity by lowering of body fat.

It is another object of the present invention to provide a method for extracting such a polysaccharide from plants belonging to the *Panax* family.

It is a further object of the present invention to provide a pharmaceutical composition for treating and preventing obesity containing such a polysaccharide as an active ingredient.

It is still further object of the present invention to provide a method for treating and preventing obesity using such a polysaccharide or pharmaceutical composition.

[Technical Solution]

In accordance with an aspect of the present invention, the above and other objects can be accomplished by the provision of a polysaccharide that is extracted from plants belonging to the *Panax* family and can be used for treating and preventing obesity by lowering of body fat.

As can be seen from Experimental Examples that will be described below, the polysaccharides extracted from plants belonging to the *Panax* family in accordance with the present invention lower the amount of body fat without causing significant side effects, and thus can be safely used for treating and preventing obesity.

As examples of plants belonging to the *Panax* family, mention may be made of *Panax ginseng C.A. Mayer*, *Panax quinquefolium*, *Panax notoginseng*, *Panax pseudoginseng*, *Panax japonicum*, and *Panax vietnamensis* Ha et Grushv. and they may be used alone or in any combination thereof. Among them, *Panax ginseng C.A. Mayer*

is particularly preferred.

Polysaccharides in accordance with the present invention may be obtained from roots, leaves, stems and fruits of plants belonging to the *Panax* family, respectively, and it is particularly preferably obtained from leaves and stems from an economical point of view. This is because since, unlike roots of ginseng of which pharmacological efficacy was already verified, leaves and stems thereof were annually wasted without use, it is possible to obtain desired polysaccharides of the present invention at very low cost of raw material.

Among polysaccharides in accordance with the present invention, polysaccharides of ginseng roots have a molecular weight distribution of about 6000 to 520,000 Da, and an average molecular weight of around 140,000 Da. In addition, polysaccharides of ginseng leaves and stems have a molecular weight distribution of about 6000 to 340,000 Da, and an average molecular weight of around 120,000 Da.

Polysaccharides in accordance with the present invention contains 30 to 80% neutral saccharide, 10 to 60% acidic saccharide and 0.01 to 10% protein, and the saccharide composition thereof is made up of rhamnose, fucose, arabinose, xylose, mannose, glucose, galactose, galacturonic acid, glucuronic acid, 3-deoxy-D-manno-2-octulosonic acid (KDO) and 3-deoxy-D-lyxo-2-heptulosaric acid (DHA). Specific contents of the respective saccharides may exhibit slight variation depending on kinds of plants belonging to the *Panax* family, extraction parts (such as roots, leaves and stems), extraction methods and the like. Among those saccharides, it was confirmed that KDO and DHA are polysaccharides contained only in the leaves and stems of ginseng.

Specifically, when ginseng extracts obtained through solvent extraction were

dissolved in triple distilled water in the concentration of 1 mg/mL and analyzed, it was confirmed that roots of *Panax ginseng C.A. Mayer* contain 32.8% neutral saccharide, 57.3% acidic saccharide and 6.4% protein, and leaves and stems thereof contain 68.9% neutral saccharide, 15.9% acidic saccharide, and 8.7% protein. In addition, when extracts of ginseng, which were precisely separated and purified using ion exchange resins, adsorption resins and the like, were dissolved in triple distilled water in the concentration of 1 mg/mL and analyzed, it was confirmed that roots of the same *Panax ginseng C.A. Mayer* contain 35.4% neutral saccharide, 64.5% acidic saccharide, and 0.09% protein, and the saccharide composition is made up of 58.3% glucuronic acid, 6.2% galacturonic acid, 26.8% glucose, 6.3% galactose, 1.4% arabinose and 0.9% rhamnose. Whereas, it was confirmed that leaves and stems of ginseng contain 51.3% neutral saccharide, 46.8% acidic saccharide, and 0.1% protein, and the saccharide composition is made up of 5.97% rhamnose, 1.22% fucose, 14.86% arabinose, 0.44% xylose, 1.93% mannose, 3% glucose, 22.7% galactose, 31.4% galacturonic acid, 14.4% glucuronic acid, 1.38% KDO and 1.02% DHA.

In accordance with another aspect of the present invention, there is also provided a method for extracting such polysaccharides from plants belonging to the *Panax* family.

The method for extracting a polysaccharide in accordance with the present invention, comprises:

(a) placing roots, leaves, stems and/or fruits of a plant belonging to the *Panax* family in water, cold precipitating or heating the mixture at pH of 4 to 10 and a temperature of 0 to 180°C for 0.5 to 20 hours, and separating remnants including roots, leaves, stems and/or fruits of the plant to obtain an extract;

(b) concentrating the extract, and precipitating and separating polysaccharide using an organic solvent; and

(c) removing the organic solvent from the separated polysaccharide to obtain a polysaccharide.

In step (a), if the reaction pH is outside the above-mentioned range, some polysaccharides may be undesirably altered. If the reaction temperature is too low, an extraction amount is very small and the process does not smoothly progress. Conversely, if the reaction temperature is too high, some polysaccharides may be undesirably altered. Further, if the reaction time is too short, an extraction amount is very small, while if the reaction time is too long, the process is uneconomical and thus is undesirable. The term "cold precipitation" refers to dipping at a temperature of about 0 to 25°C. There is no particular limit to the used amount of water, but for easy extraction, a 10 to 20-fold excess of water may be used based on the volume of raw materials such as roots of ginseng.

In step (b), the extract is concentrated for effective recovery of polysaccharides, and for example, the extract may be concentrated by heating it to a range of 20 to 90°C under reduced pressure between vacuum and 760 mmHg until it reached more than 20 brix, as determined by a saccharometer.

In order to settle polysaccharides by adding an organic solvent to the concentrated extract, 0.1 to 1M sodium chloride and lower alcohols or acetone are preferably used. The organic solvent is used in a 1 to 5-fold, preferably 2 to 4-fold excess amount based on the extract volume. As the lower alcohols, alcohols containing up to 5 carbon atoms such as methanol, ethanol, propanol, butanol and pentanol may be used and ethanol is preferably used.

Separation of polysaccharides in step (b) and/or step(c) may be performed by various methods such as filtration (including ultrafiltration), centrifugation, ion exchange resins, adsorption resins and dialysis, and there is no particular limit to separation methods as long as they can separate polysaccharides in accordance with the present invention. Furthermore, such separation processes may be performed by repetition of various methods. An example of more effective separation (purification) method will be described below.

The pH of the extract obtained in step (a) was adjusted to about 8.0, 0.4M NaCl/10mM Tris-HCl was added and passed through an anion exchange resin column at a rate of 30 mL/min. This anion exchange resin was previously equilibrated with 0.4M NaCl/10mM Tris-HCl so as to adsorb only pigments or impurities and prevent polysaccharides in a sample from adsorbing. This anion exchange resin column was sufficiently washed with a buffer, pH of the passed liquid was adjusted to a range of about 6 to 7 and then passed through an adsorbent resin column at a rate of 30 to 50 mL/min. The column was sufficiently washed with distilled water, and a 3-fold ethanol precipitation method was used to confirm that all of the sample had passed through the column. About a 3-fold excess of ethanol (95%) was added to the passed liquid so as to precipitate polysaccharides. This was followed by centrifugation (5000 rpm, 15 min) and dialysis in order to recover precipitated polysaccharides. The pH of this solution was adjusted to 8, a 10mM Tris-HCl (pH 8) buffer was added thereto and then the solution was flowed through the anion exchange resin column at a rate of 15 to 20 mL/min to be adsorbed thereon. This column was sufficiently washed with a buffer and eluted with 0.5M NaCl. About a 3-fold excess of ethanol was added to the resulting eluted liquid to precipitate polysaccharides, centrifuged (5000 rpm, 15 min)

to recover polysaccharides, washed with 2 x 95% ethanol to remove impurities, and dialyzed with a molecular weight of less than 6000 Da to remove the remaining ethanol.

In step (c), after removing an organic solvent, a drying process may be performed to obtain polysaccharides in the form of powder. The drying process may be performed using hot air, drying under vacuum, lyophilization and spray drying.

Although the method for extracting polysaccharides in accordance with the present invention was described, variation or addition of some processes may be made in order to enhance extraction efficiency or purity of polysaccharides and it should be construed that those variations or additions will fall within the scope of the present invention.

In accordance with further aspect of the present invention, there is provided a pharmaceutical composition comprising a therapeutically effective amount of the above-mentioned polysaccharides as an active ingredient, and a pharmaceutically acceptable carrier, and more specifically a composition for treating and preventing obesity.

The polysaccharides may be formulated in various pharmaceutical dosage forms depending on desired purpose. In preparing the pharmaceutical composition in accordance with the present invention, an active ingredient, specifically polysaccharide of plants belonging to the *Panax* family is mixed with a variety of pharmaceutically acceptable carriers that can be selected depending on formulation to be prepared.

The active ingredient may be, depending on desired purpose, formulated into injectable preparations, transdermal preparations or oral preparations and for easy

administration and uniform dose, it is preferably prepared in a unit dosage form.

In preparing formulations intended for oral administration, a conventional pharmaceutically acceptable carrier may be used. For solution preparations for oral administration such as suspensions, syrups, elixirs and solutions, water, glycol, oil and alcohol may be used as the carrier. For solid forms of preparations such as powders, pills, capsules and tablets, starch, sugar, kaoline, lubricant, binding agent and disintegrant may be used as the carrier. For easy administration, the most convenient dosage forms are tablets and capsules, and tablets and pills are preferably prepared in enteric coating. In addition, in order to prevent oral preparations from being degraded by gastric acid, an acid scavenger may be used or solid preparations for oral administration such as tablets may be formulated into the enteric film coated preparation and then administered.

In the case of parenteral preparations, sterilized water is conventionally used as the carrier, and other ingredients such as dissolution aids can be included. Injectable preparations, for example aqueous or oily suspensions for sterile injection may be prepared using suitable dispersants, wetting agents or suspending agents by known techniques. Solvents used herein may include water, Ringer's solution and isotonic NaCl solution, and sterile fixed oil is also conventionally used as the solvent or suspending medium. Any non-irritable fixed oil including mono- and di-glyceride may be used for such purpose and further, fatty acids such as oleic acid may be used in the injectable preparation.

In the case of transdermal preparations, penetration promoters and/or suitable wetting agents as the carrier may be optionally used in combination with a suitable additive that is non-irritable to skin. The additive is selected from those that promote

administration through skin and/or assist in preparing a desired composition. Transdermal preparation may be administered in various manners such as transdermal patches, creams or ointments.

In order to prevent active ingredients from being rapidly removed within the body, the composition in accordance with the present invention may be formulated into sustained-release preparations. In this connection, usable carriers include implants, microencapsulated delivery systems, and biodegradable/biocompatible polymers, which are known in the art.

The term "therapeutically effective amount" means an amount of active ingredient effective to alleviate or reduce symptoms of a disease in need of treatment, or to reduce or retard initiation of clinical markers or symptoms of a disease in need of prevention. The therapeutically effective amount may be empirically determined by experimenting with the above-mentioned polysaccharides in known in vivo and in vitro model systems for a disease in need of treatment.

For humans, a dose of the active ingredient, i.e., polysaccharide of plants belonging to the *Panax* family is appropriately determined depending on absorptivity, inactivation and excretion rate of the active ingredient in the body, age, sex and conditions of patients. Generally, the active ingredient may be administered in an amount of 100 μg to 6000 mg/day, and preferably 20 to 5000 mg/day, for adults.

The pharmaceutical composition in accordance with the present invention may further include other ingredients that do not inhibit action of the active ingredient or assist action of the active ingredient, and may be formulated into various forms known in the art.

In accordance with still further aspect of the present invention, there is

provided a method for treating or preventing a disease caused by obesity, using a polysaccharide of plants belonging to the *Panax* family as an active ingredient. Examples of obesity-causing diseases include primary obesity as obesity itself and secondary obesity, diseases due to increased load to circulation system (for example, hypertension, coronary disease), diseases due to metabolism disorder (for example, diabetes, hypertriglyceridemia), disorder of the liver or biliary system (for example, cholelithiasis), a lowering of respiratory function, diseases caused by excessive loading to bones or articular system (for example, degenerative articular diseases) and the like. Therefore, administration of the therapeutically effective amount of the polysaccharide of plants belonging to the *Panax* family in accordance with the present invention to humans lowers body fat, thereby effective for treating and preventing diseases due to obesity. The term "treating" means stopping or retarding progresses of a disease when used in a subject exhibiting onset of the disease, and the term "preventing" means stopping or retarding symptoms of disease development when used in a subject who does not exhibit onset of disease but is at a high risk for such disease onset.

[Description of the Drawings]

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

Figs. 1 through 4 are, respectively, graphs showing changes in body weight with respect to the passage of time, in a mouse group to which **a** polysaccharide of *Panax ginseng C.A. Mayer* in accordance with the present invention was administered

and a mouse group to which no polysaccharide of *Panax ginseng C.A. Mayer* was administered, based on the experimental results of Examples 1 through 4.

[Best Mode]

Now, the present invention will be described in more detail with reference to the following Examples. These examples are provided only for illustrating the present invention and should not be construed as limiting the scope and sprit of the present invention.

EXAMPLES

Preparation Example 1: Preparation of crude polysaccharide extract of ginseng root

100 g of roots of *Panax ginseng C.A. Mayer* was placed in 1 L of water and heated to extract at 100°C for 5 hours. The extract thus obtained and ginseng roots were separated using an unwoven fabric, centrifuged at 7,000 rpm for 30 min to remove insoluble foreign materials, and the resulting supernatant containing polysaccharides was concentrated at 760 mmHg and 70°C to 20 brix so as to obtain 100 mL of concentrate. Sodium chloride was dissolved in the concentrate thus obtained to 1M concentration, 300 mL of ethanol was added and mixed, the mixture was allowed to stand for 1 hour to separate supernatant and precipitate. The precipitate thus obtained was washed with 100 mL of 95% ethanol twice to remove impurities, dialyzed and lyophilized to obtain crude polysaccharides of ginseng roots.

Preparation Example 2: Preparation of crude polysaccharide of ginseng root

Crude polysaccharide of ginseng leaves was obtained using the same procedure as Preparation Example 1, except that leaves of *Panax ginseng C.A. Mayer* were used instead of roots thereof.

Preparation Example 3: Preparation of crude polysaccharide of ginseng stems

Crude polysaccharide of ginseng stems was obtained using the same procedure as Preparation Example 1, except that stems of *Panax ginseng C.A. Mayer* were used instead of roots thereof.

Preparation Example 4: Preparation of polysaccharide of ginseng roots

500 g of roots of *Panax ginseng C.A. Mayer* was crushed with a chopper, and the crushed material was placed in 5 L of distilled water followed by extraction at 4°C for about 15 hours. Gauze was used to separate the extract and sludge, and the extract was centrifuged at 700 rpm for 20 min to recover supernatant. The pH of the supernatant containing polysaccharide was adjusted to about 8.0, 0.5M NaCl/10 mM Tris-HCl was added and passed through an anion exchange resin (trade name: PA312, a styrene based strong basic anion exchange resin) column at a rate of 30 mL/min. This anion exchange resin was previously equilibrated with 0.5M NaCl/10mM Tris-HCl so as to adsorb only coloring materials or impurities and prevent polysaccharides in sample from adsorbing. This anion exchange resin column was sufficiently washed with a buffer, the pH of the passed liquid was adjusted to a range of about 6 to 7 and then passed through an adsorbent resin (trade name: Hp 20, Diaion) column at a rate of 30 to 50 mL/min. The column was sufficiently washed with distilled water, and a 3-

fold volume ethanol precipitation method was used to confirm that all of the sample had passed through the column. About a 3-fold excess of ethanol (95%) was added to the passed liquid so as to precipitate only polysaccharides. This was followed by centrifugation (5000 rpm, 15 min) and dialysis in order to recover polysaccharides. The pH of this solution was adjusted to 8, 10mM Tris-HCl (pH 8) buffer was added thereto and then the solution was flowed through an anion exchange resin (trade name: Q Sepharose, Pharmacia) column equilibrated with the buffer at a rate of 15 to 20 mL/min to be adsorbed thereon. Liquid that passed through without adsorption was pooled and precipitated by addition of a 3-fold excess of ethanol and separated so as to recover neutral polysaccharides. This column was sufficiently washed with 10 mM Tris-HCl buffer and eluted with 0.5M NaCl. About a 3-fold excess of ethanol was added to the resulting eluate to precipitate polysaccharides, centrifuged (5000 rpm, 15 min) to recover polysaccharides, washed with 2 x 95% ethanol to remove impurities, and dialyzed with a molecular weight of less than 6000 Da to remove remaining ethanol, followed by lyophilization to obtain polysaccharides of ginseng roots.

Preparation Example 5: Preparation of polysaccharide of ginseng leaves

500 g of leaves of *Panax ginseng C.A. Mayer* was placed in 5 L of distilled water followed by extraction with hot water for about 3 hours. Gauze was used to separate the extract and sludge, and the extract was centrifuged at 7000 rpm for 20 min to recover supernatant. The pH of the supernatant was adjusted to about 8.0, 0.4M NaCl/10 mM Tris-HCl was added and passed through an anion exchange resin (trade name: PA312, a styrene based strong basic anion exchange resin) column at a rate of 30 mL/min. This anion exchange resin was previously equilibrated with 0.4M

NaCl/10mM Tris-HCl so as to adsorb only pigments or impurities and prevent polysaccharides in sample from adsorbing. This anion exchange resin column was sufficiently washed with a buffer, pH of the passed liquid was adjusted to a range of about 6 to 7 and then passed through an adsorbent resin (trade name: Hp 20, Diaion) column at a rate of 30 to 50 mL/min. The column is sufficiently washed with distilled water, and a 3-fold ethanol precipitation method was used to confirm that all of the sample had passed through the column. About a 3-fold excess of ethanol (95%) is added to the passed liquid so as to precipitate only polysaccharides which were then centrifuged (5000 rpm, 15 min) and dialyzed. The pH of this solution was adjusted to 8, 10mM Tris-HCl buffer was added thereto and then the solution was flowed through the anion exchange resin (trade name: Q Sepharose, Pharmacia) column at a rate of 15 to 20 mL/min to be adsorbed thereon. This column was sufficiently washed with 10 mM Tris-HCl buffer and eluted with 0.5M NaCl. About a 3-fold excess of ethanol was added to the resulting eluate to precipitate polysaccharides, centrifuged (5000 rpm, 15 min) to recover polysaccharides, washed with 2 x 95% ethanol to remove impurities, and dialyzed with a molecular weight of less than 6000 Da to remove remaining ethanol, followed by lyophilization to obtain polysaccharides of ginseng leaves.

Preparation Example 6: Preparation of polysaccharide of ginseng stems

Polysaccharide of ginseng stems was obtained using the same procedure as Preparation Example 5, except that stems of *Panax ginseng C.A. Mayer* were used instead of leaves thereof.

Preparation Example 7: Preparation of sample for injectable preparation

In order to remove pyrogenic materials from polysaccharides obtained in Preparation Examples 4 through 6, respectively, polysaccharides were passed through a column packed with silica gel, 3-fold excess of ethanol was added thereto to precipitate polysaccharides followed by centrifugation and recovery, and washed with 2x 95% ethanol to remove impurities. The resulting materials were dissolved in triple distilled water, dialyzed with a molecular weight of 6,000 Da to remove ethanol, passed through a 0.2 μm filter to prepare a sterile sample containing no pyrogenic materials. A predetermined amount of the sterilized sample was pipetted into a 3 mL vial, frozen and then lyophilized. The dried sample was dissolved in physiological saline to prepare a sample for injection.

Example 1: Composition analysis of ginseng extract

Composition analysis of ginseng extracts obtained in Preparation Examples 1 through 7 was carried out as follows. Quantitative analysis was performed for total saccharide content using galactose as a standard with a phenol-sulfuric acid method (Dubois et al., Anal. Chem., 28, 350, 1956), for acidic saccharide content using β-D-galacturonic acid as a standard with a m-hydroxybiphenyl method (Blumenkrantz et al., Anal. Biochem., 54, 484, 1973), for protein content using bovine albumin as a standard with a Lowry method (Lowry et al., J. Biol, Chem., 193, 265, 1951), and for KDO (3-deoxy-D-manno-2-octulosonic acid) material with a thiobarbituric acid method, respectively.

Analysis of constituting saccharides was performed with a gas-liquid chromatography (GLC) method by alditol acetate derivatization according to the Jones method (Plant Physiol., 49, 926, 1972).

Crude polysaccharides obtained in Preparation Examples 1 through 3 were dissolved in triple distilled water in a concentration of 1 mg/mL, respectively and analyzed according to the above-mentioned methods. Based on the results, roots of *Panax ginseng C.A. Mayer* were found to contain 32.8% neutral saccharide, 57.3% acidic saccharide and 6.4% protein. Leaves and stems of *Panax ginseng C.A. Mayer* were found to contain 68.9% neutral saccharide, 15.9% acidic saccharide and 8.7% protein.

Purified polysaccharides obtained in Preparation Examples 4 through 6 were dissolved in triple distilled water in a concentration of 1 mg/mL, respectively, and analyzed according to the above-mentioned methods. Based on the results, roots of *Panax ginseng C.A. Mayer* were found to contain 35.4% neutral saccharide, 64.5% acidic saccharide and 0.09% protein, and the saccharide composition thereof was made up of 58.3% glucuronic acid, 6.2% galacturonic acid, 26.8% glucose, 6.3% galactose, 1.4% arabinose and 0.9% rhamnose. Whereas, leaves and stems of *Panax ginseng C.A. Mayer* were found to contain 51.3% neutral saccharide, 46.8% acidic saccharide and 0.1% protein, and saccharide composition thereof was made up of 5.97% rhamnose, 1.22% fucose, 14.86% arabinose, 0.44% xylose, 1.93% mannose, 3% glucose, 22.7% galactose, 31.4% galacturonic acid, 14.4% glucuronic acid, 1.38% 3-deoxy-D-manno-2-octulosonic acid (KDO) and 1.02% 3-deoxy-D-lyxo-2-heptulosaric acid (DHA).

Molecular weight was confirmed by HPLC (Young Lin Instrument Co., Ltd., Korea) and determination was performed by sequentially connecting columns GS-520HQ, GS-320HQ and GS-220HQ (Shodex Asahipack GS series, Showa Denko K.K.). As a result, roots of ginseng had a molecular weight distribution of about 6,000 to 520,000 Da and an average molecular weight of about 140,000 Da, while leaves and

stems of ginseng had a molecular weight distribution of about 6,000 to 340,000 Da and an average molecular weight of about 120,000 Da

Example 2: Experiment on anti-obesity effect of ginseng root polysaccharide

An experiment on an anti-obesity effect of crude polysaccharide extracted from ginseng roots was performed in the db/db (10 weeks, male) mouse as the obesity model. Crude polysaccharide of ginseng roots obtained in Preparation Example 1 was dissolved in PBS, sterile filtered through a 0.2 μm filter, and the resulting sterilized sample was administered to the peritoneal cavity of the db/db mouse, at concentrations of 500 μg/mouse and 1 mg/mouse (amount of polysaccharide), respectively, every day. Body weight of animals was measured at intervals of 5 days and feed intake was confirmed every day.

After a 15-day experiment, the db/db normal group, which was not administered polysaccharides, showed about 2.7% gain in body weight, while the group in which 500 μg of polysaccharide of ginseng roots was administered showed 4.2% loss in body weight and the group in which 1 mg of polysaccharide was administered showed 10.5% loss in body weight (see Fig. 1).

Example 3: Experiment on anti-obesity effect of ginseng leave polysaccharide

An experiment on an anti-obesity effect of crude polysaccharide extracted from ginseng leaves was performed in the db/db (10 weeks, male) mouse as the obesity model. Crude polysaccharide of ginseng roots obtained in Preparation Example 2 was dissolved in PBS, sterile filtered through a 0.2 μm filter, and the resulting sterilized sample was administered to the peritoneal cavity of the db/db

mouse, at concentrations of 200 μg/mouse and 500 μg/mouse, respectively, every day. Body weight of animals was measured at intervals of 5 days and feed intake was confirmed every day.

After a 15-day experiment, the normal group showed about 2.7% gain in body weight, while the group in which 200 μg of crude polysaccharide of ginseng leaves was administered showed 5.6% loss in body weight and the group in which 500 μg of crude polysaccharide was administered showed 12.7% loss in body weight (see Fig. 2). Upon observing changes in dietary intake, the polysaccharide administered group exhibited relatively low dietary intake compared to a control group, at the early stage of administration, but exhibited a dietary intake aspect approaching that of the control group, with respect to the passage of time.

Example 4: Experiment on anti-obesity effect of purified ginseng root polysaccharide

An experiment on an anti-obesity effect of polysaccharide extracted from ginseng roots was performed in the db/db (10 weeks, male) mouse as the obesity model. Polysaccharide of ginseng roots obtained in Preparation Example 4 was dissolved in PBS, sterile filtered through a 0.2 μm filter, and the resulting sterilized sample was administered to the peritoneal cavity of the db/db mouse, at concentrations of 200 μg/mouse and 500 μg/mouse, respectively, every day. Body weight of animals was measured at intervals of 5 days and feed intake was confirmed every day.

After a 15-day experiment, the normal group showed about 2.7% gain in body weight, while the group in which 200 μg of polysaccharide of ginseng roots was administered showed 3.5% loss in body weight and the group in which 500 μg of

polysaccharide was administered showed 8.6% loss in body weight (see Fig. 3). Upon observing changes in dietary intake, the polysaccharide administered group exhibited relatively low dietary intake compared to a control group, at the early stage of administration, but exhibited a dietary intake aspect approaching that of the control group, with respect to the passage of time.

Example 5: Experiment on anti-obesity effect of purified ginseng stem polysaccharide

An experiment on an anti-obesity effect of polysaccharide extracted from ginseng stems was performed in the db/db (10 weeks, male) mouse as the obesity model. Polysaccharide of ginseng stems obtained in Preparation Example 6 was dissolved in PBS, sterile filtered through a 0.2 μm filter, and the resulting sterilized sample was administered to the peritoneal cavity of db/db mouse, at concentrations of 200 μg/mouse and 500 μg/mouse, respectively, every day. Body weight of animals was measured at intervals of 5 days and feed intake was confirmed every day.

After a 15-day experiment, the normal group showed about 2.7% gain in body weight, while the group in which 200 μg of polysaccharide of ginseng stems was administered showed 4.2% loss in body weight and the group in which 500 μg of the polysaccharide was administered showed 9.7% loss in body weight (see Fig. 4). Upon observing changes in dietary intake, the polysaccharide administered group exhibited relatively low dietary intake compared to a control group, at the early stage of administration, but exhibited a dietary intake aspect approaching that of the control group, with respect to time lapse.

[Industrial Applicability]

As described above, a polysaccharide in accordance with the present invention can be easily extracted from plants belonging to the *Panax* family and use of such a polysaccharide as an active ingredient can provide treatment and prevention of obesity. In addition, when extracting polysaccharides from leaves or stems of plants belonging to the *Panax* family, it is possible to prepare polysaccharides at very low production cost, thus being advantageous from point of economical respect. Further, since plants belonging to the *Panax* family have been used as the raw material of medicines for a long period of time, general problems associated with use thereof as medicines, including toxicity can be naturally solved.

Although the preferred embodiments of the present invention have been disclosed for illustrative purposes, those skilled in the art will appreciate that various modifications, additions and substitutions are possible, without departing from the scope and spirit of the invention as disclosed in the accompanying claims.

WHAT IS CLAIMED IS:

1. A polysaccharide extracted from a plant belonging to the *Panax* family for treating and preventing obesity by lowering of body fat.

2. The polysaccharide as set forth in claim 1, wherein the plant belonging to the *Panax* family is at least one selected from the group consisting of *Panax ginseng C.A. Mayer*, *Panax quinquefolium*, *Panax notoginseng*, *Panax pseudoginseng*, *Panax japonicum*, and *Panax vietnamensis* Ha et Grushv. and the polysaccharide is extracted from roots, leaves, stems and/or fruits thereof.

3. The polysaccharide as set forth in claim 2, wherein the plant belonging to the *Panax* family is *Panax ginseng C.A. Mayer*, and the polysaccharide is extracted from leaves and/or stems thereof.

4. The polysaccharide as set forth in claim 1, wherein the polysaccharide extracted from roots of the plant belonging to the *Panax* family has a molecular weight distribution of 6000 to 520,000 Da and an average molecular weight of about 140,000 Da, and the polysaccharide extracted from leaves and stems thereof has a molecular weight distribution of 6000 to 340,000 Da and an average molecular weight of about 120,000 Da.

5. The polysaccharide as set forth in claim 1, wherein the polysaccharide contains 30 to 80% neutral saccharide, 10 to 60% acidic saccharide and 0.01 to 10% protein, and

the saccharide composition thereof contains rhamnose, fucose, arabinose, xylose, mannose, glucose, galactose, galacturonic acid, glucuronic acid, 3-deoxy-D-manno-2-octulosonic acid (KDO) and 3-deoxy-D-lyxo-2-heptulosaric acid (DHA).

6. A method for extracting a polysaccharide of Claim 1 from the plant belonging to the *Panax* family, comprising:

(a) placing roots, leaves, stems and/or fruits of a plant belonging to the *Panax* family in water, cold precipitating or heating the mixture at a pH of 4 to 10 and a temperature of 0 to 180°C for 0.5 to 20 hours, and separating remnants including roots, leaves, stems and/or fruits of the plant to obtain an extract;

(b) concentrating the extract, and precipitating and separating polysaccharide using an organic solvent; and

(c) removing the organic solvent from the separated polysaccharides to obtain a polysaccharide.

7. The method as set forth in claim 6, wherein the organic solvent for precipitating the polysaccharide is 0.1 to 1M sodium chloride, and a lower alcohol or acetone and is used in an 1 to 5-fold excess amount based on the extract volume.

8. The method as set forth in claim 7, wherein the lower alcohol is ethanol.

9. The method as set forth in claim 6, wherein in step (b) and/or step(c), separation of the polysaccharide is performed by filtration including ultrafiltration, centrifugation, an ion exchange resin, an adsorption resin or dialysis.

10. A pharmaceutical composition for treating and preventing obesity, comprising a therapeutically effective amount of polysaccharide of the plant belonging to the *Panax* family according to Claim 1 as an active ingredient, and a pharmaceutically acceptable carrier.

11. The composition as set forth in claim 10, wherein the composition is formulated into an injectable preparation, transdermal preparation or oral preparation.

12. A method for treating and preventing an obesity-causing disease using the polysaccharide of the plant belonging to the *Panax* family according to Claim 1 as an active ingredient.

13. The composition as set forth in claim 12, wherein the obesity-causing disease is primary obesity as obesity itself or secondary obesity, diseases due to increased load of circulation system (for example, hypertension, coronary disease), diseases due to metabolism disorder (for example, diabetes, hypertriglyceridemia), disorder of the liver or biliary system (for example, cholelithiasis), a lowering of respiratory function, or diseases caused by excessive loading to bones or articular system (for example, degenerative articular diseases).

DRAWINGS

FIG. 1

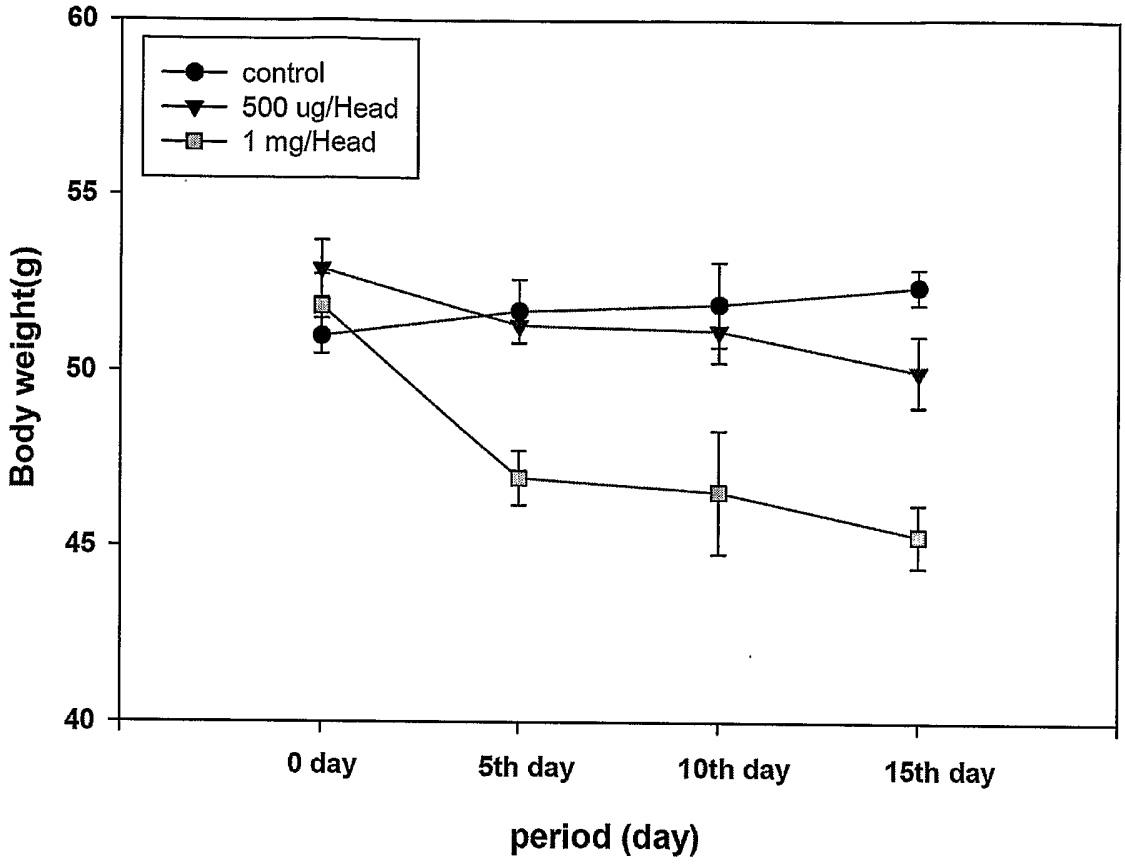


FIG. 2

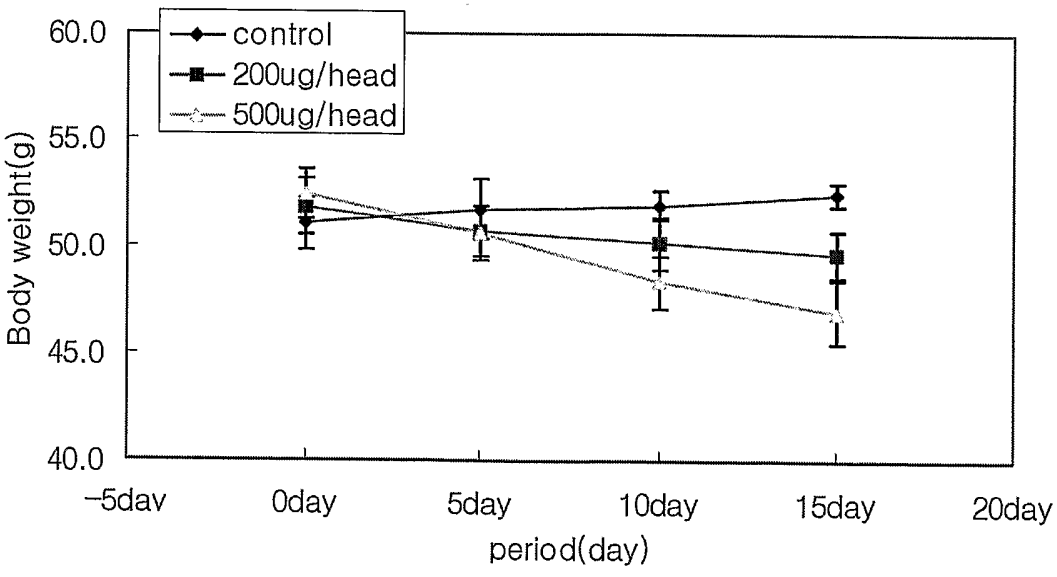
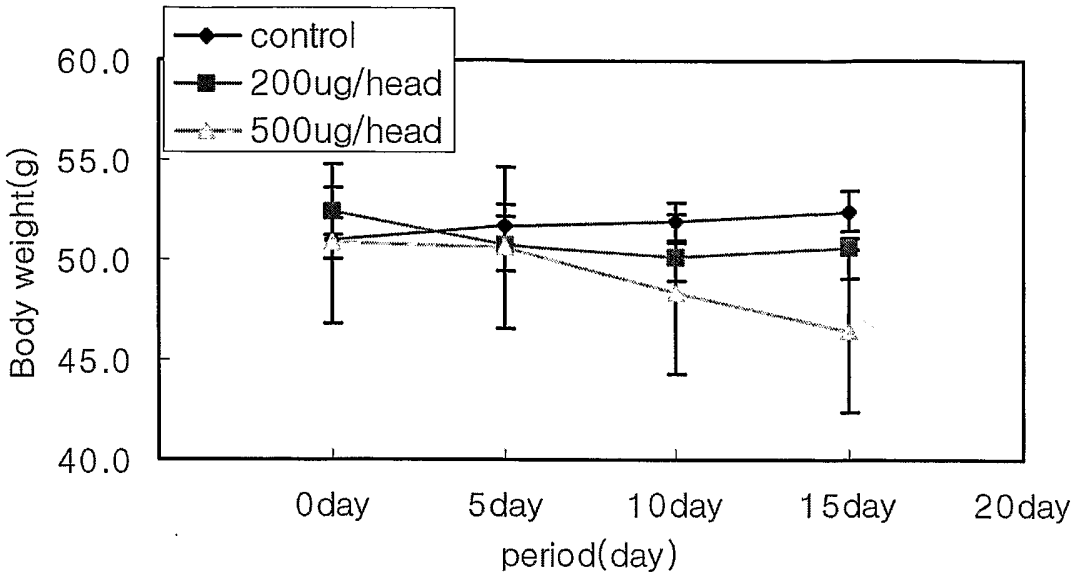


FIG. 3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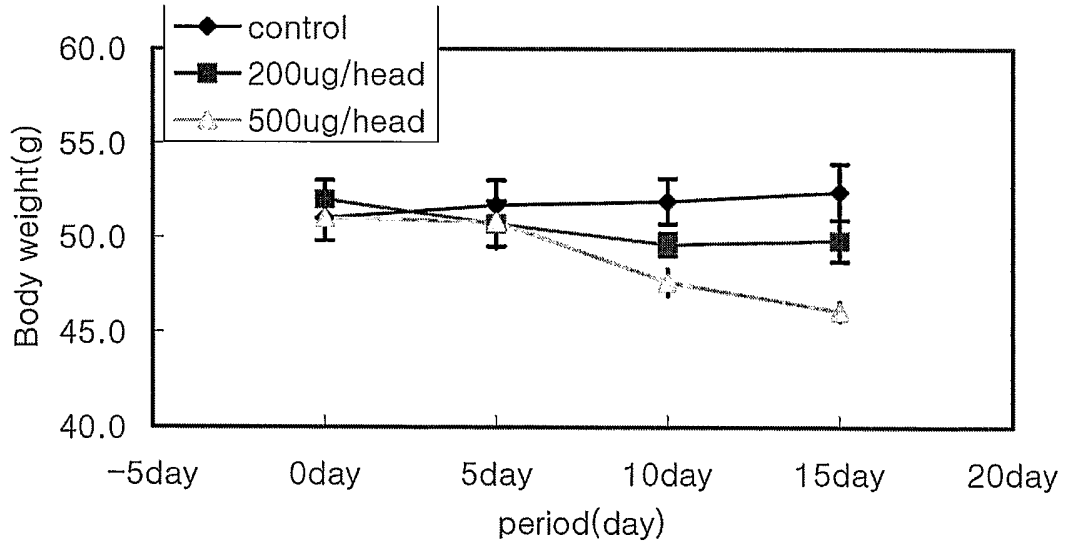
period(day)

FIG. 4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45.0
40.0
Body weight(g)
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0day
5day
10day
15day
20day
period(day)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2004/002763

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A61K 31/715

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

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
KOREAN PATENTS AND APPLICATIONS FOR INVENTIONS SINCE 1975

Electronic data base consulted during the internationl search (name of data base and, where practicable, search terms used)
PubMed, WPI, USPTAFULL, JAPIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Yang et al., 'Effects of ginseng polysaccharides on reducing glucose and liver glycogen' In Zhongguo Yao Li Xue Bao., Vol. 11(6), pp. 520-4 (1990)	1-11
Y	Xie et al., 'Ginseng berry reduces blood glucose and body weight in db/db mice' Phytomedicine, Vol. 9(3), pp. 254-8 (2002)	1-11
Y	Ng TB & Yeung HW, 'Hypoglycemic constituents of Panax ginseng' In Gen. Pharmacol., Vol 16(6), pp. 549-52 (1985)	1-11

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document 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
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents,such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
07 FEBRUARY 2005 (07.02.2005)	**07 FEBRUARY 2005 (07.02.2005)**

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Telephone No. 82-42-481-5605

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2004/002763

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. [X] Claims Nos.: 12-13
because they relate to subject matter not required to be searched by this Authority, namely:
The subject-matter of claims 12 and 13 does not require an international examination with respect to industrial applicability, as it is directed to a method for treatment of the human or animal body by therapy under PCT Article 17(2)(a)(i) and Rule 39.1(iv).

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:

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).

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 addtional 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 addition 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.

[ ] No protest accompanied the payment of additional search fees.