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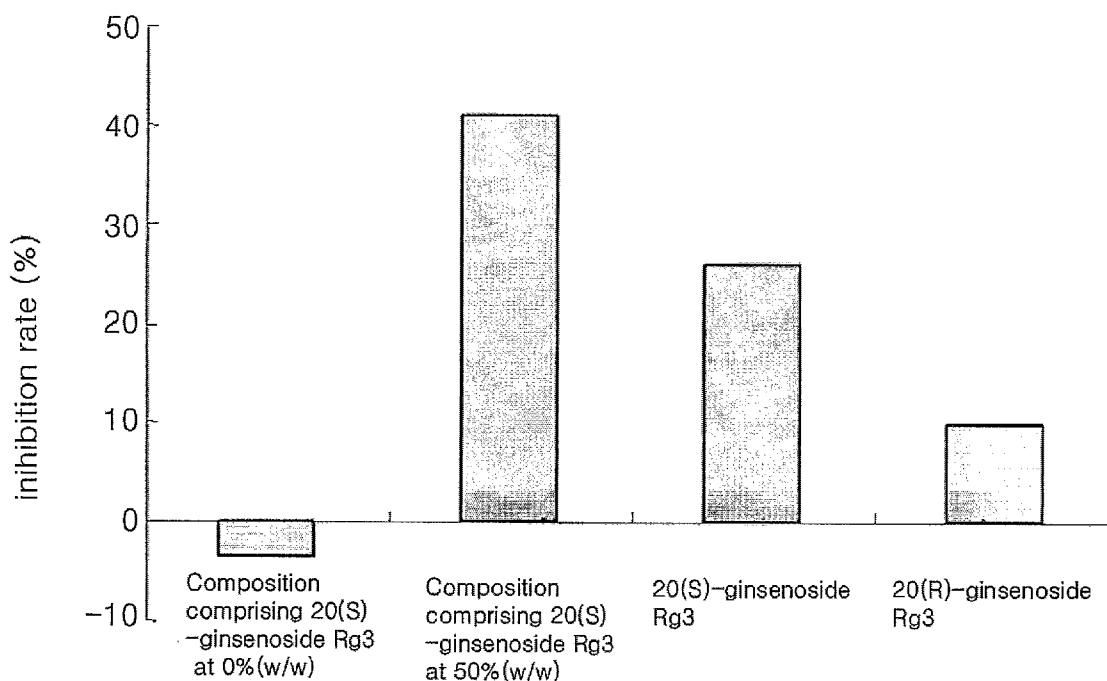
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[Continued on next page]

(54) Title: 20(S)-GINSENOSIDE Rg3 AS AN ANTI-ANGIOGENIC COMPOSITION



(57) Abstract: The present invention relates to an anti-angiogenic composition comprising ginsenoside Rg3. In particular, the present invention relates to an anti-angiogenic composition comprising 20(S)-ginsenoside Rg3 at a concentration of from 40 to 60 % (w/v) which shows excellent inhibitory effect on angiogenesis and tumor growth by inhibiting the activity of vascular endothelial growth factor receptor-1 (VEGFR-1) and lowering in vivo level of vascular endothelial growth factor (VEGF).

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20(S -ginsenoside Rg3 as an anti-angiogenic composition**FIELD OF THE INVENTION**

5 The present invention relates to an anti-angiogenic composition comprising ginsenoside Rg3. In particular, the present invention relates to an anti-angiogenic composition comprising 20(S)-ginsenoside Rg3 at a concentration of from 40 to 60 %(w/v) having excellent inhibitory activity against angiogenesis and tumor growth by inhibiting the activity of vascular
10 endothelial growth factor receptor-1 (VEGFR-1) and lowering *in vivo* level of vascular endothelial growth factor (VEGF).

BACKGROUND OF THE INVENTION

15 There has been a growing incidence of cancer due to the increase in aged population, percentage of smoking people, changes in dietary habits, lacking of exercise, etc., and therefore various attempts have been made to develop an anti-cancer agent for the treatment of cancer. However, most anti-cancer agents developed so far have drawbacks such as toxicity to normal
20 cells, drug resistance and risk of recurrence, and thus there is still a need to

develop a new innovative anti-cancer agent having a different mechanism from the pre-existing anti-cancer agents. Therefore, extensive researches have been conducted to discover causes for cancer pathogenesis and progression and suggest more fundamental solutions on the cancer treatment.

5 In this regard, J. Folkman suggested in 1971 an anti-angiogenic agent as a new alternative solution for preventing tumor growth and metastasis [Folkman et al., *N. Engl. J. Med.*, 285, p1182, 1971].

Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels, and it is believed to be responsible for
10 various kinds of diseases such as tumor growth and metastasis, diabetic retinopathy, psoriasis, chronic inflammation and ulcer [Carmeliet et al., *Nature*, 407, p249, 2000]. In particular, it has been reported that in the case of occurring excessive angiogenesis such as cancer, it is possible to effectively prevent the growth and metastasis of tumor by merely inhibiting such
15 angiogenesis [Garcea et al., *European Journal of Cancer* 40, p1302, 2004].

Representative examples of a target for developing an anti-cancer agent through the inhibition of angiogenesis include vascular endothelial growth factor (VEGF), vascular endothelial growth factor receptor (VEGFR), basic fibroblast growth factor (bFGF), TGF- α and - β , epidermal growth factor
20 (EGF), platelet-derived growth factor (PDGF) and the like [Presta et al.,

Cytokine Growth Factor Rev 16, p159, 2005; Hoeben et al., *Pharmacol Rev* 56, p549, 2004; Shannon et al., *Clin Colorectal Cancer* 4, S74, 2004].

A front runner of anti-cancer agents based on the inhibition of angiogenesis is Avastin®, which is a monoclonal antibody to VEGF. Avastin® has been used as a primary drug for treating metastatic colon carcinoma patients in combination with a chemotherapy agent including 5-Fluorouracil (5-FU) and approved as an anti-angiogenic inhibitor for the first time in February, 2004 from the U.S. Food and Drug Administration (FDA) [Hurwitz et al., *Clin Colorectal Cancer* 4, S62, 2004]. Further, in order to add non-small cell lung cancer and breast cancer to the list of diseases for which Avastin is efficacious, clinical test and approval procedures are actively underway [Miller et al., *Clin Breast Cancer* 3, 421, 2003; Lynch et al., *Lung Cancer* 50, S25, 2005]. In addition, Nexavar® which is an oral targeting anti-cancer agent co-developed by Bayer and Onyx has received FDA approval for kidney cancer in 2005, and SU11248 of Pfizer approved by FDA as a primary drug for progressive kidney cancer and gastrointestinal stromal tumor (GIST) in January, 2006 has been commercially available in the name of Sutent®. Besides, many anti-angiogenic inhibitors including PTK787/ZK222584 of Novartis are in progress of clinical tests [Kim, *Biochem. Mol. Biol. News*, 12, p263, 2005; Thomas et al., *Semin Oncol* 30, p32, 2003].

It has been reported that ginseng and red ginseng exhibit various pharmacological activities including anti-cancer activity, and a representative major ingredient showing anti-cancer activity is ginsenoside. Especially, ginsenoside Rg3 ($C_{42}H_{72}O_{13}$: 785.03) is a characteristic saponin of red ginseng.

5 It is believed that ginsenoside Rg3 is generated by heat-degradation of Protopanaxadiol saponin included in fresh ginseng during the preparation of red ginseng. Red ginseng generally contains a trace amount of ginsenoside Rg3, i.e., 0.006% of 20(S)-ginsenoside Rg3 and 0.014% of 20(R)-ginsenoside Rg3 [Kitagawa et al., *The Ginseng Review*, Vol 1, p21, 1983].

10 Since the first isolation of 20(R)-ginsenoside Rg3 from red ginseng and subsequent characterization of its structure by Kaku et al. in 1980 [Kaku et al., *Arzneim. Forsch. Drug Res.* 30, 936-943, 1980], it has been known to exhibit inhibitory actions against tumor growth and metastasis, blood vessel relaxation and inhibitory action against anti-cancer drug resistance [Back et al.,
15 *Arch. Pharm. Res.*, 18, p164, 1995; Hasegawa et al., *Planta Med.*, 61, p409, 1995; Mochizuki et al., *Biol. Pharm. Bull.*, 18, p1197, 1995; Ginseng Res., 25, p107, 2001; Shinkai et al., *Jpn. J. Cancer Res.*, 87, p357, 1996; Tao et al., *Chin. J. Surg.*, 40, p606, 2002; Tao et al., *Chin. J. Surg.*, 40, p606, 2002].

Further, it has been also found that ginsenoside Rg3 can inhibit
20 metastasis by suppressing cancer cell adhesion, invasion and angiogenesis

[Mochizuki et al., *Biol. Pharm. Bull.*, 18, p1197, 1995], and the inhibitory action against angiogenesis of 20(R)-ginsenoside Rg3 is due to the inhibition of VEGF [Zhang et al., *Biochem Biophys. Res. Commun.*, 342, p824, 2006; Chen et al., *Sichuan Da Xue Xue Bao Yi Xue Ban*, 37, p60, 2006; Chen et al., *Yao Za Zhi*, 30, p357, 2005; Pan et al., *Zhonghua Fu Chan Ke Za Zhi*, 37, p227, 2002].

As described above, there have been many reports regarding the inhibitory activities of ginsenoside Rg3 against angiogenesis and VEGF activity. However, they were all related to 20(R)-ginsenoside Rg3, an isomer of 20(S)-ginsenoside Rg3, or they did not provide enough information to classify them into "S-type" or "R-type" according to optical activity at a 20th carbon position of ginsenoside Rg3. Knowing the difference in inhibitory activity on metastasis between 20(S)-ginsenoside Rg3 and 20(R)-ginsenoside Rg3 that belong to stereoisomers of ginsenoside Rg3 [Korean Patent Application No. 2005-53631, SK Chemicals], the inventors of the present inventions have concluded that it is worthwhile examining inhibitory activity against angiogenesis and anti-cancer activity of 20(S)-ginsenoside Rg3 and a composition comprising the same. Based on the information as such, the present inventors have conducted the experiments described herein below, and as a result, they have confirmed that 20(S)-ginsenoside Rg3 exhibits

stronger inhibitory activity on VEGF than 20(R)-ginsenoside Rg3, whose antagonism on VEGF was already reported. In particular, the present inventors have found that a composition comprising 20(S)-ginsenoside Rg3 at a concentration of from 40 to 60 %(w/w) shows better inhibitory activity
5 against angiogenesis than that of a single administration of 20(S)-ginsenoside Rg3 or 20(R)-ginsenoside Rg3.

SUMMARY OF THE INVENTION

10 Accordingly, in an embodiment of the present invention, there is provided a composition comprising 20(S)-ginsenoside Rg3 at a concentration of from 40 to 60 %(w/w) which shows excellent inhibitory effect against angiogenesis, and is thus capable of preventing or treating cancer by continued administration of the same.

BRIEF DESCRIPTION OF THE DRAWINGS

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

accompanying drawings, in which:

Fig. 1 is a high pressure liquid chromatogram of the composition comprising 20(S)-ginsenoside Rg3 at a concentration of 50 % (w/w);

Fig. 2 is a graph showing inhibitory effect of the composition comprising 20(S)-ginsenoside Rg3 on *in vivo* activity of vascular endothelial growth factor (VEGF);

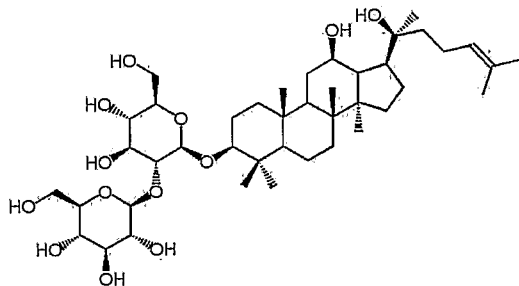
Fig. 3 is a graph showing inhibitory effect of the composition comprising 20(S)-ginsenoside Rg3 at a concentration of 50 % (w/w) on *in vivo* angiogenesis of non-small cell lung cancer; and

Fig. 4 is a photograph taken on the last day of *in vivo* tumor growth inhibition experiment of the composition comprising 20(S)-ginsenoside Rg3 at a concentration of 50 % (w/w) on non-small cell lung cancer.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with one aspect of the present invention, there is provided a composition for the prevention and treatment of cancer which comprises 20(S)-ginsenoside Rg3 represented by Formula 1 at a concentration of from 40 to 60 % (w/w).

[Formula 1]



Ginseng used as a source material for extraction in the present invention may include *Panax* plants, for example, *Panax ginseng*, *Panax notoginseng*, *Panax quinquefolium*, and *Panax japonicus*, or tissue culture products thereof.

The composition comprising 20(S)-ginsenoside Rg3, more particularly 20(S)-ginsenoside Rg3 at a concentration of from 40 to 60 %(w/w) as an effective ingredient, can be effectively used for the prevention or treatment of cancer through its excellent inhibitory effect against angiogenesis.

The typical daily dose of the composition of the present invention may range from 1 to 1000 mg/kg of body weight. However, it should be understood that the amount of the effective ingredient actually administered ought to be determined in light of various relevant factors including the absorption, inactivation and excretion rates of the effective ingredient, the chosen route of administration, the age, sex and body weight of the individual

patient, and the severity of the patient's symptom. Therefore, the above dose should not be construed as a limitation to the scope of the invention in any way.

The composition of the present invention may be prepared into health foods or pharmaceuticals and can be administered in a single dose or in a divided dose according to the dose as described above.

The composition of the present invention may be prepared into a drink, tablet, pill or capsule according to a conventional procedure in the art and can be administered orally. Alternatively, the composition of the present invention can be administered in the form of an injection.

Pharmaceutical preparations may be manufactured using the composition of the present invention according to the conventional procedures in the art. In doing so, it is preferable to mix, dilute or encapsulate the composition of the present invention with a suitable carrier, an excipient and a diluent in the form of a capsule, a sachet or other forms of a container. Thus, the preparations may be manufactured in the form of tablets, pills, powders, sachets, elixirs, suspensions, emulsions, solutions, syrups, aerosols, soft and hard gelatin capsules, sterile injectable solutions, sterile packaged powders and the like. In particular, it is more preferable to have the composition formulated into an agent for oral administration.

Further, in order to prevent the pharmaceutical preparation from being degraded by gastric acid when administered orally, it may be referable to administer it along with an antacid. In addition, a solid form of preparation for oral administration such as a tablet may be protected via enteric coating.

5 Meanwhile, the use of the composition of the present invention is not limited to medical products being approved as pharmaceuticals, and should be understood as a comprehensive concept including conventional functional foods or health supporting foods.

10 When the composition comprising 20(S)-ginsenoside Rg3 at a concentration of from 40 to 60 %(w/w) as an effective ingredient prepared as such is administered, it exhibits excellent therapeutic effect against tumor growth and angiogenesis without any known side effects, and is therefore expected to be safely used for the prevention and treatment of cancer.

15 Embodiments of the present invention will now be described in more detail with reference to the following examples. However, the examples are given for the purpose of illustration and are not to be constructed as limiting the scope of the invention.

Example 1: Isolation of panaxadiol and panaxatriol saponin fractions

The root hair of ginseng in the amount of 1 kg was extracted twice with 7 L of 70% methanol at 80°C for 5 hours and concentrated, and the extract was suspended in water to adjust its solid content to 25%. The suspension was
5 processed by adding 50 g of sodium hydroxide followed by extracting four times with 1.2 L of water-saturated butanol, thereby obtaining 39.6 g of panaxatriol saponin. The water phase obtained after the extraction was neutralized by adding 180 mL of 18% hydrochloric acid while stirring and extracted three times with 1.4 L of water-saturated butanol, thereby obtaining
10 46.3 g of panaxadio saponin.

Example 2: Preparation of panaxadiol saponin fraction form an acid degradation product

After the panaxadiol saponin containing ginsenoside Ra, Rb1, Rb2, Rc,
15 Rd and the like obtained in Example 1 was completely dissolved in 2.4 L of water, 23 g of lactic acid was added thereto at 70°C for 36 hours and reacted while stirring. The reaction mixture was neutralized by adding 20 g of sodium hydroxide, extracted twice with 1.4 L of water-saturated butanol and concentrated under reduced pressure, thereby obtaining 27 g of panaxadiol
20 saponin of the acid-hydrolyzed product.

Example 3: Preparation of 20(R)-ginsenoside Rg3

20 g of the panaxadiol saponin of the acid-hydrolyzed product obtained in Example 2 was mixed with 380 mL of methanol or ethanol and reacted under reflux at 80°C for 2 hours, thereby obtaining 4 g of 20(R)-ginsenoside Rg3 as a white powder of an insoluble material. 20(R)-ginsenoside Rg3 thus obtained showed the same ¹³C-NMR spectrum and mass spectrum data as previously reported in the literature [*Pharmacology* Vol. 35(5), p432, 1991].

Example 4: Preparation of 20(S)-ginsenoside Rg3 and fractions containing variable amounts of 20(S)-ginsenoside Rg3

The filtrate remained after the recovery of 20(R)-ginsenoside in Example 3 was concentrated under reduced pressure to obtain 15 g of panaxadiol saponin of the acid degradation product. The acid degradation product was passed through silica gel column chromatography using a mixture of chloroform/methanol/water (14:2:0.1~12:3:0.3) as an effluent with varying concentration gradient, thereby obtaining a 20(S)-ginsenoside Rg3 fraction having a desired ratio. As a representative drawing for each composition, the composition comprising 50% of 20(S)-ginsenoside Rg3 is shown in Fig. 1.

The fraction comprising 20(S)-ginsenoside Rg3 at a concentration of greater than or equal to 80 %(w/w) was purified with high pressure liquid chromatography using semi-fractionating column (250×20 mm, YMC-ODS H80, s-4 µm/ effluent = 43% methyl chloride), to obtain 500 mg of pure 20(S)-ginsenoside Rg3. 20(S)-ginsenoside Rg3 thus obtained shows the same ¹³C-NMR spectrum and mass spectrum data as previously reported in the literature [Pharmacology Vol. 35(5), p432, 1991], similar to 20(R)-ginsenoside Rg3 obtained in Example 3.

Experimental Example 1: Inhibitory action of the compositions comprising variable concentrations of 20(S)-ginsenoside Rg3 on *in vitro* VEGFR-1 activity

In order to select a composition comprising 20(S)-ginsenoside Rg3 which shows excellent inhibitory activity against angiogenesis, the compositions comprising of 20(S)-ginsenoside Rg3 at various concentrations were prepared by using column chromatography as described in Example 4.

For comparing inhibitory activities of the compositions comprising 20(S)-ginsenoside Rg3 at various concentrations prepared above on VEGFR-1 which plays an important role in angiogenesis, the following experiment was conducted. In this experiment, insect cell sf21 being engineered to express

human recombinant VEGFR-1 was employed. Each of the compositions, comprising 0, 20, 40, 50, 60, 80 and 100 %(w/w) of 20(S)-ginsenoside Rg3, respectively, was dissolved in dimethyl sulfoxide at a concentration of 5 $\mu\text{g/mL}$ and preincubated in pH 7.4 of HEPES buffer together with 0.2 $\mu\text{g/mL}$ of enzyme in a 37°C incubator for 15 minutes. 0.2 mg/mL of poly(Glu:Tyr), 10 μM of ATP and 0.25 μCi [$\gamma^{32}\text{P}$]ATP were then added to the culture solution and reacted for 30 minutes. After 30 minutes, the reaction was stopped by adding 3% of H_3PO_4 to the reaction mixture. After the reaction product was removed, inhibition rate of each composition on VEGFR-1 activity was determined by measuring the amount of [$\gamma^{32}\text{P}$]poly(Glu:Tyr) formed during the reaction.

The inhibition rate (%) of the composition on VEGFR-1 activity was represented as the ratio of the amount of [^{32}P]poly(Glu:Tyr) formed in each composition to 1% dimethylsulfoxide as a negative control [see Table 1] (each value was obtained by averaging the values of two wells).

[Table 1]

Inhibition rate (%) on VEGF-1 activity at a concentration of 5 $\mu\text{g/mL}$

Concentration of 20(S)-ginsenoside Rg3 (%)	0 % (w/w)	20 % (w/w)	40 % (w/w)	50% (w/w)	60 % (w/w)	80 % (w/w)	100% (w/w)
VEGFR-1	7	5	18	22	19.8	8	9

As a result, it was confirmed that the composition comprising 20(S)-ginsenoside Rg3 at a concentration of from 40 to 60%(w/w) exhibits excellent inhibitory effect on VEGF-1 activity, and in particular, the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 shows strongest inhibition effect (22%).

Experimental Example 2: Inhibitory action of the composition comprising 20(S)-ginsenoside Rg3 on *in vivo* VEGF activity

It was confirmed in Experimental Example 1 that the composition comprising 20(S)-ginsenoside Rg3 inhibits the activity of VEGFR-1. Further, the present inventors carried out the following experiment to compare *in vivo* inhibitory activities of the compositions comprising 20(S)-ginsenoside at various concentrations Rg3 on VEGF with that of 20(R)-ginsenoside Rg3, which is a stereoisomer of 20(S)-ginsenoside Rg3. Mouse high metastatic skin cancer cells, B16F10 melanoma, and specific pathogen-free (SPF) female C57BL/6 mouse were employed in this experiment. As an experimental group, drug samples were prepared by formulating each composition at a dose of 10 mg/kg using 0.5 % carboxy methyl cellulose as an excipient.

B16F10 melanoma cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and separated by using trypsin-EDTA. The culture solution was then diluted with PBS, and 0.2 mL of each diluent was subcutaneously injected into the scapular region of SPF female C57BL/6 mouse at a concentration of 5×10^5 cells/mouse. After cell transplantation to 10 mice per group, the drug sample was orally administered to the mouse for 7 days at a dose of 10 mL/kg, and toxicity of the drug sample was determined by monitoring changes in body weight. After the administration was completed, tumors were cut off to have the weight of 20 to 30 mg, dissolved in PBS. In order to examine the inhibitory activity on VEGF, ELISA (Enzyme-Linked ImmunoSorbent Assay) was carried out. First, 100 μ L of each tumor sample was added to each well of a 96-well plate of a ELISA kit and reacted at room temperature for 2.5 hours. The plate was washed and reacted with 100 μ L of a biotinylated antibody for 1 hour. After the plate was washed again, 100 μ L of HRP-Streptavidin was added to each well and reacted for 45 minutes. The plate was then washed and reacted with a reaction solution for 10 minutes. After the reaction was stopped by adding a stop solution, an absorbance of each well was measured at 450 nm to determine *in vivo* level of VEGF.

Inhibition rate (%) of the drug sample on VEGF activity was represented as the ratio of VEGF activity measured in each sample to that of the negative control to which 0.5% carboxy methyl cellulose was administered [see Table 2 and Fig. 2] (each value was obtained by averaging the values of 10 mice).

[Table 2]

Inhibition rate (%) on VEGF activity of B16F10 melanoma cells at a concentration of 10 mg/kg

Concentration of 20(S)-ginsenoside Rg3 (%)	0 %(w/w)	50 %(w/w)	100 %(w/w)	20(R)-Rg3
B16F10	-3.4	41	26	10
- 100 %(w/w): 20(S)-ginsenoside Rg3				
- 20(R)-Rg3 : 20(R)-ginsenoside Rg3				

As described in Table 2, it has been found that 20(S)-ginsenoside Rg3 exhibits about 2.6-fold more powerful inhibitory activity than its stereoisomer 20(R)-ginsenoside Rg3, and the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 shows most powerful inhibitory activity by 41%.

Experimental Example 3: Inhibitory action of the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 on *in vivo* angiogenesis of melanoma cell lines

In order to compare inhibitory activities of the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3, which shows most powerful inhibitory activity on VEGFR-1 and VEGF in Experimental Examples 1 and 2, and its major ingredient 20(S)-ginsenoside Rg3 alone on *in vivo* angiogenesis, the following experiment was conducted. B16F10 melanoma cells and specific pathogen-free (SPF) female C57BL/6 mouse were employed in this experiment. As an experimental group, drug samples were prepared by formulating 20(S)-ginsenoside Rg3 and the composition comprising the same at a concentration of 50 %(w/w) at a dose of 10 mg/kg using 0.5 % carboxy methyl cellulose as an excipient, respectively, and a negative control was administered with the excipient alone.

B16F10 melanoma cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and separated by using trypsin-EDTA. The culture solution was then diluted with PBS, and 0.2 mL of each diluent was subcutaneously injected into the scapular region of SPF female C57BL/6 mouse at a concentration of 5×10^5 cells/mouse. Each group consisted of 8 mice. After cell transplantation, the drug sample was orally administered to the mouse once a day for 6 days at a dose of 10 mL/kg. During the experiment, body weight was measured everyday, and toxicity of the drug sample was determined by monitoring changes in body weight. After the

administration was completed, the skin tissue was isolated from the mouse and taken a picture with a digital camera. Subsequently, the number of blood vessels formed around a carcinoma was calculated with the naked eye. Statistical significance of the experiment was examined by Student's t-TEST.

5 When the experiment was carried out according to the method as described above, inhibition rate (%) of the drug samples on angiogenesis was represented as the ratio of the number of blood vessels observed in each experimental group to that of the negative control to which 0.5% carboxy methyl cellulose was administered, and the results are shown in Table 3 (each
10 value was obtained by averaging the number of blood vessels observed in 8 mice).

[Table 3]

Inhibition rate (%) on angiogenesis of B16F10 melanoma cells at a concentration of 10 mg/kg

	20(S)-ginsenoside Rg3	Composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3
B16F10	23.6***	37.1***
(***) p<0.001)		

15

As a result, both 20(S)-ginsenoside Rg3 alone and the composition comprising the same at a concentration of 50 %(w/w) showed excellent

inhibitory activity. In particular, the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 exhibited superior inhibitory activity against angiogenesis to 20(S)-ginsenoside Rg3 alone. These results demonstrated that antagonism of VEGF is a major mechanism responsible for the inhibitory activity of the composition comprising 20(S)-ginsenoside Rg3 on angiogenesis as confirmed in Experimental Examples 1 and 2.

Experimental Example 4: Inhibitory action of the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 on *in vivo* angiogenesis of non-small cell lung cancer

In order to compare *in vivo* angiogenesis inhibitory activities of (S)-ginsenoside Rg3 and the composition comprising the same at a concentration of 50 %(w/w), whose inhibitory activity against angiogenesis of B16F10 melanoma were confirmed in Experimental Example 3, on human-derived cell lines, the following experiment was conducted. Human-derived non-small cell lung cancer cell line NCI-H460 and specific pathogen-free (SPF) female BALB/c nude mouse were employed in this experiment. As an experimental group, drug samples were prepared by formulating 20(S)-ginsenoside Rg3 with 0.5% carboxy methyl cellulose as an excipient at a maximum dose of 10 mg/kg and diluting the maximum dose sample up to 5 mg/kg with a

common ratio of 2, thereby obtaining the lower dose samples. The composition comprising 50 % (w/w) of 20(S)-ginsenoside Rg3 was prepared to be its maximum dose to 40 mg/kg, and the lower dose was prepared by diluting the maximum dose sample up to 5 mg/kg with a common ratio of 2.

5 A negative control was administered with the excipient alone.

NCI-H460 cells were cultured in DMEM supplemented with 10 % fetal bovine serum (FBS) and separated by using trypsin-EDTA. The culture solution was then diluted with PBS, and 0.3 mL of each diluent was subcutaneously injected into the scapular region of SPF female BALB/c nude
10 mouse at a concentration of 9×10^6 cells/mouse. Each group consisted of 6 mice. After cell transplantation, the drug sample was orally administered to the mouse once a day for 14 days at a dose of 10 mL/kg, and toxicity of the drug sample was determined by monitoring changes in body weight. After the drug administration was completed, tumors were separated and fixed
15 with 10% neutral formalin, to prepare a tissue block. Thus prepared tissue block was sliced with a ultramicrotome in a thickness of 5 μm , thereby obtaining a tissue section. The tissue section was subjected to immunostaining for CD31, to prepare a stained tissue section. After 5 spots were selected on the stained tissue section by means of an optical microscope,
20 the number of blood vessels observed by the optical microscope were counted

and taken as an average. Statistical significance of the experiment was examined by Student's t-TEST.

Inhibition rate (%) of the drug sample on angiogenesis was represented as the ratio of the number of blood vessels observed in each experimental group administered with 20-(S)-ginsenoside Rg3 alone and the composition comprising 50%(w/w) of the same at various concentrations to that of the negative control to which 0.5% carboxy methyl cellulose was administered, and the results are shown in Table 4 and Fig. 3 (each value was obtained by averaging the number of blood vessels observed in 6 mice).

[Table 4]

Inhibition rate (%) on angiogenesis of NCI-H460 non-small cell lung cancer cell line at various concentrations

	20(S)-ginsenoside Rg3	Composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3
5 mg/kg	23.8***	18.4***
10 mg/kg	24.8***	30.3***
20 mg/kg	-	35.9***
40 mg/kg	-	38.9***
(*** p<0.001)		

As a result, it has been found that the composition comprising 20(S)-ginsenoside Rg3 at a concentration of 50 %(w/w) shows inhibitory activity

against angiogenesis in human-derived non-small cell lung cancer cell line NCI-H460 at a dose of 10 mg/kg equal to or greater than the single administration of 20(S)-ginsenoside Rg3.

5 **Experimental Example 5: Inhibitory action of the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 on *in vivo* tumor growth of non-small cell lung cancer**

10 In order to examine *in vivo* tumor growth inhibitory effect of the composition comprising 50%(w/w) of 20(S)-ginsenoside Rg3 at a concentration of 5 and 10 mg/kg, respectively, which showed concentration dependent inhibitory effect in Experimental Example 4, the following experiment was conducted.

15 Human-derived non-small cell lung cancer cell line NCI-H460 and specific pathogen-free (SPF) female BALB/c nude mice were employed in this experiment, and doxifluridine (40 mg/kg) was used as a positive control. As an experimental group, drug samples were prepared by formulating 20-(S)-ginsenoside Rg3 at a concentration of 5 mg/kg using 0.5 % carboxy methyl cellulose as an excipient. Further, the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 was prepared to be its maximum dose to 10 mg/kg, 20 and the lower dose samples were prepared by diluting the maximum dose

sample up to 5 mg/kg with a common ratio of 2. Mice for negative control were administered with the excipient alone.

NCI-H460 cells were cultured in DMEM supplemented with 10% fetal bovine serum (FBS) and separated by using trypsin-EDTA. The culture solution was then diluted with PBS, and 0.3 mL of each diluent was subcutaneously injected into the scapular region of SPF female BALB/c nude mouse at a concentration of 9×10^6 cells/mouse. Each group consisted of 6 mice. After cell transplantation, the drug sample was orally administered to the mouse once a day for 2 weeks at a dose of 10 mL/kg.

Tumor volume (TV) was calculated with a calipers three times per week according to the following formula:

$$TV = \text{the major axis (mm)} \times \text{the minor axis (mm)}^2 \times 0.5$$

After the administration was completed, the mouse was sacrificed by blood-letting, tumors were separated and measured. The body weight was measured every day during the experiment, and toxicity of the drug sample was determined by monitoring changes in body weight. Statistical significance of the experiment was examined by Student's t-TEST.

Inhibition rate (%) of the drug sample on tumor growth was represented as the ratio of the tumor volume measured in each experimental group to that of the negative control to which 0.5% carboxy methyl cellulose

was administered, and the results are shown in Table 5 and Fig. 5 (each value was obtained by averaging the number of blood vessels observed in 6 mice).

[Table 5]

Inhibition rate (%) on tumor growth of NCI-H460 non-small cell lung cancer

5 cell line

	Composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3	doxifluridine
5 mg/kg	21.0*	-
10 mg/kg	39.2**	-
40 mg/kg	-	18.3
(* p<0.05, ** p<0.01)		

As a result, it was found that the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 shows inhibitory activity on tumor growth of human-derived non-small cell lung cancer cell line NCI-H460 equal to or
 10 greater than that of the positive control doxifluridine, and in particular, it showed a 2-fold or higher inhibitory activity against tumor growth at a concentration of 10 mg/kg. There was observed no toxicity related to the administration of the drug sample.

15 Based on the above results obtained from Experimental Examples, it was confirmed that 20(S)-ginsenoside Rg3 and the composition comprising

the same exhibited excellent inhibitory effect on angiogenesis and tumor growth through VEGF relating antagonism.

Experimental Example 6: Toxicity test of the composition comprising

5 50 %(w/w) of 20(S)-ginsenoside Rg3 on SD rats

In order to examine toxicity of the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 50 %(w/w), the following experiment was performed. SD (Sprague-Dawley) type specific pathogen-free rats were used in this toxicity test, and each group was composed of 5 female and male rats.

10 The composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 was prepared by using 0.5% carboxy methyl cellulose as an excipient, and its maximum dose was determined to be 200 mg/kg based on the result of a preliminary experiment. The lower dose samples were prepared by diluting the maximum dose sample up to 1000 mg/kg with a common ratio of 2, and

15 thus prepared drug samples were orally administered once a day to rats with a dose of 20 mg/kg per rat. All animals were monitored whether any abnormalities are observed immediately after the injection and hour after hour until 6 hours after that at the administration day. From the following day on, the presence of abnormalities was observed once or more every other

20 day for 14 days. Body weight was measured at before the administration, 1st,

3rd, 7th and 14th day after the administration. 14 days after the administration, all animals were anesthetized with ether, cut the abdomen open, sacrificed by blood-letting according to arteriotomy, and the abnormalities of all organs were observed with the naked eye. If the animal died one day after the
5 administration, the tissue obtained from the telescopic abnormal organ was fixed with 10% neutral formalin and subjected to histopathologic test as occasional demands. Statistical significance of the experiment was examined by a one-way analysis of variance using SPSS 10.1.

As a result, all experimental groups of female and male SD rats
10 administered with the composition comprising 50 %(w/w) of 20(S)-ginsenoside_Rg3 survived, and there was observed no striking clinical abnormalities, change in body weight or other toxic effect. From these result, it has been found that a minimal lethal dose (MLD) of the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 is 2000 mg/kg or more.

Preparation Example 1: Preparation of powder and capsule

100 mg of the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 was mixed with 14.8 mg of lactose, 3 mg of crystalline cellulose and 0.2 mg of magnesium stearate. The mixture was filled into a No.5 gelatin
20 capsule by means of a suitable apparatus.

Preparation Example 2: Preparation of ointment

An ointment was prepared by using the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 according to the following formulation of ingredients.

[Formulation of ingredients]

Effective ingredient 5 g, cetyl palmitate 20 g, cetanol 40 g, stearyl alcohol 40 g, myristan isopropyl 80 g, mono stearic acid sorbitan 20 g, polysorbate 60 g, paraoxy propyl benzoate 1 g, paraoxy methyl benzoate 1 g, phosphoric acid, and distilled water with adequate amount

Preparation Example 3: Preparation of injection

An injection was prepared by using the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3 according to the following formulation of ingredients.

[Formulation of ingredients]

Effective ingredient 100 mg, mannitol 180 mg, sodium phosphate dibasic 25 mg, and distilled water for injection 2974 mg

Preparation Example 4: Preparation of health food

Based on a daily dose, 0.3 g of the composition comprising 50 %(w/w) of 20(S)-ginsenoside Rg3, powdered vitamin E, ferrous lactate, zinc oxide, nicotinamide, vitamin A, vitamin B1 and vitamin B2 were mixed.

The formulation of ingredients of the health food is as follows (based on a daily dose for an adult).

Effective ingredient	300 mg
Ginseng extract	100 mg
Green tea extract	100 mg
Vitamin C	100 mg
Powdered vitamin E	120 mg
Ferrous lactate	2 mg
Zinc oxide	2 mg
Nicotinamide	20 mg
Vitamin A	5 mg
Vitamin B1	2 mg
Vitamin B2	2 mg
Corn starch	200 mg
Magnesium stearate	20 mg

INDUSTRIAL APPLICABILITY

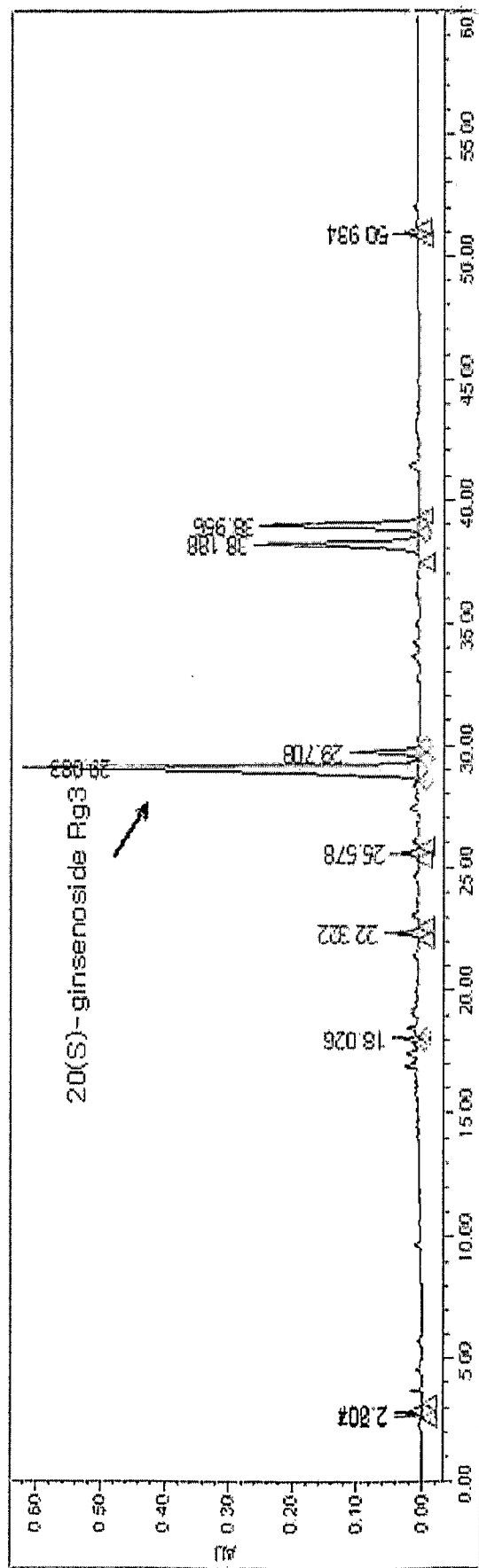
As described above, the composition of the present invention exhibits higher inhibitory effect against angiogenesis and tumor growth than
5 ginsenoside Rg3 alone and is also known to have no side-effects, and thus it is expected to be useful for the prevention and treatment of cancer.

What is claimed is:

1. A composition for the prevention and treatment of cancer by inhibiting angiogenesis which comprises 20-(S)-ginsenoside Rg3 at a
5 concentration of from 40 to 60 % (w/w).
2. In claim 1, said cancer is skin cancer or lung cancer.
3. In claim 1, said composition is formulated into an injectional
10 preparation or a preparation for oral administration.
4. In claim 1, its daily dose for an adult is in the range of from 1 to 1000 mg in total.

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FIG. 1



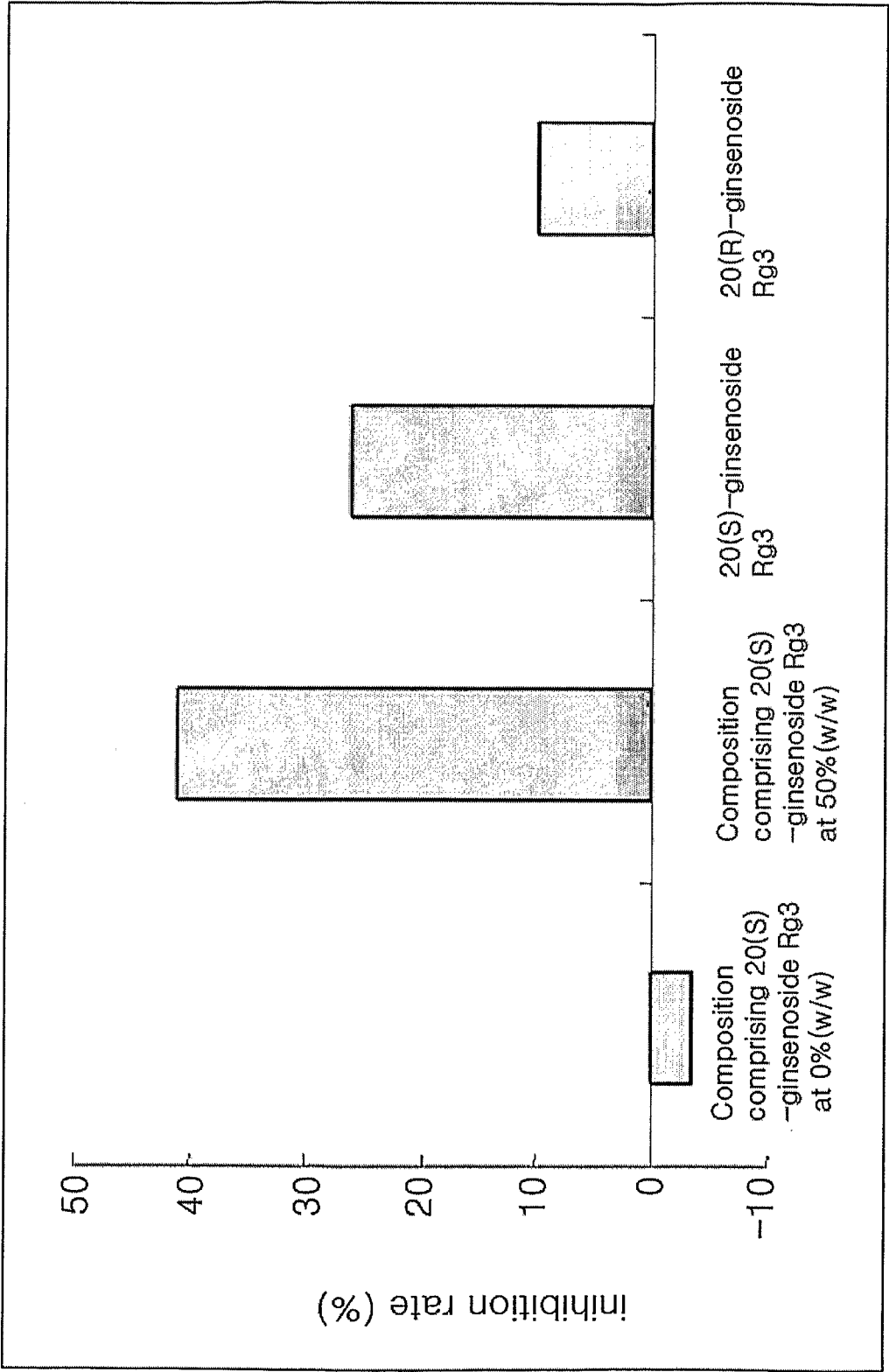
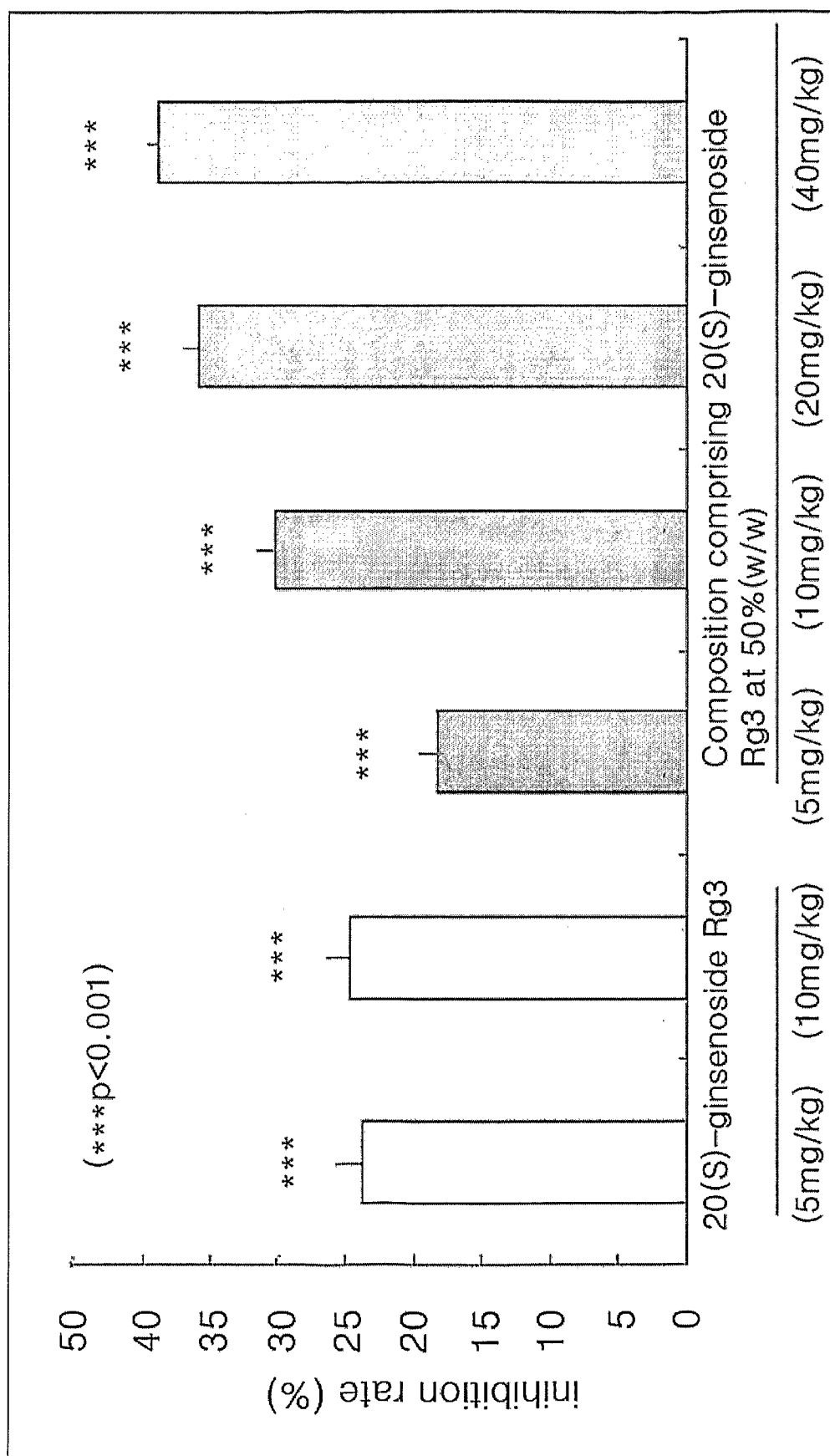


FIG. 2

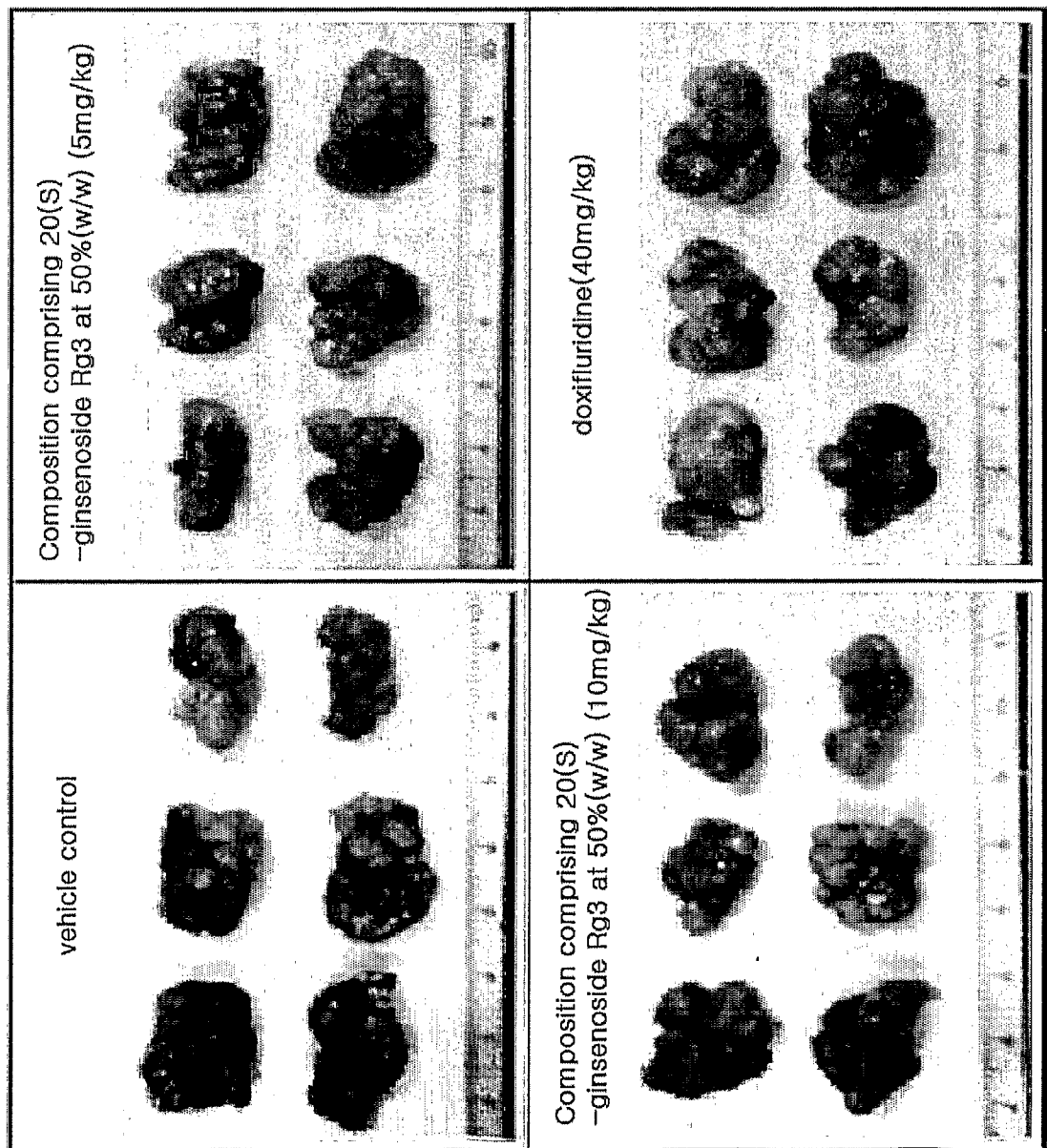
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FIG. 3



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FIG. 4



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 2007/003473

A. CLASSIFICATION OF SUBJECT MATTER

IPC⁸: **A61K 36/258** (2006.01); **A61K 31/704** (2006.01); **A61K 31/56** (2006.01);
A61P 35/00 (2006.01)

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)

IPC⁸: A61K

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

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

EPODOC, WPI, TXTE, PAJ, MedLine

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 1243128 A (BASIC MEDICAL COLLEGE SCIENCE) 2 February 2000 (02.02.2000) <i>abstract (EPODOC)</i>	1

Y	CN 1623553 A (SHANDONG LUYE NATURAL PHARMACE) 8 June 2005 (08.06.2005) <i>abstract (EPODOC)</i>	1

Y	WO 2005/034963 A1 (PANAGIN PHARMACEUTICALS INC) 21 April 2005 (21.04.2005) <i>claims 1, 9</i>	1

☒ 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
17 October 2007 (17.10.2007)Date of mailing of the international search report
30 October 2007 (30.10.2007)Name and mailing address of the ISA/ AT
Austrian Patent Office
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Telephone No. +43 / 1 / 534 24 / 363

Continuation of first sheet**Continuation 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:

Claims Nos.: 2-4 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:

The product can be claimed by its composition, shape, and structure. The characterisation of the medical application in claim 2 refers to an irrelevant and unsearched part of claim 1. A product can not be claimed by its mode of action as in claim 3. The product can not be described by its mode of application by delineating the mode of administration or dose. The product's concentration can be claimed, not the daily needed dose as in claim 4. It is adverted, that the pharmaceutical composition should be described as a clear first medical indication claim and clearly characterised by its novelty.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR 2007/003473

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Mochizuki M, et al. Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2, 20(R)- and 20(S)-ginsenoside Rg3, of red ginseng. Biol Pharm Bull. 1995 Sep; 18(9): 1197-202 <i>abstract</i>	1
A	Shinkai K, et al. Inhibition of in vitro tumor cell invasion by ginsenoside Rg3. Jpn J Cancer Res. 1996 Apr; 87(4): 357-62 <i>abstract</i>	1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR 2007/003473

Patent document cited in search report			Publication date		Patent family member(s)		Publication date	
CN	A	1243128			CN	A	1243128	2000-02-02
CN	A	1623553			CN	A	1623553	2005-06-08
WO	A	2005034963			WO	A1	2005034963	2005-04-21