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(54) Title: COMPOSITION FOR PREVENTING OR TREATING INFLAMMATORY DISEASES OR PAIN



(57) Abstract: The present invention relates to a composition for preventing, treating or improving inflammatory diseases or pain, and the composition comprising, as an active constituent, a complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein a weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more has not only an excellent anti-inflammation and analgesic effects, but also very excellent arthritis or periodontal disease treatment effects, and thus, can be usefully used in the pharmaceutical composition or health functional foods for preventing, treating or improving inflammatory diseases or pain.

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Description

Title of Invention: COMPOSITION FOR PREVENTING OR TREATING INFLAMMATORY DISEASES OR PAIN

Technical Field

[1] The present invention relates to a composition for preventing or treating inflammatory diseases or pain, more specifically it relates to the composition for preventing or treating inflammatory diseases or pain, which comprises a complex extract of Notoginseng Radix Et Rhizoma (*Panax notoginseng*) and Rehmanniae Radix Preparata (*Rehmannia glutinosa*) having the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 of 0.20 or more as an active constituent.

Background Art

[2] Although it is known that Non-Steroidal Anti-inflammatory Drugs (NSAIDs) such as phenylbutazone, diclofenac, aceclofenac, etc. have excellent anti-inflammatory analgesic effects, it is also known that many side effects are occurred in a long-term use.

[3] Therefore, there has been made a lot of studies for preparing the pharmaceutical preparation by extracting the bioactive substances, effective for anti-inflammatory analgesic effect, etc. from oriental herbal medicine, as one which can be alternative for such NSAIDs.

[4] Meanwhile, diseases relating to arthritis are representative degenerative, intractable diseases that about 12% of the populations of the whole world suffer and there are about 2 million of patients in South Korea. Arthritis is a disease name generally called as those systemic symptoms are occurred in muscular skeletal system due to the inflammatory change in the musculoskeletal and connective tissues.

[5] The said diseases are characterized by the chronic inflammation often causing permanent tissue damage, malformation, degeneration and disorder by affecting on joint, bone, cartilaginous tissue or spinal cord (Hofbause, L. C., Heufelder, A. E.: The role of osteoprotegerin and receptor activator of nuclear factor kappa B ligand in the pathogenesis and treatment of rheumatoid arthritis, Arthritis and Rheumatism 44:253-259, 2001). Arthritis is classified into degenerative arthritis (osteoarthritis), rheumatoid arthritis, extraarticular rheumatism or collagen disease.

[6] The degenerative arthritis is a disease exhibiting the topical degenerative change that the articular cartilage is worn out and disappeared. Although a cause of it is unclear, it has been known that it is closely related to signs of decrepitude or the excessive weight, and degenerative changes of articular cartilage are primarily appeared in this disease. The degenerative changes are started from the articular cartilage, cartilage

cells are necrotized, and substrates are destroyed by Cathepsin B, Cathepsin D, Collagenase and the like. As a generation of proteoglycan and collagen can not be kept up the degree of the destruction and adaptability of the cartilage against external force is gradually decreased, and finally there is a point of view such as microfracture, etc in subchondral bone tissue. As the diseases are progressed, stiffening of subchondral bone, excessive formation of the bone in paraarticular, deformation of the articular, and the like are occurred, the surface of the articular becomes rough and the inflammatory reactions in joint cavity wrapped with joint membrane are repeatedly occurred. Therefore, repetitive pain, the stiffness of articular, gradual motor disturbance, etc. in articular are occurred.

[7] Rheumatoid arthritis among arthritis is systemic chronic inflammatory diseases which morbid symptoms are symmetrically occurred in diathrosis, and is known as autoimmune diseases which are occurred by abnormality of the immune system and the cause of which has not been clearly identified yet. The diseases are characterized by continuous inflammatory synovitis causing the cartilage destruction and bone erosion, which leads to the structural abnormality of articular. Symptoms relating to the rheumatoid arthritis include arthredema, joint tenderness, inflammation, morning stiffness and in particular pain when bending joints. The structural damages including articular destruction together with bone erosion are occurred in a subject developing the arthritis (Firestein, G.S.: Evolving concept of rheumatoid arthritis, Nature 423:356-361, 2003). In addition, the patient may have other clinical symptoms such as damages of various organs including damages of skin, kidney, heart, lung, central nervous system and eye due to angiitis relating to an autoimmune process.

[8] The treatment methods of degenerative arthritis and rheumatoid arthritis being used at present are as follows. The drug which is used a lot in the early treatment stage is non-steroidal anti-inflammatory drugs (NSAIDs), and such NSAIDs can improve the symptoms a little but can not prevent the cartilage loss in parts of articulation or the progress of disease. In addition, about a half of patients using such treatment should discontinue the treatment within 1 year since side effects in gastroenteric system, central nervous system, blood-forming organ, kidney, liver, etc. are occurred when taken for a long period(Langenegger, T., Michel, B. A. : Drug treatment for rheumatoid arthritis., Clin Orthop. 366:22-30,1999). As the next step, compound drugs comprising gold (gold drugs; gold sodium thiomalate, gold sodium thiosulfate) or antiarthritis agent (disease modifying anti-rheumatic drugs, DMARDs) such as penicillamine, anti-malarials, and the like are used. However, although such agents can reduce the progress of arthritis a little, since they can accompany the serious side effects, only 5-15% of patients use such drugs after 5 years from the beginning of the DMARD therapy. If said treating agents do not exhibit the effects any more, there is

even the case that the rheumathritis occurred articulation has to be replaced with an artificial joint by extraarticular procedure (Simon L. S., Int. J. Clin. Pract., 54:243-249, 2000).

[9] Therefore, there is an urgent need for developing new materials effective for treating and preventing arthritis, which have new action and protective effect of the skeletal structure, as well as minimizing toxicity and side effects.

[10] Meanwhile, a periodontal disease refers to a disease occurred in periodontium consisting of alveolar bone, gingiva, periodontal ligament and cement, and is classified into gingivitis, localized in gum, and periodontitis that periodontal ligament and alveolar bone are destructed by deepening of gingivitis.

[11] The causes of the periodontal disease are various, but the representative cause is due to bacteria. The periodontium is directly destructed by the cellular toxins such as hydrogen sulfate, ammonia and toxic amine which are a metabolite of anaerobic gram-negative bacteria, or the destruction of periodontium and inflammation are caused by various cytokines such as active oxygen, prostaglandin, leukotriene, histamine, interleukin, etc., which are secreted by the action of immune system activated by lipopolysacchride (LPS), the constitutive component of the cell wall of anaerobic gram-negative bacteria. In addition, a gingival retraction is occurred by the depomposition of collagen, the substrate of periodontium by enzyme such as collagenase from bacteria and leukocyte, and if it is neglected continuously, the periodontal disease is deteriorated. Therefore, an anti-bacterial action, cytostatic action and removal of the toxic products against anaerobic gram-negative bacteria, which is the basic pathogenetic cause of periodontal disease, and recovery of the lossed periodontium, etc. are important factors for preventing or treating periodontal disease.

[12] At present, an improvement of oral hygiene of the patient, surgical care (scaling, root planning, gingival curettage, periodontal regeneration) and non-surgical care (medicinal therapy) are used in the treatment of periodontal disease. The surgical care, which can be the most effective method, has a problem that the treatment for periodontal disease is not effectively made and mostly proceeded into the chronic disease due to the discomfort that the patient should visit the dentist for the treatment and the limitation that the patient should visit the dentist after proceeding the disease rather than the prevention of disease. In addition, antibiotics, which are used in the additional treatment, have the side effects due to the excessive transfer of drugs even to unnecessary parts and also the problem being reported the case that bacteria of periodontal disease exhibiting a resistance are isolated recently.

[13] In order to supplement the limitation of the surgical care and the problem regarding the use of antibiotics to improve the prevention and treatment effects of periodontal disease, the medicine with the efficacy of recovering the destructed and lossed peri-

odontium is needed.

[14] Accordingly, there is urgently needed the development of a composition for preventing or treating an inflammatory disease (for example, osteoarthritis, rheumatoid arthritis, periodontitis) or pain, which can minimize the side effects of conventional inflammatory analsegic drugs, the arthritis treating agent and the periodontal disease treating agent.

[15] [Prior Art Technical References]

[16] [Non-Patent References]

[17] Hofbause, L. C., Heufelder, A. E.: The role of osteoprotegerin and receptor activator of nuclear factor kappa B ligand in the pathogenesis and treatment of rheumatoid arthritis, Arthritis and Rheumatism 44:253-259, 2001.

[18] Firestein, G.S.: Evolving concept of rheumatoid arthritis, Nature 423:356-361, 2003.

[19] Langenegger, T., Michel, B. A.: Drug treatment for rheumatoid arthritis., Clin Orthop. 366:22-30, 1999.

[20] Simon L. S.: DMARDs in the treatment of rheumatoid arthritis: current agents and future developments., Int. J. Clin. Pract., 54:243-249, 2000.

Disclosure of Invention

Technical Problem

[21] Inventors of the present invention have identified that the composition having the certain ratio between ginsenoside Rb1 and ginsenoside Rg3, by the heat treatment, etc. of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata, has an excellent anti-inflammatory activity, as well as the effect of inhibiting pain in arthritis, protecting cartilage, inhibiting the expression of inflammatory cytokine and treating periodontal disease, and then completed the present invention.

[22] Therefore, the object of the present invention is to provide the composition for preveneting or treating inflammatory disease or pain.

[23] In addition, another object of the present invention is to provide a health functional food for preventing or improving the inflammatory disease or pain.

Solution to Problem

[24] In order to achieve the above objects, the present invention provides a composition for preventing or treating inflammatory diseases or pain, which comprises, a complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein a weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more, as an active constituent.

[25] Notoginseng Radix Et Rhizoma, as used herein, means a root and/or Rhizoma of *Panax Notoginseng*. *Panax Notoginseng* is a perennial herb of Araliaceae, and is smaller than ginseng, has seven (7) leaflets, form of small spindle shape, and is

cultivated at Unnam, Sacheon, etc. of South of China. Its root has 3 to 8% of saponin, and its main component is ginsenosides Rb1, Rg1, Rd, and notoginsenosides R1, R2, Fa, Fc, and comprises small amount of ginsenosides F1, F2, Ra1, Ra2, Rb2, Rb3, Re, Rc, Rf, etc. The essential oil component is lower than ginseng and comprises oleanolic acid, etc. The root has haemostatic action and cardiotonic action, and has been found as having the efficacy that increases blood flow amount of coronary artery, reduces oxygen consumption amount in heart and reduces contents of lipid and cholesterol in blood.

[26] Rehmanniae Radix Preparata, as used herein, is one dried after steaming roots of medicinal plant belonging to Scrophulariaceae, is used as medicine in oriental medicine clinic, and the original one is referred to as the raw *Rehmannia glutinosa*, the dried one is referred to as the dried *Rehmannia glutinosa*, and the one which is made by soaking Rehmanniae glutinosa into alcohol and then steaming and drying it in nine times repeatedly is referred to as Rehmanniae Radix Preparata and considered as having the greatest medicinal effect.

[27] In the present invention, the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 may be 0.20 to 30, and preferably 0.20 to 25. More preferably, it is 0.20 to 21.

[28] In the present invention, the said complex extract can further comprise ginsenosides Rd, Rh1, Rh4, Rk1, Rk3 and Rg5, in addition to gensenoside Rb1 and Rg3.

[29] In the present invention, the weight ratio of the total amounts of said ginsenosides Rh1, Rh4, Rk3, Rg5, Rk1 and Rd to ginsenoside Rb1 may be 1.1 to 75.

[30] In the present invention, 7.0 % or more by weight of ginsenoside Rg3 compared to the total contents of the said ginsenosides Rb1, Rg3, Rd, Rh1, Rh4, Rk1, Rk3 and Rg5 can be comprised. Further, said ginsenoside Rg3 can be comprised in 7.0 to 25.0 % by weight, and in 9.0 to 23.0 % by weight.

[31] In the present invention, the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata can be one extracted from Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata, or a mixture of single extract of Notoginseng Radix Et Rhizoma and single extract of Rehmanniae Radix Preparata. Further, it is preferable that the said complex extract is the extract of the mixture of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata, but it is not limited to such extract.

[32] In the present invention, the complex extract of the present invention can be extracted from the botanical raw material itself as its original form or as a finely cutting or powdery form by the conventional extraction method such as, maceration, digestion, heating, and etc. In the present invention, it is preferable that Notoginseng Radix Et Rhizoma is used by powdering, and it is preferable that Rehmanniae Radix Preparata is used by itself or by finely cutting.

[33] In addition, in the present invention, the extraction solvent can be water, alcohol or a mixture thereof, and it is preferable to use the mixed solvent of water/alcohol. The said mixed solvent of water/alcohol may be aqueous alcohol solution of 30 to 95 % by volume (v/v), preferably aqueous alcohol solution of 50 to 70 % by volume (v/v), more preferably aqueous alcohol solution of 50 % by volume (v/v). Further, the said alcohol may be methanol, ethanol or isopropanol, preferably methanol or ethanol, more preferably ethanol.

[34] Specifically, Notoginseng Radix Et Rhizoma, Rehmanniae Radix Preparata or their mixture are extracted in water/alcohol-mixed solvent, preferably water/methanol-mixed solvent or water/ethanol-mixed solvent, more preferably water/ethanol-mixed solvent at the temperature of 70 ~ 85 ℃ for 2 ~ 4 hours to obtain the extract. In order to increase the extraction efficiency of the said botanical raw material, the above procedure can be repeatedly performed several times, and the said extract can be concentrated under reduced pressure in vacuum codition, at the temperature of 60℃ or less. At this time, the concentrate can be treated by heat, acid, enzyme, or ultrasonic treatment, etc. to obtain the composition wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more.

[35] According to one embodiment of the present invention, the complex extract of the present invention may be one extracted from Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata mixture. At this time, the weight ratio of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata comprised in the said mixture may be 3:1 to 18:1. Preferably, the said weight ratio may be 4:1 to 16:1, more preferably may be 8:1 to 16:1, most preferably may be 8:1.

[36] According to other embodiment of the present invention, the complex extract may be the mixture of single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract. When preparing the single Notoginseng Radix Et Rhizoma extract or the single Rehmanniae Radix Preparata extract, it is preferable to extract it by using the mixed solvent of water/alcohol, preferably the mixed solvent of water/methanol or the mixed solvent of water/ethanol, more preferably the mixed solvent of water/ethanol, and the single Notoginseng Radix Et Rhizoma extract or single Rehmanniae Radix Preparata extract can be prepared by treating a heat, acid, enzyme, or ultrasonic, etc. after the concentration. At this time, if the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 can be 0.20 or more in the mixture of the single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract, the process of heat treatment, acid treatment, enzyme treatment, ultraviolet treatment, etc. can be omitted in the preparation of the single Notoginseng Radix Et Rhizoma extract or single Rehmanniae Radix Preparata extract.

[37] In addition, if the single Notoginseng Radix Et Rhizoma extract and single

Rehmanniae Radix Preparata extract are prepared by using same weight of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata, respectively, the weight ratio of the mixture of the single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract may be 1.5 to 9:1, preferably 3 to 6:1, more preferably 3:1.

[38] In addition, yields of the single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract may be different to each other by the extraction method. Therefore, regulating the mixing ratio of the original botanical raw materials by considering the yield, the weight of the mixture of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata may be 3:1 to 18:1, preferably 4:1 to 16:1, more preferably 8:1 to 16:1, and the most preferably 8:1 like the extract of the mixture of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata.

[39] More specifically, the preparation method for the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention can be explained as below.

[40] Notoginseng Radix Et Rhizoma was used as a powdered. If it is not powdered, the extraction efficiency of the active constituent may be lowered. Each of Notoginseng Radix Et Rhizoma powder and Rehmanniae Radix Preparata, or the mixture of them was prepared, extracted with reflux by water or the mixed solvent of water/alcohol in 5 to 10 folds of the total botanical raw materials as the extraction solvent at the temperature of 75 ~ 85 ℃ for 4 ~ 6 hrs and filtered. Water or water/alcohol-mixed solvent in 5 to 10 folds of the total weight of the botanical raw material was added to each of the residue, warmed at the temperature of 75 ~ 85 ℃ to re-extraction and then filtered. It was mixed with previous filtrate solution, and concentrated in 10 brix or more, for example, about 20 brix. If the used amount of the extraction solvent is too low, since the stirring becomes difficult and a solubility of the extracts becomes low, the extraction efficiency becomes lower. Also, if the used amount of the extracts is too much, since the cost used in the next concentration and purification steps raised great. Therefore, it is not economic and the problems in handling them may be occurred.

[41] As above, the extract obtained by extracting with water or water/alcohol-mixed solvent over the first and second steps are filtered and concentrated, and then some of ginsenoside Rb1 were converted into ginsenoside Rg3 by heat-treatment at 90 ℃ or more to obtain the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata where the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more. The extract obtained by such process can be additionally concentrated to obtain wet extract, and the obtained extract can be lyophilized (freeze-dried) to obtain extract in the powder state, and excipients are added and spray-dried to obtain extract in the powder state.

[42] The term, "inflammatory diseases" in the present invention can be arthritis or periodontal disease.

[43] The term, "arthritis" in the present invention can be degenerative arthritis (osteoarthritis) or rheumatoid arthritis.

[44] The term "periodontal disease" in the present invention can be gingivitis or periodontitis.

[45] The "room temperature" in the present invention means 15 to 25 ℃.

[46] The term "prevention" in the present invention means all behaviors inhibiting or delaying inflammatory diseases or pain by administering the composition of the present invention.

[47] The composition of the present invention can further comprise the known natural products or extracts thereof, or active functional substances, etc., in addition to the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more. Such components can be used alone or in combination.

[48] In order to administer the pharmaceutical composition for preventing or treating inflammatory diseases or pain, which comprises the complex extract according to the present invention, the complex can additionally comprises one or more kinds of the pharmaceutically acceptable carriers, in addition to the active constituent mentioned above. The pharmaceutically acceptable carrier can be saline, sterile water, Ringer's solution, buffered saline, dextrose solution, maltodextrine solution, glycerol, ethanol and mixtures of one or more of these components, and if necessary, other customary additives such as an anti-oxidant agent, buffering agent, bacteristatic agent can be added. In addition, it can be formulated as an injectable formulation such as an aqueous solution, suspension and emulsion, a pill, a capsule, a granule or tablet by additionally adding a diluent, dispersing agent, surfactant, binding agent and lubricant. Further, it can be preferably formulated by the proper method according to the respective diseases or the components by using the method disclosed in Remington's Pharmaceutical Science (a recent edition), Mack Publishing Company, Easton PA.

[49] The composition of the present invention can be administered parenterally (for example, by intravenous, subcutaneous, peritoneous or topical administration) or orally, and the range of the dosage may be various according to the weight, age, sex, health state of the patient, diet, administration time, administration mode, excretion rate, and severity of the disease, etc.

[50] In the present invention, the composition of the present invention can be administered by an oral administration, intraperitoneal administration, intraveneous administration, intramuscular administration, subcutaneous administration, intraendotheial administration, intranasal administration, intrapulmonary administration, en-

dorectal administration, intracavitary administration, intrathecal administration, and is not limited thereto.

[51] The daily dosage is 0.1 to 1000.0 mg/kg based on the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata, preferably 0.1 to 500.0 mg/kg, and it is more preferably administered in one time or by dividing several times per day.

[52] The present invention can be provided in the form of oral composition for effectively preventing or treating inflammatory diseases or pain, and the formulation of the oral composition is not particulary limited and can have conventional formulation. Specifically, it can have the formulation such as toothpaste, mouth washes or mouth refreshener, etc. The oral composition provided by the present invention can comprise the various bases and additives necessary for formulating depending on the formulation, and the kinds and amounts of such components can be selected by a person skilled in the art. For example, in the case that the formulation of the oral composition is tooth paste, it can be prepared by adding an abrasive, wetting agent, foaming agent, binding agent, sweetening agent, pH regulator, preservative, active ingredient, perfume, whitening agent, colorant, resolvent, etc.

[53] The known natural product or extract thereof that effective to inflammatory disease or pain, may be an Trichosanthes kirilowii Maxim extract, Aralia cordata extract, Magnolia officinalis extract, Acyranthes bidentata extract, Kalopanax Pictus extract, Citrus reticulata extract, Cnidium japonicum extract, Foeniculum vulgare extract, Liriope platyphylla extract, Smilax china extract, Clematis mandshurica extract, Gynostemma pentaphyllum extract or Actnidia Polygammae extract, etc.

[54] Although the ratio of the known natural product, extract thereof and active functional material which are known to be effective for the above inflammatory diseases or pain is not so important, it is general to be selected in the range of 1 to about 90 parts by weight per 100 parts by weight.

[55] In order to achieve the above object, the present invention provides the health functional food for preventing or improving inflammatory diseases or pain, which comprises, the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more as an active constituent.

[56] The term “improvement” as used herein means all behaviors which improves or advantageously changes inflammatory diseases or pain by administering the composition according to the present invention.

[57] The health functional food of the present invention includes the foods defined in the FUNCTIONAL HEALTH FOODS ACT of Korea, established on August 26, 2002. Specifically, “the health functional food” includes foods which are prepared and

processed in the form of a tablet, capsule, powder, granule, liquid, pill, etc., by using raw materials or ingredients having the functionality useful for human body.

[58] The health functional food for preventing or improving the said inflammatory diseases or pain can additionally comprise sitologically acceptable additives.

[59] The complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more according to the present invention can be added to the health functional food for improving inflammatory diseases or pain, and if the extract according to the present invention is used as the food additive, the mixed botanical raw material extract can be used by itself or together with other foods or food components, and can be properly used according to the conventional method. The mixed amount of the active constituents can be determined according to the object to be used (prevention, health or therapeutic treatment). In general, the extract of the present invention is added in the amounts of 100 wt% or less, preferably 50 wt% or less to the raw materials in preparing foods or beverages. But, in the case of the object for the health and hygiene, or in the case of taking it for a long period to regulate the health, the amount can be less than the above range, and since there is no problem in light of safety, the active constituents can be used in the amounts of the above range or more.

[60] There is no limitation for the kinds of the above foods. The examples of the food to which the above materials can be added include meat, sausage, bread, chocolate, candy, snack, cracker, pizza, ramen, other noodles, gum, diary products including ice creams, various soups, beverage, tea, health drink, alcohol beverage and vitamin complex, etc. and include all health foods in the common sense.

[61] The health beverage composition of the present invention can comprise various flavoring agent or natural carbohydrate, etc., as an additional component like the conventional beverage. The said natural carbohydrate is monosaccharide such as glucose, fructose, disaccharide such as maltose, sucrose, polysaccharide such as dextrine, cyclodextrine, sugar alcohol such as xylitol, sorbitol, erythritol, etc. As the sweetening agent, the natural sweetening agent such as taumatin, stevia extract, or the synthetic sweetening agent such as saccharin, aspartame, etc. can be used. The ratio of the natural carbohydrate is generally about 0.1 ~ 20.0 g, preferably about 1 ~ 10.0 g per 100 ㎖ of the composition of the present invention.

[62] The health functional food composition of the present invention can comprise various nutrients, vitamin, electrolyte, flavoring agent, colorant, pectic acid and salts thereof, alginic acid and salts thereof, organic acid, protective colloidal thickening agent, pH regulator, stabilizer, preservative agent, glycerin, alcohol, carbonization agent, etc., in addition to the above components. In addition to that, the composition of the present invention can comprise flesh for preparing the natural fruit juice, fruit juice

beverage and vegetable beverage. Such components can be used alone or in combination. Although the ratio of such additives is not important, it is general that they are selected in the range of 0.05 ~ 50 parts by weight per 100 parts by weight of the composition of the present invention.

[63] In addition, the present invention provides the method for preventing or treating inflammatory diseases or pain by administering the composition for preventing or treating inflammatory diseases or pain, which comprises the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more, as the active constituent, to the subject in need thereof including mammal.

[64] The present invention provides the use of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more for preparing the pharmaceutical preparation for preventing or treating inflammatory diseases or pain.

Advantageous Effects of Invention

[65] Since the composition according to the present invention has very excellent anti-inflammatory and analgesic effects, as well as the excellent treatment effects for arthritis or periodontal disease, it can be used in the pharmaceutical composition or health functional food for preventing, treating or improving inflammatory diseases or pain.

Brief Description of Drawings

[66] Figs. 1a and 1b represent the chemical fingerprint results of the high performance liquid chromatogram exhibiting each complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of preparation example 1-1, and Comparative preparation example 1.

[67] Fig. 2 is a histopathological photograph of knee joint and represents the effects that the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention affects the inhibition of cartilage destruction. In Fig. 2, A represents a normal control (NC group), B represents MIA group, C represents Celecoxib group, D represents preparation example 1-1 group, and E represents comparative preparation example 1 group, respectively.

[68] Fig. 3 is a figure exhibiting the influences of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention on arthritis index of type 2 collagen-induced arthritis model. In Fig. 3, 1 represents the normal group, 2 represents the control group, 3 represents preparation example 1-1 150 mg/kg group, 4 represents preparation example 1-1 300 mg/kg group, 5 represents celecoxib 100 mg/kg group, respectively.

[69] Fig. 4 is a figure exhibiting the influences of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention on the improvement of clinical attachment level (CAL) in the ligature-induced periodontal disease model. In Fig. 4, 1 represents the normal control group, 2 represents the negative control group, 3 represents preparation example 1-1 7.5 mg/kg administration group, 4 represents preparation example 1-1 30 mg/kg administration group, 5 represents preparation example 1-1 90 mg/kg administration group, respectively.

[70] Fig. 5 is a figure exhibiting the influence of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention on the inhibition of thermal pain. In Fig. 5, 1 represents the control group, 2 represents preparation example 1-1 25 mg/kg group, 3 represents preparation example 1-1 100 mg/kg group, 4 represents preparation example 1-1 300 mg/kg group, and 5 represents celecoxib 50 mg/kg group, respectively.

[71] Fig. 6 is a figure representing results of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention on Tail-flick test. In Fig. 6, 1 represents control group, 2 represents preparation example 1-1 50 mg/kg group, 3 represents preparation example 1-1 200 mg/kg group, 4 represents preparation example 1-1 600 mg/kg group, and 5 represents celecoxib 100 mg/kg group, respectively.

[72] Fig. 7 is a figure representing the result of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention on acetic acid-writhing test. In Fig. 7, 1 represents control group, 2 represents preparation example 1-1 50 mg/kg group, 3 represents preparation example 1-1 200 mg/kg group, 4 represents preparation example 1-1 600 mg/kg group, and 5 represents celecoxib 100 mg/kg group, respectively.

[73] Fig. 8 is a figure representing the result of the complex Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention on rotarod test. In Fig. 8, 1 represents rat control, 2 represents preparation example 1-1 100 mg/kg group, 3 represents preparation example 1-1 300 mg/kg group, 4 represents mouse control group, 5 represents preparation example 1-1 300 mg/kg group, and 6 represents preparation example 1-1 600mg/kg group, respectively.

Best Mode for Carrying out the Invention

[74] Hereinafter, the present invention will be described in detail by the following examples. But, the following examples do not limit the scope of the present invention, and are to be construed for helping the understanding of the present invention.

[75] Preparation Example 1. The preparation of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata (the extract of the mixture of No-

toginseng Radix Et Rhizoma and Rehmanniae Radix Preparata)

[76] Preparation Example 1-1. Preparation of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[77] Notoginseng Radix Et Rhizoma was obtained from China and purchased from Myohyang Ibc. and Pharmaline Inc. Rehmanniae Radix Preparata was used after purchasing the distributed products in Korea via Puremind. After identifying the presence or absence of foreign matters on each of the botanical raw materials of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata, only botanical raw materials in the clear state were selected and used in the Experiments. Notoginseng Radix Et Rhizoma was used after finely grinding. That is, the powder of Notoginseng Radix Et Rhizoma was used. Rehmanniae Radix Preparata was used as the original form itself. 540 grams of mixture wherein the weight ratio of 8:1 of Notoginseng Radix Et Rhizoma to Rehmanniae Radix Preparata was placed in the 5 L flask, extracted by using 3.78 L of 50 %(v/v) ethanol at 84 ℃ for 4 hrs with stirring under reflux and filtered. Then the residue was stirred under reflux and extracted by using 2.7 L of 50 %(v/v) ethanol at 84 ℃ for 2 hrs. The resulting extract was concentrated to about 20 brix and then refluxed at 100 ℃ for 16 hrs. After filtering it again, it was concentrated under reduced pressure in vacuum condition with rotary vacuum condensing evaporator (N-12, EYELA) at 60 ℃ or lower, powdered by lyophilization (freeze-drying) to use it in the experiments. The yield was 30%.

[78] Preparation Example 1-2. Preparation of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[79] Preparation example 1-2 was prepared by the same method of the above preparation 1-1, except for refluxing at 100 ℃ for 8 hrs instead of 16 hrs in the preparation example 1-1.

[80] Preparation 1-3. Preparation of complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[81] Preparation example 1-3 was prepared by the same method of the above preparation 1-1, except for refluxing at 100 ℃ for 32 hrs instead of 16 hrs in the preparation example 1-1.

[82] Preparation 1-4. Preparation of complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[83] Preparation example 1-4 was prepared by the same method of the above preparation 1-1, except for refluxing at 100 ℃ for 64 hrs instead of 16 hrs in the preparation example 1-1.

[84] Preparation Example 1-5. Preparation of complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[85] Preparation example 1-5 was prepared by the same method of the above preparation

1-1, except for the weight ratio of Notoginseng Radix Et Rhizoma powder to Rehmanniae Radix Preparata of 16:1 instead of 8:1 in the preparation example 1-1.

[86] Preparation Example 1-6. Preparation of complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[87] Preparation example 1-6 was prepared by the same method of the above preparation 1-1, except for the weight ratio of Notoginseng Radix Et Rhizoma powder to Rehmanniae Radix Preparata of 4:1 instead of 8:1 in the preparation example 1-1.

[88] Preparation Example 2. Preparation of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata (The mixture of the single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract)

[89] Preparation Example 2-1. Preparation of complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[90] Preparation of single Notoginseng Radix Et Rhizoma extract

[91] Notoginseng Radix Et Rhizoma was used by finely grinding. 200 g of Notoginseng Radix Et Rhizoma was placed in 3 L flask, was extracted by 1.4 L of 50%(v/v) ethanol at 84 ℃ with stirring under reflux for 4 hrs, and filtered and then the residue was extracted by stirring under reflux by 1.0 L of 50 %(v/v) ethanol at 84 ℃ for 2 hrs. After gathering the resulting extract and filtering it, it was concentrated under reduced pressure in vacuum condition to be about 20 brix and then was refluxed at 100 ℃ for 16 hrs. After filtering it again, it was concentrated under the vacuum-decompression by rotary vacuum condensing evaporator (N-12, EYELA) at 60 ℃ or less, powdered by lyophilization (freeze-drying) to use it in the Experiment. The yield was 25.0%.

[92] Preparation of single Rehmanniae Radix Preparata extract

[93] Rehmanniae Radix Preparata was used as original form itself. 200 g of Rehmanniae Radix Preparata was placed in 3 L flask, was extracted by 1.4 L of 50%(v/v) ethanol at 84 ℃ with stirring under reflux for 4 hrs and filtered and then the residue was extracted with stirring under reflux by 1.0 L of 50 %(v/v) ethanol at 84 ℃ for 2 hrs. After gathering the said extract and filtering it, it was concentrated under the vacuum-decompression by rotary vacuum condensing evaporator (N-12, EYELA) at 60 ℃ or less, powdered by lyophilization (freeze-drying) to use it in the Experiment. The yield was 67%.

[94] The preparation of the mixture of single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract

[95] The complex extract of single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract was prepared by mixing the weight ratio of 6:1 of single Notoginseng Radix Et Rhizoma extract to single Rehmanniae Radix Preparata extract, as prepared in the above.

[96] Preparation Example 2-2. The preparation of the complex extract of Notoginseng

Radix Et Rhizoma and Rehmanniae Radix Preparata

[97] The complex extract of the single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract was prepared by mixing the weight ratio of 3:1 of the single Notoginseng Radix Et Rhizoma extract to the single Rehmanniae Radix Preparata extract, as described in the above preparation example 2-1.

[98] Comparative Preparation Example 1. The preparation of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[99] After identifying the presence or absence for foreign matters for each Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata, only the botanical raw material with the clear state was selected and used in the experiment. Notoginseng Radix Et Rhizoma was used by finely grinding. Rehmanniae Radix Preparata was used as the original form itself. 540g of the mixture wherein the weight ratio of 8:1 of Notoginseng Radix Et Rhizoma to Rehmanniae Radix Preparata were mixed and placed in 5 L flask, was extracted by 3.78 L of 50%(v/v) ethanol at 84 ℃ with stirring under reflux for 4 hrs and filtered and then the residue was extracted with stirring under reflux by 2.7 L of 50 %(v/v) ethanol at 84 ℃ for 2 hrs. After gathering the said extract and filtering it, it was concentrated under reduced pressure in vacuum condition by rotary vacuum condensing evaporator (N-12, EYELA) at 60 ℃ or less, powdered by lyophilization (freeze-drying) to use it in the Experiment. The yield was 29.7%.

[100] Comparative Preparation Example 2. The preparation of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[101] Comparative Preparation example 2 was prepared by the same method of the above preparation example 1-1, except for refluxing at 100 ℃ for 2 hrs instead of 16 hrs in the preparation example 1-1.

[102] Comparative Preparation Example 3. The preparation of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[103] Comparative Preparation example 3 was prepared by the same method of the above preparation example 1-1, except for refluxing at 100 ℃ for 4 hrs instead of 16 hrs in the preparation example 1-1.

[104] Experimental Example 1. Assay of the content of ginsenoside and chromatogram

[105] Each of the complex-extracts of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of preparation examples 1 to 2 and Comparative preparation examples 1 to 3 was used as a sample. 500 mg of sample was precisely weighed, was placed in 50 mL volume of flask with 25 mL of water, shaked by ultrasonic for 1 hr with swaying from time to time and then 20 mL of methanol was added and shaked by ultrasonic for 30 minutes. After allowing this liquid to be at a room temperature, it was adjusted into the marked line with methanol and was used as a test solution after filtering it with 0.45 μm membrane filter. Separately, about 10 mg of ginsenoside Rb1

reference standard preparation was precisely weighted, and was concisely adjusted into 50 mL by melting it in methanol. 5 mL of this liquid was placed in a flask with 10 mL volume, was adjusted into the marked line with water, filtered with 0.45 μm membrane filter and then was used as the standard solution. 20 μL of each the test solution and standard solution was tested according to the liquid chromatographic method under the following condition and then the peak areas for the test solution and standard solution were determined. The reference standard product for analytical marker component was purchased from Sigma-Aldrich, chromadex, Embolaboratory. Zorbax SB C18(3.5 μm, 4.6×150 mm) column was used and the mixed solvent of water and acetonitrile was used as the mobile phase. The ratio of the organic solvent was gradually increased to be from 1 % to 80 % of ratio of acetonitrile at 40 minute. The determination wavelength is 203 nm.

[106] The results of the high performance liquid chromatography (HPLC, High Performance Liquid Chromatography) analyzed by the above method were represented in Figs. 1a to 1b and Table 1. The contents of ginsenoside of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation examples 1 to 2 and Comparative preparation examples 1 to 3, as determined by the above high performance liquid chromatography, were as shown in Table 1, and the relative contents of various ginsenosides to ginsenoside Rb1 were as shown in the below Table 2.

[107] Table 1. the content of ginsenoside (mg/g)

[108]

Ginsenoside / Samples	Rb1	(S)Rh1	Rd	Rh4	Rk3	(S)Rg3	(R)Rg3	Rg5	Rk1
Preparation Ex. 1-1	40.8	21.4	12.0	22.2	43.0	17.2	8.8	13.0	20.0
Preparation Ex. 1-2	56.4	9.1	13.7	8.9	17.2	8.3	4.2	6.8	10.9
Preparation Ex. 1-3	12.3	29.9	6.0	31.1	55.9	30.1	15.4	22.8	35.0
Preparation Ex. 1-4	2.8	33.4	1.2	33.1	59.3	38.1	19.0	29.5	45.4
Preparation Ex. 1-5	46.53	24.35	12.87	24.62	42.58	17.07	8.70	13.33	24.59
Preparation Ex. 2-1	45.0	24.0	13.2	24.3	45.8	20.4	10.0	16.1	23.4
Preparation Ex. 2-2	39.4	21.0	11.5	21.3	40.2	17.9	8.7	14.1	20.5
Comparative Preparation Ex. 1	66.7	1.3	15.4	0	0	0	0	0	0
Comparative Preparation Ex. 2	60.2	2.2	13.8	1.3	2.7	1.0	0.8	1.6	2.1
Comparative Preparation Ex. 3	60.1	4.2	13.2	3.6	6.9	1.8	1.2	2.8	4.1

[109] Table 2. The relative contents of various ginsenosides to ginsenoside Rb1

[110]

Ginsenoside / Sample	(S)Rh1	Rd	Rh4	Rk3	Rg3	Rg5	Rk1
Preparation Ex. 1-1	0.52	0.29	0.54	1.05	0.64	0.32	0.49
Preparation Ex. 1-2	0.16	0.24	0.16	0.30	0.22	0.12	0.19
Preparation Ex. 1-3	2.44	0.49	2.54	4.56	4.01	1.86	2.85
Preparation Ex. 1-4	11.93	0.43	11.82	21.28	20.39	10.54	16.21
Preparation Ex. 1-5	0.52	0.28	0.53	0.92	0.55	0.29	0.53
Preparation Ex. 2-1	0.53	0.29	0.54	1.02	0.67	0.36	0.52
Preparation Ex. 2-2	0.53	0.29	0.54	1.02	0.67	0.36	0.52
Comparative Preparation Ex. 1	0.02	0.23	0.00	0.00	0.00	0.00	0.00
Comparative Preparation Ex. 2	0.04	0.23	0.02	0.05	0.03	0.03	0.03
Comparative Preparation Ex. 3	0.07	0.22	0.06	0.11	0.05	0.05	0.07

[111] As represented in Fig. 1b, it can be seen that the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Comparative Preparation Example 1 which was not performed with separate heat treatment does not comprise ginsenoside Rg3. In addition, it can be seen that notoginsenoside R1 (NG.R1), ginsenoside Rg1 (G.Rg1), ginsenoside Rb1 (G.Rb1) and ginsenoside Rd (G.Rd) were the main components in the extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Comparative Preparation Example 1.

[112] However, when performing the heat treatment, as represented in Fig.1a, it can be seen that ginsenoside Rg3 (G.Rg3) was generated, and in addition to that, ginsenoside Rh1 (G.Rh1), ginsenoside Rh4 (G.Rh4), ginsenoside Rk3 (G.Rk3), ginsenoside Rg5 (G.Rg5), ginsenoside Rk1 (G.Rk1), ginsenoside Rg2 (G.Rg2), etc. were also generated.

[113] Experimental Example 2. Acute toxicity test of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[114] Since the botanical raw materials used in the present invention are classified into food materials, it is considered that there is no problem in the safety of the complex botanical raw material extract, but the inventors of the present invention performed the acute toxicity test, using the following method, to confirm the safety of the complex botanical raw material extract for oral administration. The acute toxicity test was performed with the specific pathogen-free (SPF) 6-week old rats as the test animals. The complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 of the present invention was dissolved in 0.5 % carboxymethyl cellulose (CMC) and then orally administered once to the rats (ten rats / group) fasted for 17 hrs in a volume of 5 g/kg. After the administration of the test material, a fate of animals, the clinical symptoms and the weight change were observed, and abnormality for the abdominal organs and thoracic organs was observed with the naked eye after a necropsy.

[115] As results of the test, there were no specific clinical symptoms or died animals in all

animals administered by the test material, and the toxicity changes in the weight change, the blood test, an autopsy's opinion, etc. were not observed. As a result of the above test, the mixed botanical raw materials extract of the present invention did not exhibit up to 5 g/kg in rats and had 5 g/kg of the minimal lethal dose (LD_{50}) in the oral administration, and thus, it was determined as being safe.

[116] Experimental Example 3. Chronic toxicity test of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[117] Since the botanical raw materials used in the present invention are classified into food materials, it is considered that there is no problem in the safety of the complex botanical raw material extract, but the inventors of the present invention performed the repetition toxicity test, using the following method, to confirm the safety of the complex botanical raw material extract for oral administration. The chronic toxicity test was performed with the specific pathogen free (SPF) 6-week old rats as the test animals. The complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 of the present invention was dissolved in 0.5 % CMC and then orally administered to the rats (ten rats / group) in a maximal concentration of 2400 mg/kg and middle volume of 1200 g/kg and low volume of 600 mg/kg in a geometric ratio of 0.5 for 26 weeks. After administering the test materials, a fate of animals, the clinical symptoms, the intake of an edible water and food, the eye examination and the weight change were observed, and abnormality for the abdominal organs and thoracic organs was observed with the naked eye after a necropsy. In addition, organ weight, hematological assay, blood biochemical assay, histopathological assay, urine analysis, and etc. are gathered, and safety was evaluated.

[118] The repetitive toxicity test was performed to estimate the toxic response and its safety exhibited for male and female, 6-weeks Sprague-Dowley rat orally administered by the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 of the present invention for 26 weeks. Also, having 4 weeks recovery period to confirm whether the reversibility for the toxic change was present. 2,400 mg/kg of the test material was set as high volume, and the middle volume (1,200 mg/kg) and low volume (600 mg/kg) were set in 0.5 of the geometric ratio. Twelve animals per group were set, and the recovery group was set by adding six animals to the high volume group and control group. There was no observation for death in the present experiment and since the toxic response relating to the repeated administration of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 in the general symptoms, weight, an intake of food, an ophthalmologic examination, urine examination, a gross finding in autopsy, the weight of organs, the hematological assay, a blood biochemical assay, a pathological test, it was determined that No Observed Adverse Effect Level

(NOAEL) was 2,400 mg/kg or more. When the value was convert into a human's volume, based on the body surface area, it corresponds to 23,339 mg/day60·kg·person.

[119] Example 1. Identification of NO inhibitory effect, an index relating to the inflammation

[120] In order to determine an influence affecting NO generation, the important index (factor) in the inflammation, of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata, 2 x 10^5 cells of mouse macrophage cell line RAW 264.7 were plated into 24 well plate, were treated with 100 μg/mL of complex extracts of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Examples 1 to 2 and Comparative Preparation Examples 1 to 3 on the next day, and 1hr later, cells were activated to generate NO by adding lipopolysaccharide (LPS) to be 2 μg/mL. Nitrite (NO) standard product included in Griess reagent system kit(Promega,USA) was prepared into 100 μM with DMEM. After incubating 24hrs, medium collected from each well of 24 well plates and the standard liquid for calibration curve were added to each well of 96 well plates for 50 μL. 100 μM of Nitrite(NO) standard liquid was diluted into 1/2 with DMEM, respectively to prepare the standard liquid for calibration curve as 50, 25, 12.5, 6.25, 3.13 and 1.56 μM. 0 μM of the standard liquid for calibration curve used DMEM. 50 μL of Sulfanilamide solution in Griess reagent system kit was added to each well of 96 well plates, and then left at the room temperature for 10 minutes while blocking light. 50 μL of NED solutionin Griess reagent system kit was added to each well of 96 well plates and then left at the room temperature for 10minutes while blocking light. An absorbance was determined on 540 nm with Multiskan spectrum. The control group used the group treated with only LPS. The amount of NO generation was determined, and inhibition rate of the generation to the control group was calculated and then represented in Table 3.

[121] The results of IC_{50} obtained from the tests performed by the same procedure as above after treating with each concentration of the complex extracts of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Examples 1 to 2 and Comparative Preparation Examples 1 to 3 were represented in Table 4.

[122] Table 3. Inhibition rate of NO generation

[123]

Test drug	Inhibition rate of NO generation (%)
Preparation Ex. 1-1	85.4±2.7**
Preparation Ex. 1-2	77.6±5.6*
Preparation Ex. 1-3	94.1±3.5**
Preparation Ex. 1-4	92.4±6.5**
Preparation Ex. 1-5	92.2±2.10*
Preparation Ex. 2-1	88.4±5.96**
Preparation Ex. 2-2	84.6±1.01**
Comparative Preparation Ex. 1	58.4±7.7
Comparative Preparation Ex. 2	55.7±2.1
Comparative Preparation Ex. 3	59.0±4.0
*P<0.05, **P<0.01 compared to Comparative Preparation Ex. 1	

[124] As represented in the above Tables 2 and 3, as the results obtained after estimating the inhibition of No generation for the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata having various weight ratios of ginsenoside Rg3 to ginsenoside Rb1, the inhibition activity for NO generation was remarkably high in the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 was 0.20 or more.

[125] Table 4. Inhibition rate of NO generation (IC_{50})

[126]

Test drug	NO IC_{50} (μg/mL)
Preparation Ex. 1-1	126.4
Comparative Preparation Ex. 1	180.8

[127] As represented in the above Tables 2 and 4, when treating the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 was 0.20 or more, 50% inhibition concentration (IC_{50}) of NO was 126.4 μg/mL, and it was remarkably lower than 180.8 μg/mL of 50% inhibition concentration of NO when treating the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of comparative Preparation Example1 wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 was notbelonged to 0.20 or more.

[128] Example 2. Identification of anti-inflammation, analgesic and cartilage protection effects in MIA-induced osteoarthritis model

[129] 2-1. Disease induction and administration of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata

[130] 12 Weeks-old Sprague dawley rat(Dae Han Bio Link CO., Chungbuk, Korea) was placed in anesthesia chamber (Matrix, US/VME-2) and was anesthetized with diethyl

ether. Monosodium iodoacetate (MIA) (I2512, Sigma, Poole, UK) was dissolved in saline for injection in a concentration of 60 mg/mL and this MIA was injected to right knee joint cavity via infrapatella ligament with gauge 0.5 inch needle (model 750; Hamilton Company, Reno, NV) in an amounts of 50 μL (MIA 3 mg/body). Naive (Normal Control group) was injected the same area of the right knee joint with 50 μL of saline. The test materials (Preparation Example 1-1, Comparative Preparation Example 1, respectively) were dissolved in 0.5 % CMC solution and used after homogenizing to the max with sonicator. The Test material and Control material (Celecoxib, 20 mg/kg) were orally administered once per day for total 21 days from 7 days (day 7) after MIA injection to 28 day (day 27). Normal Control (NC) and Osteoarthritis-Induced Aminals (MIA) were orally administered with 0.5% CMC solvent agent in the volume of 10 mL/kg once everyday for the same period as the test material. The numbers of animals were 10 per each, and each test and control materials were administered at the constant time everyday. The test drug was administered in the volume of 25 mg/kg.

[131] 2-2. Determination of Thermal hyperalgesia in plantar (Plantar test)

[132] The threshold of thermal pain was determined using thermal pain determination device (IITC life science, USA). The plantar of the right rear paw was adapted at 30 ℃ for 10 minutes , and then the rat was placed on a foothold raised to 50 ℃, and the time the licking the rear paw or lifting the paw from the bottom (paw withdrawal latency, PWL) was measured. The determination time for the response was limited to 30 seconds to prevent the damage of the tissue, and the mean value for the determination values for three times determination was used. The determinations were made at the constant time before administration of the drug for degenerative arthritis (day 7) and once a week after the administration (day 14, day 21, day 27) in normal control group, MIA-induced group and test group (Hargreaves K, Dubner R, Brown F, Flores C, Joris J. A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 32: 77-88, 1988). The variation of PWL at the respective time after administering the drug was calculated in relation to PWL before administering the drug, and student t-test was performed to make the statistical treatment (significance level $p < 0.05, 0.01$).

[133] As can be seen from the below Table 5, the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 was the most excellent one in the analgesic effect and was significant in compared to MIA group. In addition, when comparing with the group administered by Celecoxib, it exhibited the statistically significant analgesic effect. The complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 exhibited the effect greater than the complex extract of Notoginseng Radix Et Rhizoma and

Rehmanniae Radix Preparata of Comparative Preparation Example 1.

[134] Table 5. The change in paw withdrawal latency (PWL) (second)

[135]

Group	Day 14	Day 21	Day 27
NC	-1.26 ± 1.16	0.179 ± 1.85	0.14 ± 1.64
MIA	-1.63 ± 0.72	-1.47 ± 1.12*	-1.84 ± 1.43*
Celecoxib	3.41 ± 1.38**	1.01 ± 1.30	1.16 ± 1.50
Preparation Ex. 1-1	3.53 ±3.12**##	3.90 ±1.73**##	4.46 ±1.52**#
Comparative Preparation Ex. 1	1.76 -1.49**#	2.54 +/.58**	2.07 +1.38*#
Data represent mean±SD. *$P < 0.05$, **$P < 0.001$ compared to MIA, #$P < 0.05$, ##$P < 0.001$ compared to Celecoxib.			

[136] 2-3. Analysis of the inflammation and anti-inflammation cytokine in the blood

[137] The rat was fasted for 16 hrs before autopsy, laparotomized under anesthesia with diethyl ether and collected the blood from ventral aorta. The gathered blood was left at the room temperature for 30 minutes, put in the Centrifugation Tube (SST tube) which was not treated with heparin, centrifuged with 3000 rpm at 4 ℃ for 20 minutes to isolate serum and then stored at -70 ℃ before using in the experiment. The contents of tumor necrosis factor (TNF)-α, interleukin(IL)-1β, IL-10, which were known as cytokine relating to the inflammation in the serum, were determined with Sandwich analysis, using Enzyme-Linked ImmunoSorbent Assay (ELISA) kit (R&D Systems Inc. Minneapolis, USA). The quantitative procedure used the protocol provided by the manufacturer. Student t-test was performed to proceed with the statistical processing (Significant level $p < 0.05$, 0.01).

[138] As represented in the following Table 6, when treating the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 was 0.20 or more, the blood concentration of TNF-α and IL-1β was remarkably lower than that of TNF-α and IL-1β when treating the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Comparative Preparation Example 1. In addition, when treating the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 was 0.20 or more, the blood concentration of IL-10 was remarkably higher than that of IL-10 when treating the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Comparative Preparation Example 1 wherein the ratio of ginsenoside Rg3 to ginsenoside Rb1 does not belong to 0.20 or more.

[139] Table 6. Cytokine concentration in blood (pg/mL)

[140]

Group	TNF-α	IL-1β	IL-10
NC	32.34±2.90	8.16±1.43**	1.31±0.09**
MIA	71.21±22.12**	15.81±3.43**	0.90±0.11**
Celecoxib	51.46±16.14**#	13.03±3.09**	1.17±0.22**##
Preparation Ex. 1-1	44.88±10.79**##	10.87±2.60*##	1.36±0.18##
Comparative Preparation Ex. 1	61.68±1.47**	14.35±2.34**	0.97±0.13**
Data represent mean±SD. *$P < 0.05$, **$P < 0.001$ compared to MIA, #$P < 0.05$, ##$P < 0.001$ compared to Celecoxib.			

[141] 2-4. Knee joint histopathological analysis

[142] In order to perform the histopathological analysis of the right knee joint of each animal, the animal was conducted autopsy at the end day of the experiment and the knee joint was taken. The right hip joint and an ankle joint were isolated and extracted not to damage the part of knee joint and then trimming for the soft tissue around the proximal tibia and distal femur knee joint was performed with sterilized surgical knife. The obtained knee joint was immobilized with 10 % formalin for 24 hrs, decalcification was performed while changing the solution with 10 % formic acid at intervals of 24 hrs for 72 hrs before the procedure of the tissue treatment. Sagittal section of knee joint was made via coronal section so that the medial condyle articular surface of the hip joint and tibia was well identified. The tissue-processing procedure of general dehydration, transparency and penetration was performed with tissue processor (VIP-5Jr, Sakura Finetek, Japan), the paraffin embedding process was performed and then 5 μm of thickness of the thin section of the knee joint was prepared with Rotary Microtome (RM2245, Leica 2040, DE). Hematoxylin & Eosin staining and Safranin O staining were performed for each of slices as made. H&E stain: the thin section of the knee joint was performed with deparaffin and moisturization, stained with hematoxylin and eosin, and then dehydrated and clarificated to embed. The tissue slide was observed for the presence or absence of infiltration of the inflammatory cells, destruction level of cartilage and bone, and the histopathological change of articular cartilage. Safranin-O fast green (SOFG) stain: The thin section of the knee joint was performed with deparaffinization and moisturization, and then was reacted in Weigert's iron hematoxylin (HT110232, Sigma, St Louis, USA) solution for 10 minutes, washed, and stained with 0.02 % fastgreen (FCF) solution (F7258, Sigma, St Louis, USA) for 5 minutes. After reacting it in 1 % acetic acid solution, it was stained with 0.1 % Safranin O (S2255, Sigma, St Louis, USA) solution. The slide stained with Safranin O-fastgreen was washed for 5 minutes so that a nucleus was clear color, and embedded after dehydration and clarification. It was identified as to whether the cartilage tissue was damaged, by using an optical microscope (Olympus BX51, Olympus Optical Co. Japan).

[143] In order to estimate the progression level for degenerative arthritis, osteoarthritis index was obtained by dividing and digitalizing the damage degree thereof, based on

the histopathological observation results for the depth and degree of articular cartilage damage, according to the method of Bar-Yehuda, et al. Student t-test was performed and then statistics processing was performed (significance level $p < 0.05, 0.01$).

[144] In the histopathological opinion, all animals of NC group administered saline in the glenoid cavity had a homogeneous cartilage layer, and the well-done maturation and arrangement of cartilage cells, and kept the normal state coated with one layer of synovial cells in synovial joint. When staining it with Safranine-O, all layers of the articular cartilage were stained with dark violet-red and were observed in the state that the content of proteoglycan was riched (See Fig. 2). Meanwhile, an osteoarthritis-induced MIA group had very thin cartilage layer, most of articular cartilages of femur, tibia and kneecap were denatured and necrotized, the form of cartilage cells was disappeared, an erosion was progressed to the radial zone and tidemark, and a granulation tissue carrying an infiltration of the inflammatory cell was found in the surface of the damaged cartilage tissue (See Fig. 2). Together with the opinion for hypertrophic synovial joint, and denaturation and degradation of the articular cartilage, the subchondral osseous tissue is exposed in joint cavity due to the elimination of cartilage layer, and a reconstruction process was frequently observed due to the fibrous proliferation. Since the articular cartilage layer of MIA group was mostly destructed, it was very lightly stained or was not stained with Safranine-O staining. Most animals of Comparative Preparation Example 1 group had the thin layer together with the middle level of the damage in the middle zone of the articular cartilage, the destruction of the subchondral osseous tissue and the proliferation degree of the fibrous tissue were observed in the same appearance similar to the lesion in MIA group. In addition, the elimination of the denatured articular cartilage layer was observed in some area, and thus, the effect for inhibiting the articular degenerative damage was weakly identified (See Fig. 2). Marginal area and some depth part of articular cartilage were stained with a pale tone. However, in Celecoxib group and Preparation Example 1-1 group, the range of articular cartilage that the denature necrosis was progressed, observed as being reduced, the exposure degree of subchondral osseous tissue due to the elimination of cartilage layer was reduced and the fibrosis progression around the subchondral osseous tissue and hypertrophic degree of synovial membrane were also observed very lightly. When staining with Safranine-O, since the stained range and stained status were observed as being increased compared to MIA group, it could be identified that the progression of osteoarthritis was effectively inhibited by the administration of MIA (See Fig. 2).

[145] The right knee joint was stained with H & E and Safranin-O fast green and observed. The results of the osteoarthritis index according to the damage level and depth of the articular cartilage in each group and the histopathological opinion based on the above

observation was represented in Table 7. The osteoarthritis indexes were identified as 0.0 ± 0.00 and 4.5 ± 0.85 in NC group and MIA group, respectively, and were identified as 3.1 ± 1.52 in Celecoxib group, 2.5 ± 0.85 in Preparation Example 1-1 group and 3.7 ± 1.34 in Comparative Preparation Example 1 group in the test group. Since all MIA group and test groups exhibited the significant increase in osteoarthritis index ($p < 0.01$) compared to the normal control group, NC group, and Celecoxib group ($p < 0.05$) and Preparation Example 1-1 group ($p < 0.01$) in test groups exhibited the significant decrease compared to MIA group. It could be seen that the damage degree of articular cartilage tissue was improved in the treatment dose of such test materials. Since preparation Example 1-1 group exhibited the lowest arthritis index compared to MIA group, it could be determined that it could inhibit the degenerative change and damage progress of articular cartilage by MIA most effectively.

[146] Table 7. Histopathological arthritis index

[147]

Group	Histology score
NC	0.0 ± 0.00
MIA	4.5 ± 0.85**
Celecoxib	3.1 ± 1.52**#
Preparation Ex. 1-1	2.5 ± 0.85**##
Comparative Preparation Ex. 1	3.7 ± 1.34**
Data represent mean±SD. *$P < 0.05$, **$P < 0.001$ compared to NC, #$P < 0.05$, ##$P < 0.001$ compared to MIA.	

[148] Example 3. Estimation of the pharmaceutical effect in collagen-induced rheumatoid arthritis model

[149] 3-1. Type 2 collagen-induced rheumatoid arthritis test animal

[150] 5 ~ 6 Week-old male DBA1/1J mice were purchased from Central Experimental Animal Co. and the mice were bred under the condition at the temperature of 21 ~ 23 ℃, humitidy of 40 ~ 60 %. Type 2 collagen of bovine (Chondrex, WA, USA) was dissolved in the concentration of 4 mg/mL in 0.1 N acetic acid and then type 2 collagen of bovine was mixed with the same amount of complete Freund's adjuvant (Chondrex, WA, USA). The homogeneous suspension was made with T-connector connected to 3 mL syringe while cooling it with ice. After identifying as to whether the suspension was well made, strunt of the mouse was sterilized with alcohol cotton and 100 μg of collagen was injected into the dermis of strunt part of the mouse. After 2 weeks, 100 μg of the type 2 collagen of bovine was dissolved, mixed with incomplete Feund's adjuvant (Difco, MI, USA) in the ratio of 1:1 and then administered to the plantar of the mouse.

[151] 3-2. Administration of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention (Preparation Ex. 1-1)

[152] The complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata prepared in Preparation Example 1-1 was dissolved in water to obtain 30 mg/mL and 60 mg/mL, and directly administered to the mouth of the mouse with a 1 mL syringe connected to 1 mL oral gavage once per day from day 4 after the second administration of type 2 collagen of bovine.

[153] 3-3 Observation of the pathological progression of arthritis: Examination with the naked eye and determination

[154] The examination with the naked eye was performed with the following scores, based on the reference documents.

[155] 0: No swelling and rubor, 1: Slight swelling and rubor on Articular, 2: Clear swelling and rubor on articular part, 3: Severe swelling and rubor including a phalangeal joint, 4: Very severe swelling over all area of articular.

[156] Therefore, the highest score for the articular lesion was 16 by combining the left and right of fore-paw and rear-paw per a mouse, and the score per a leg was at most 4 (Courtenay JS, Dallman MJ, Dayan AD, et al. : Immunization against heterologous type II collagen induces arthritis in mice. Nature 283: 666-668. 1980).

[157] As represented in Fig. 3, when orally administering the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of the present invention (Preparation Ex. 1-1) to the mouse having type 2 collagen-induced arthritis, the pathological progress of arthritis was greatly inhibited from 6 weeks, compared with the control group.

[158] <u>Example 4. Identification of the effect for treating periodontitis in periodontitis model</u>

[159] Periodontal disease is the chronic disease due to the complex causes. The most important cause of such periodontal disease can be mentioned as an action of the bacteria. There is a bacterial membrane formed by proteins, foods, etc. in saliva in the surface of teeth, and many bacteria attach to it and be activate. Since most of periodontal patients have defects in the function of defensive cells of the body, they can not resist to various products made by such bacteria, and thus severe destruction of the tissue was occurred.

[160] The experimental model of the present experiment was made based on the principle that teeth of beegle dog were tied with silk suture and wire to naturally occur Plaque and Calculus which were the main causes of periodontal disease and to generate inflammation and to finally occur the absorption of alveolar bone. Molar (cheek tooth) of beegle dog was tied with silk wire (6 of teeth per a dog), was fed with liquid feed to induce periodontal disease for 8 weeks, the ligation was lift and then, the tablet comprising 37.5 mg, 150 mg and 450 mg of Preparation Ex. 1-1 was administered to about 10 kg of the dog (three dogs per group) in every one table twice a day to see as

to what effect the extract of the present invention affect. Three dogs of the negative control group were administered the tablet made with excipient in every one tablet twice a day, as the same above. One dog of the normal dog was not administered. Clinical Attachment Level (CAL), The clinical index, was determined before administering the drug and after administering the drug for 1 week. The result was represented in Fig. 4. 1 represents the normal control group, 2 represents the negative control group, 3 represents the administration group of 7.5 mg/kg of Preparation Example 1-1, 4 represents the administration group of 30 mg/kg of Preparation Example 1-1, 5 represents the administration group of 90 mg/kg of Preparation Example 1-1.

[161] As represented in Fig. 4, CAL of the negative control group was 1.62 mm, but CAL of the group administered by the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 exhibited a concentration-dependent reduction, and in the cases of groups administered 30 and 90 mg/kg, they were reduced to 1.17 mm and 1.00 mm, respectively.

[162] Example 5. Identification of hyperanalgesic response by heat stimulation (hot plate method)

[163] A mouse was placed on hot pad maintain at 30 ℃ to be adapted for about 10 minutes. The mouse was placed on hot plate heated and maintained in the constant temperature (55 ± 1 ℃) and determined the time that it licked front or hid paws or jumped off (hot plate latency) (Jones CK, Peters SC, Shannon HE. Efficacy of duloxetine, a potent and balanced serotonergic and noradrenergic reuptake inhibitor, in inflammatory and acute pain models in rodents. J Pharmacol Exp Ther. 2005, 312(2):726-732.). In determination, it was performed within 30 seconds (cut-off time: 30 seconds) to prevent the tissue damage, excluding that the mouse took its paw to move. It was performed before administration or at 60 minutes and 120 minutes after administration, but only the animal exhibited the response within 8 seconds before administration was selected and used. After elapsing 60 minutes and 120 minutes, cut-off time (30 seconds) for the mouse which did not exhibit the response within the limited time (30 seconds) was recorded (Choi JH, Cha DS, Jeon H. Anti-inflammatory and anti-nociceptive properties of Prunus padus. J Ethnopharmacol. 2012, 21; 144(2):379-386). Calculation result for analgesic effect was calculated from an avoidance time before administration (baseline), and 120 minutes (120 min.) after administration, as hot plate latency time (sec) (Patel RB1, Pawar VD, Prajapati KD, Sonara BM, Deshpande SS, Shah GB, Jain MR. Anti-nociceptive and anti-allodynic activity of aliskiren in various pain models. Eur J Pharmacol. 2013, 15; 708(1-3): 80-87), and the results of MPE(%) were calculated with a mathematical formula: MPE(%)(percentage of maximal possible effect) = 〔(post drug latency predrug latency) / (cut off time predrug latency)〕 × 100

(Demir Ozkay U1, Can OD. Anti-nociceptive effect of vitexin mediated by the opioid system in mice. Pharmacol Biochem Behav. 2013, 109:23-30). The results were represented in Fig. 5.

[164] As represented in Fig. 5, when administering 25, 100 and 300 mg/kg of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1, MPE (%) was 14.3 %, 15.2 % and 18.4 %, respectively and greatly increased compared to the control group of 2.45 %, and the group administered by 300 mg/kg of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1 exhibited the result similar to 50 mg/kg of celecoxib.

[165] Example 6. Identification of the treatment of pain via tail avoidance response test (tail-flick method)

[166] The part of about 3 cm from the end of mouse tail was measured and marked for the position to be stimulated with a heat source, and the mark was made on the side that the light was not reached so as not to affect the response (Patel RB1, Pawar VD, Prajapati KD, Sonara BM, Deshpande SS, Shah GB, Jain MR. Anti-nociceptive and anti-allodynic activity of aliskiren in various pain models. Eur J Pharmacol. 2013, 15; 708 (1-3):80-87). analgesiometer (Ugo Basile, model No. 37360, Varese, Italy) was used as the harmful thermal tail avoidance response determination device used, and the pain degree was determined by measuring the time taken to the moment being exhibited by the avoidance response after subjecting the heat source on the marked tail area of the mouse (Ben-Bassat J, Peretz E, Sulman FG. Analgesimetry and ranking of analgesic drugs by the receptacle method. Arch Int Pharmacodyn Ther. 1959; 122: 434-47). Since the lasting of the heat stimulation damages the tail tissue, the time to be exposed to the heat stimulation was limited to 10 seconds (cut-off time) to protect the tissue (Choi JH, Cha DS, Jeon H. Anti-inflammatory and anti-nociceptive properties of Prunus padus. J Ethnopharmacol. 2012, 21;144(2):379-386). The determination frequency was performed before the administration or 30 minutes, 60 minutes, and 120 minutes after the administration of the test material or vehicle (0.5% CMC). In the case of the mouse which did not exhibit the response within the limited time in the determination of avoidance response, the cut-off time (10 seconds) was recorded (Choi JH, Cha DS, Jeon H. Anti-inflammatory and anti-nociceptive properties of Prunus padus. J. Ethnopharmacol. 2012, 21; 144 (2):379-386). The calculation of the result was calculated from the avoidance response time on 0 (baseline), 30 minutes (30 min), 60 minutes (60 min) and 120 minutes (120 min), as the tail flick latency times (sec).

[167] As represented in Fig. 6, when administering 50, 200, 600 mg/kg of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1, MPE (%) was 4.7 %, 10.7 %, and 16.0 %, respectively, and

increased in the concentration-dependent manner, and the group administered by 600 mg/kg exhibited the pharmaceutical effect similar to 100 mg/kg of celecoxib.

[168] Example 7. Identification of analgesic effect by Acetic acid writhing method (acetic acid-induced writhing test)

[169] The analgesic effect was determined according to Koster and Demir Ozkay method (Koster, R., Anderson, M., Beer, E.J., 1959. Acetic acid for analgesic screening. Federation Proceeds 18, 412-416, Demir Ozkay U, Ozkay Y, Can OD. Synthesis and analgesic effects of 2-(2-carboxyphenylsulfanyl)-N-(4-substitutedphenyl)acetamide derivatives. Med Chem Res. 2011; 20:152-157). Various doses of test material or vehicle (0.5% CMC) were administered to the mouse fasted for 12 hrs at 60 minutes before administration. 0.6% acetic acid - physiological saline solution (1% in 0.9% sterile saline) was intraperitonically injected in the dose of 0.1 mL/10 g b.wt.(body weight)(10 mL/kg) at 60 minutes after the administration, and then the number of the writhing occurrence for 10 minutes in each cage were determined from 5 minute after administration (sixty-five minutes lapse after administration). The total number during the determination period was calculated from the total number of writhes (writhes/10 min) , and calculated by using the pain inhibition rate, the mathematical formula: inhibition % = [(control mean) − (treated mean) / (control mean)] × 100 (Choi JH, Cha DS, Jeon H. Anti-inflammatory and anti-nociceptive properties of Prunus padus. J Ethnopharmacol. 2012, Nov 21;144(2):379-386. Demir Ozkay U1, Can OD. Anti-nociceptive effect of vitexin mediated by the opioid system in mice. Pharmacol Biochem Behav. 2013; 109:23-30).

[170] As represented in Fig. 7, when administering 50, 200 and 600 mg/kg of the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Example 1-1, writhing syndrome due to the administration of acetic acid was identified as being inhibited by 33.3 %, 51.3 % and 61.5 %, respectively, and it was the statistically significant result, compared to the control group.

[171] Example 8. Identification of analgesic effect by Rotarod test

[172] Rotarod test is to estimate the motor coordination of the animal and it was determined by using mouse rotarod system (Panlab, Barcelona, Spain) (Bastos LF1, Prazeres JD, Godin AM, Menezes RR, Soares DG, Ferreira WC, Dutra MM, Machado RR, Coelho MM. Sex-independent suppression of experimental inflammatory pain by minocycline in two mouse strains. Neurosci Lett. 2013 Oct 11; 553: 110-4). The rotary speed of Rotarod was determined by setting 7 rpm for the mouse and 10 rpm for the rat before and after administering the test material. The mouse or rat were placed on the rotating bar before administering the test material, and the time that they come off was identified to determine the baseline value. At this time, the cut-off time was set to 1 minute. After calculating the baseline value, the test material or vehicle (0.5% CMC)

was administered to the animal according to the group assignment. The test was performed after lapsing 120 minutes after the administration of the test material.

[173] As represented in Fig. 8, it was identified that the complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata of Preparation Examination 1-1 did not affect the mobility.

[174] From the above Examples 1 to 8, It could be identified that the composition of the present invention had the excellent effects for treating and/or preventing inflammatory diseases (for example, arthritis (osteoarthritis, rheumatoid arthritis), periodontitis) or pain.

Industrial Applicability

[175] Since the complex extract of the present invention has the excellent anti-inflammatory and anti pain effects, as well as the very excellent effect for treating arthritis or periodontal disease, it can be used for preventing, treating or improving the inflammatory diseases or pain.

Claims

[Claim 1] A pharmaceutical composition for preventing or treating inflammatory diseases or pain, which comprises a complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more.

[Claim 2] The pharmaceutical composition according to claim 1, wherein the weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 to 25.

[Claim 3] The pharmaceutical composition according to claim 1, wherein the complex extract is extracted from a mixture of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata.

[Claim 4] The pharmaceutical composition according to claim 3, wherein the weight ratio of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata comprised in the mixture is 3:1 to 18:1.

[Claim 5] The pharmaceutical composition according to claim 1, wherein the complex extract is a form of mixture of single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract.

[Claim 6] The pharmaceutical composition according to claim 5, wherein the weight ratio of single Notoginseng Radix Et Rhizoma extract and single Rehmanniae Radix Preparata extract is 1.5:1 to 9:1.

[Claim 7] The pharmaceutical composition according to claim 1, wherein the complex extract further comprises ginsenosides Rd, Rh1, Rh4, Rk1, Rk3 and Rg5, in addition to ginsenosides Rb1 and Rg3.

[Claim 8] The pharmaceutical composition according to claim 7, wherein the weight ratio of total contents of the ginsenosides Rd, Rh1, Rh4, Rk1, Rk3 and Rg5 to ginsenoside Rb1 is 1.1 to 75.

[Claim 9] The pharmaceutical composition according to claim 7, wherein the ginsenoside Rg3 is comprised in an amount of 7.0 wt% or more of the total contents of the ginsenosides Rb1, Rg3, Rd, Rh1, Rh4, Rk1, Rk3 and Rg5.

[Claim 10] The pharmaceutical composition according to claim 1, wherein the inflammatory disease is arthritis or periodontal disease.

[Claim 11] The pharmaceutical composition according to claim 10, wherein the arthritis is degenerative arthritis or rheumatoid arthritis.

[Claim 12] The pharmaceutical composition according to claim 10, wherein the periodontal disease is gingivitis or periodontitis.

[Claim 13] The pharmaceutical composition preventing or treating inflammatory diseases or pain according to claim 12, wherein the composition is an

oral composition.

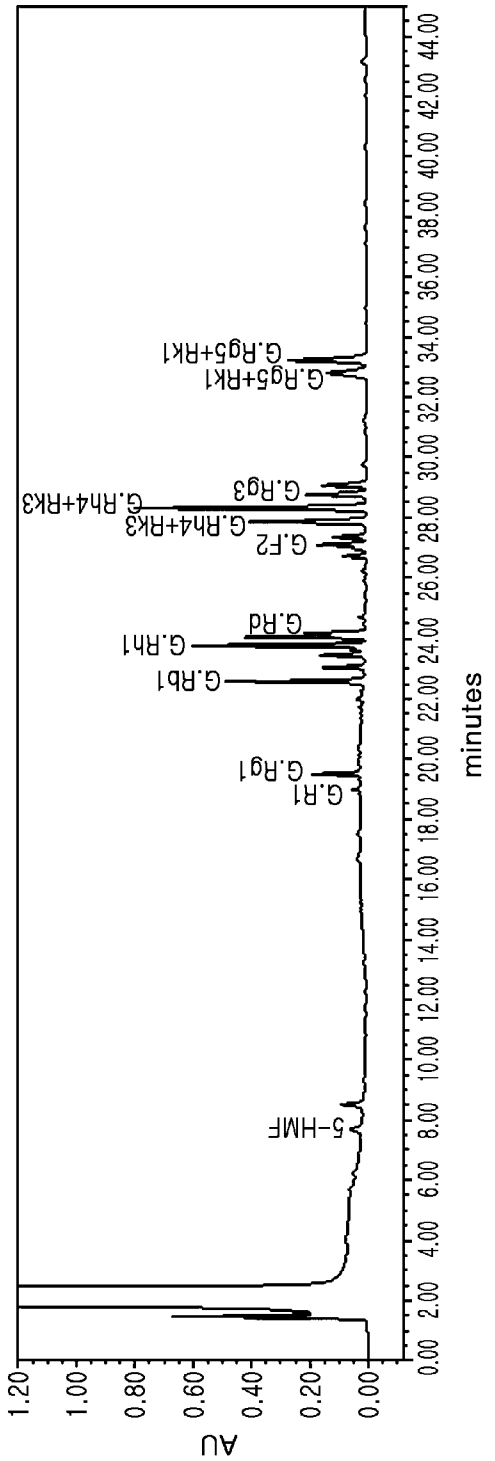
[Claim 14] The pharmaceutical composition for preventing or treating inflammatory diseases or pain according to claim 13, wherein the oral composition is one or more selected from the group consisting of toothpaste, mouth freshener and mouth washes.

[Claim 15] A health functional food for preventing or treating inflammatory diseases or pain, which comprises a complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein a weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more.

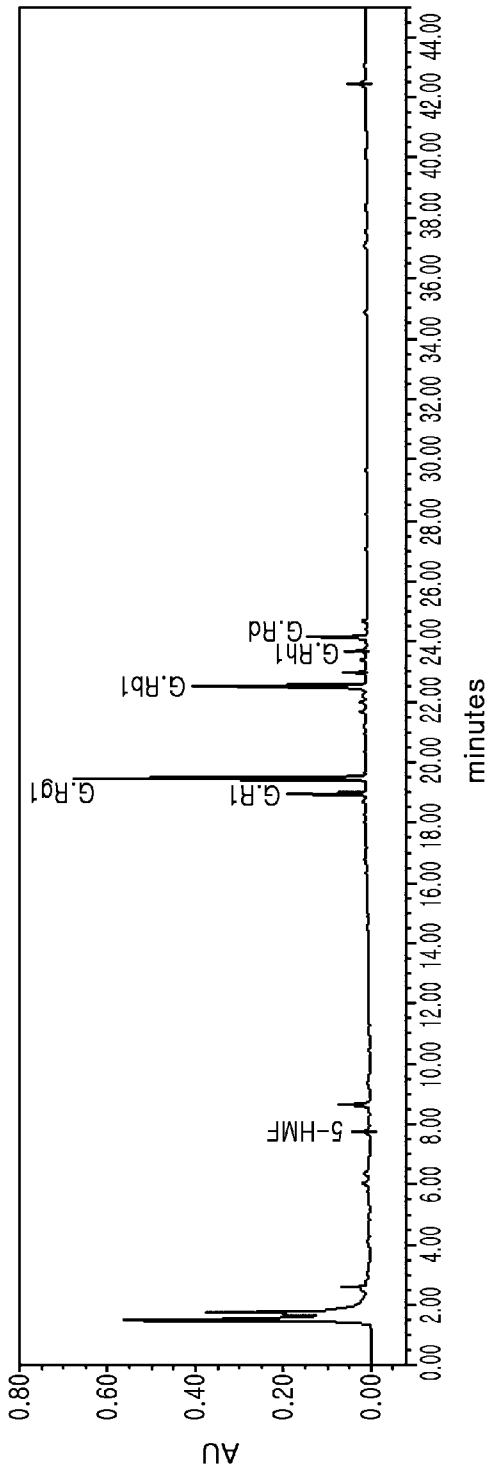
[Claim 16] A method for preventing or treating inflammatory diseases or pain, which comprises administering a complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein a weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more to a subject in need thereof.

[Claim 17] A use of a complex extract of Notoginseng Radix Et Rhizoma and Rehmanniae Radix Preparata wherein a weight ratio of ginsenoside Rg3 to ginsenoside Rb1 is 0.20 or more, for preparing a pharmaceutical preparation for preventing or treating inflammatory diseases or pain.

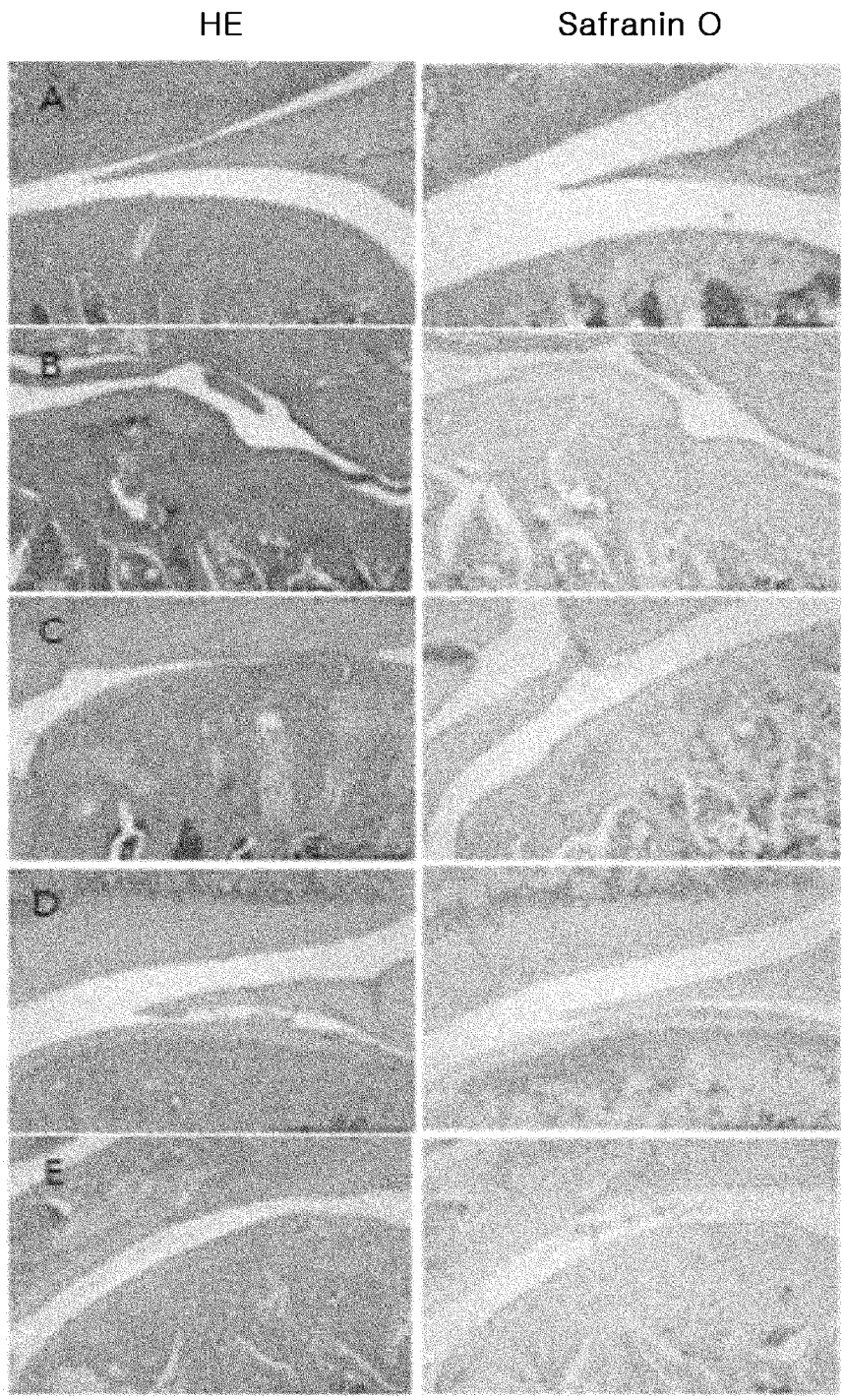
[Fig. 1a]


AU
1.20
1.00
0.80
0.60
0.40
0.20
0.00
minutes
0.00 2.00 4.00 6.00 8.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 24.00 26.00 28.00 30.00 32.00 34.00 36.00 38.00 40.00 42.00 44.00
5-HMF
G.R1
G.Rg1
G.Rb1
G.Rh1
G.Rd
G.F2
G.Rh4+Rk3
G.Rh4+Rk3
G.Rg3
G.Rg5+Rk1
G.Rg5+Rk1

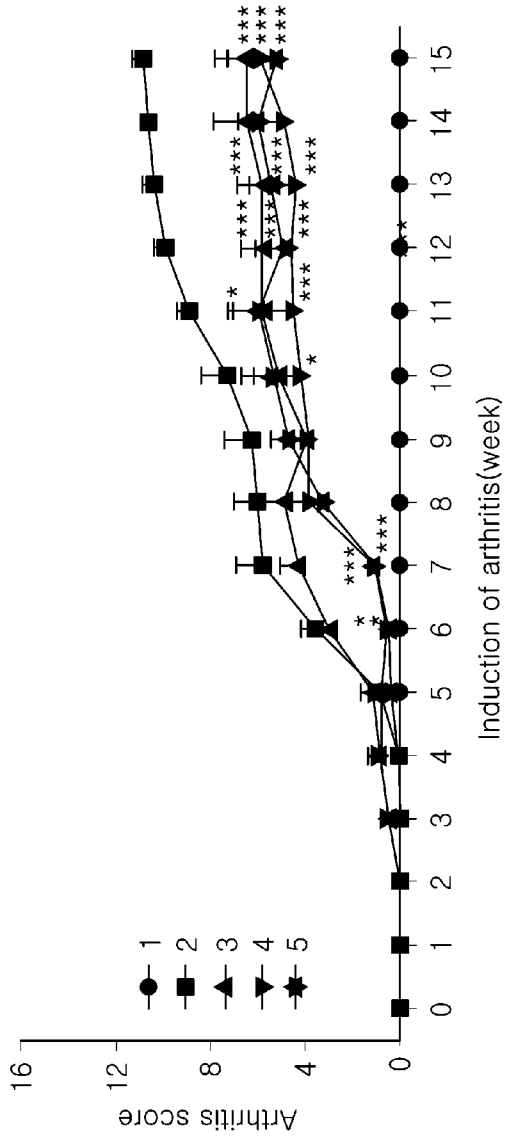
[Fig. 1b]


G.Rg1
G.Rb1
G.Rd
G.Rh1
G.R1
5-HMF
minutes
AU
0.00
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
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42.00
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0.20
0.40
0.60
0.80

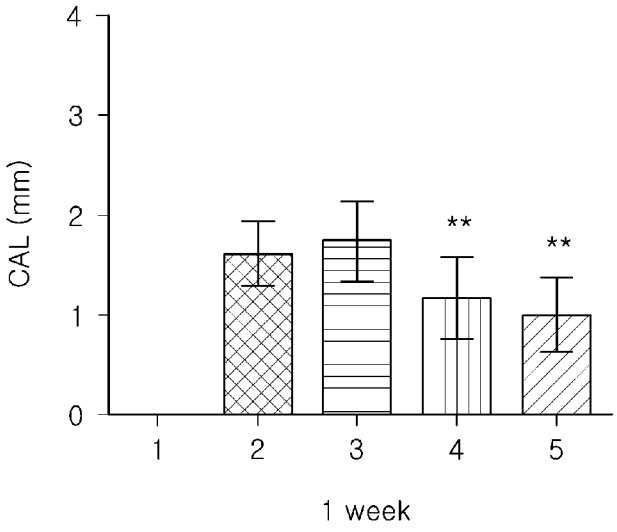
[Fig. 2]



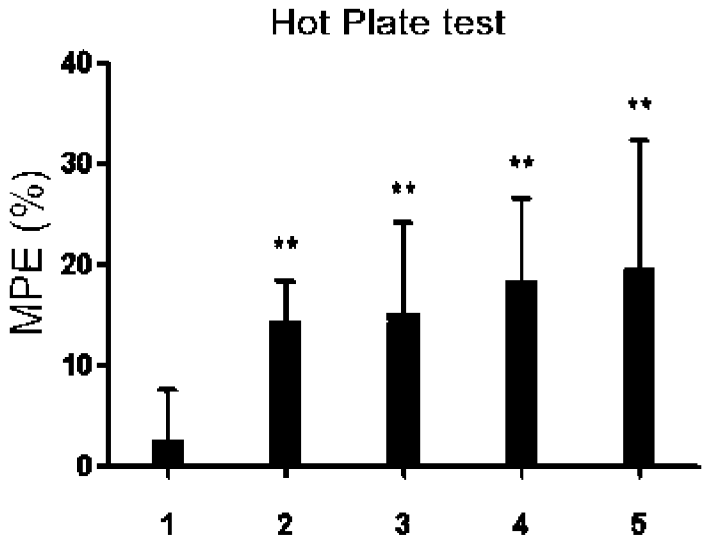
[Fig. 3]


Arthritis score
16
12
8
4
0
1
2
3
4
5
Induction of arthritis(week)
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15

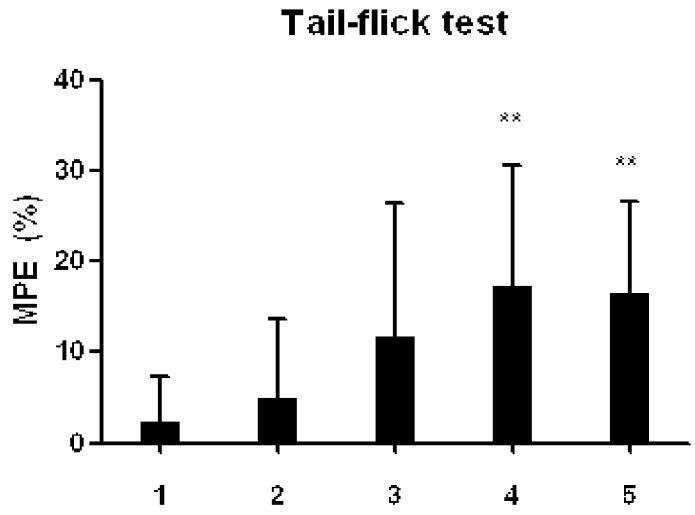
[Fig. 4]


CAL (mm)
0
1
2
3
4
**
**
1
2
3
4
5
1 week

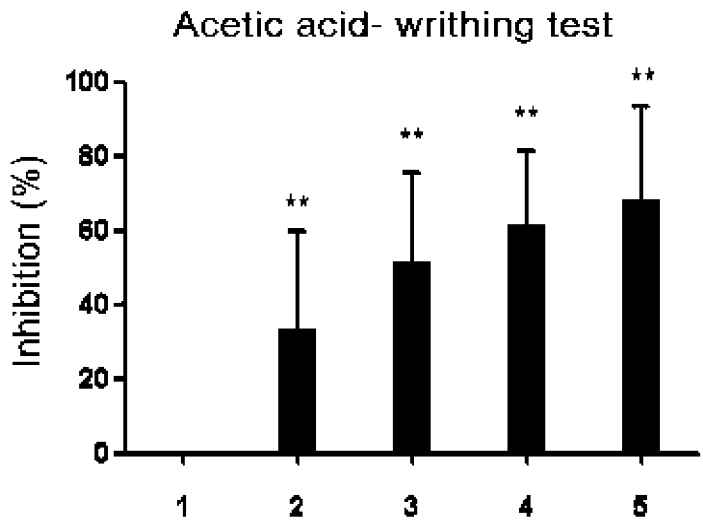
[Fig. 5]



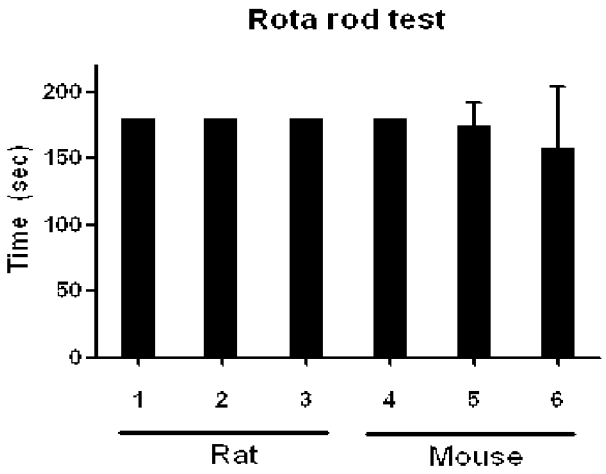
[Fig. 6]



[Fig. 7]



[Fig. 8]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2016/003903

A. CLASSIFICATION OF SUBJECT MATTER

A61K 36/254(2006.01)i, A61K 36/804(2006.01)i, A61K 31/704(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 36/254; A61K 36/00; A61K 36/90; A61K 36/258; A61P 1/02; A61K 36/804; A61K 31/704

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 data base consulted during the international search (name of data base and, where practicable, search terms used)
eKOMPASS(KIPO internal) & Keywords: panax notoginseng, rehmannia glutinosa, extract, arthritis, periodontitis

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010-0129476 A1 (KIM, JUNG-KEUN et al.) 27 May 2010 See abstract; claims 19, 26-28 and 30; paragraphs [0035] and [0046].	1-15,17
X	US 2007-0275103 A1 (KIM, JUNG-KEUN et al.) 29 November 2007 See abstract; claims 1 and 6-8; paragraphs [0020] and [0035]-[0036].	1-15,17
X	CN 101843747 B (THE GREEN SOURCE IN BEIJING SOLVES LIMITED COMPANY OF DEVELO PMENT IN SCIENCE AND TECHNOLOGY) 23 November 2011 See abstract; claim 1.	1-15,17
X	CN 102258722 A (WANG, XURI) 30 November 2011 See abstract; claims 1-2.	1-15,17
A	KR 10-2004-0108512 A (DOCTOR`S MEDICORE et al.) 24 December 2004 See abstract; claims 1 and 4-7.	1-15,17

☐ 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 another 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
27 September 2016 (27.09.2016)	**27 September 2016 (27.09.2016)**

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2016/003903

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

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

1. ☒ Claims Nos.: 16
because they relate to subject matter not required to be searched by this Authority, namely:
Claim 16 pertains to a method for treatment of the human body by therapy, and thus relate to a subject matter which this International Searching Authority is not required, under PCT Article 17(2)(a)(i) and PCT Rule 39.1(iv), to search.

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 fees, this Authority did not invite payment of any additional fees.

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

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

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/KR2016/003903

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2010-0129476 A1	27/05/2010	CN 101657209 A	24/02/2010
		CN 101657209 B	01/01/2014
		EP 2139506 A1	06/01/2010
		EP 2139506 A4	07/12/2011
		JP 2010-524921 A	22/07/2010
		JP 5427772 B2	26/02/2014
		KR 10-0852881 B1	19/08/2008
		WO 2008-130113 A1	30/10/2008
US 2007-0275103 A1	29/11/2007	CN 101119738 A	06/02/2008
		CN 101119738 B	28/11/2012
		EP 1845928 A1	24/10/2007
		EP 1845928 A4	29/12/2010
		EP 1845928 B1	12/06/2013
		JP 2008-528483 A	31/07/2008
		KR 10-0626358 B1	21/09/2006
		KR 10-2006-0084650 A	25/07/2006
		US 7811612 B2	12/10/2010
		WO 2006-078120 A1	27/07/2006
CN 101843747 B	23/11/2011	CN 101843747 A	29/09/2010
CN 102258722 A	30/11/2011	None	
KR 10-2004-0108512 A	24/12/2004	None	