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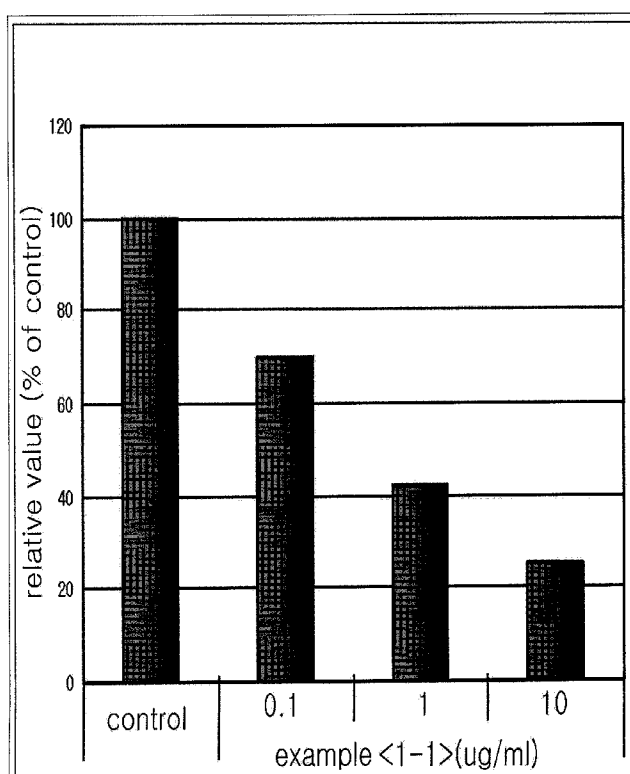
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(54) Title: A COMPOSITION COMPRISING PENTAPHYLLOIDES FRUTICOSA EXTRACT AS AN ACTIVE INGREDIENT

[Fig. 3]



(57) Abstract: The present invention relates to a composition containing *Pentaphylloides fruticosa* extract as an active ingredient. *Pentaphylloides fruticosa* extract of the present invention extracted by using water, alcohol or a mixture thereof as a solvent inhibits the activity of AP-1 (activator protein-1), a MMP-1 transcription factor, inhibits type I collagenase, a skin matrix decomposing enzyme, but increases collagen, a major component of skin, so that it can be effectively used as a preparation for skin external application including cosmetics owing to its wrinkle and elasticity improving effects. In addition, *Pentaphylloides fruticosa* extract of the present invention has radical scavenging effect, so that it can also be effectively used as an antioxidative agent.



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【DESCRIPTION】

【Invention Title】

A COMPOSITION COMPRISING PENTAPHYLLOIDES FRUTICOSA
EXTRACT AS AN ACTIVE INGREDIENT

5

【Technical Field】

The present invention relates to a composition
comprising *Pentaphylloides fruticosa* extract having anti-
skin aging and anti-oxidative activities as an active
10 ingredient.

【Background Art】

Skin aging is clinically divided into natural aging
characterized by feeble and pale skin and delicate wrinkles
15 and reduction of elasticity of skin, and photo aging
characterized by rough wrinkles by sunlight, pigmentation
and telangiectasia (Gilchrest B. A., *J. Am. Acad.
Dermatol.*, 21: 610, 1989; Ma W., et al., *Clin. Exp.
Dermatol.*, 26: 592, 2001; Jenkins G., *Mech. Ageing*
20 *Develop.*, 123: 801, 2002). The changes or decrease of
collagen which is a major component of skin has been known
as a critical reason of clinical changes of skin such as
wrinkles and reduction of elasticity. The changes or
decrease of collagen is observed in both natural aging and
25 photo aging (Lavker R. M., et al., *J. Invest. Dermatol.*,

90: 325, 1988). Collagen deficiency observed during natural aging is allegedly resulted from the decrease of collagen generation itself or the increase of type I collagenase (Matrix Metalloproteinase-1, MMP-1) decomposing collagen. UV irradiation induces type I collagenase biosynthesis in skin and the increased type I collagenase accelerates collagen decomposition in skin and further promotes extracellular matrix (ECM) decomposition (Millis A. J., et al., *Exp. Cell. Res.*, 201: 373, 1992; Chung J. H., et al., *J. Invest. Dermatol.*, 117: 1218, 2001; Fisher G. J., et al., *J. Clin. Invest.*, 106: 663, 2000; Visse R. et al., *Circ. Res.*, 92: 827, 2003; Vincenti M. P., *Crit. Rev. Eukaryot Gene Expr.*, 6: 391, 1996; Scharffetter-Kochanek K., et al., *Exp. Gerontol.*, 35: 307, 2000). So, recovering from collagen deficiency in human skin might be a way to recover from skin aging. Therefore, it is expected to prevent or recover from skin aging symptoms such as wrinkles, reduction of elasticity and skin drooping, by accelerating collagen biosynthesis or reducing MMP-1 biosynthesis.

Every aerobic organism including human survives with energy metabolism using oxygen. When oxygen *in vivo* gets physical, chemical or biological stress, it turns into harmful reactive oxygen species (ROS) such as superoxide

anion radical (O_2^-), H_2O_2 , hydroxy radical ($-OH$), etc. These reactive oxygen species cause critical physiological disorder and if worse, they cause life-threatening disease. Organism has a self defense mechanism to eliminate
5 reactive oxygen species, which is antioxidative mechanism. And lives have been evolved with the development of the antioxidative mechanism. However, generation of reactive oxygen species outmatching the defensive mechanism damage immune-related factors such as protein, DNA, enzyme and T-
10 cell to cause various diseases. The reactive oxygen species attack unsaturated fatty acid, a component of cell membrane, to cause peroxidation. As a result, the accumulated lipid peroxide *in vivo* causes aging and other diseases (Sies H., et. al., *Philos Trans R Soc Lond B Biol*
15 *Sci.* 311:617, 1985; Neuzil J., et. al., *Biochem J.* 293: 601, 1993; Steinberg D, et. al., *N Engl J Med.* 320: 915, 1989).

It was recently acknowledged that aging and adult
20 diseases are caused by reactive oxygen species. Since then, studies to develop antioxidative agents which can regulate reactive oxygen species have been actively going on, for example anti-oxidative enzymes such as superoxide dismutase, peroxidase, catalase, glutathione peroxidase,
25 and other natural low molecular antioxidants such as

tocopherol (vitamin E), ascorbic acid, carotenoid and glutathione, have been major targets of studies (Valko M., et. al., *Int J Biochem Cell Biol.* 39: 44, 2007; Terlecky SR., et. al., *Biochim Biophys Acta.* 1763-1749, 2006). In addition, a synthetic antioxidative agent such as BHA (2,6-di-tert-butyl-4-hydroxyanisole) has been developed and used in the field of medicine and food industry. However, after the report saying mass-administration of a synthetic antioxidative agent could cause cancer in animal tests (Rahimtula AD, et. al., *Br J Cancer.* 45:935, 1982; Botterweck AA, et. al., *Food Chem Toxicol.* 38: 599, 2000), consumers are tend to avoid taking a synthetic antioxidative agent, resulting in the decreasing demand of a synthetic antioxidative agent. Thus, it is required to develop a safer and more effective natural antioxidative agent (Masaki H., et. al., *Biol Pharm Bull.* 18: 162, 1995).

Pentaphylloides fruticosa is a shrub belonging to Rosaceae, which is distributed in Siberia, east of Russia, and far-east of Caucasus middle Asia. It grows on banks of river or lake or even on a rock wall of an alpine region of at least 2000 m above sea-level. Young bud is silvery gold and a mature tree is 20 ~ 150 cm in height and the leaf is thin and long in shape. It blossoms in June - August, the

flower is light yellow, and its separated bark of a stem is grey-brown. The aerial part of this plant contains vitamin C and P, carotin, phenolcarboxylic acids (caffeic acid, sinapic acid, ferulic acid, ellagic acid, cumaric acid), catechins, flavonoids and triterpenoids. Leaves and flowers have been used as a medicine, which have anti-bacterial activity, phlegm discharging activity, hemostatic activity and astringent effect. The plant has been largely used to treat digestive disease, inflammatory disease in the liver, pneumonia, fever, etc. Besides, it has been used to treat diarrhea, mental illness and blood-related disease or as a supplement to treat heart disease, or as an orexigenic agent (folk remedy in Russia).

According to the study results of *Pentaphylloides fruticosa*, it helps to recover from chronic hepatitis and lipid peroxidation in the liver (Kolpakov MA, et. al., *Bull Exp Biol Med.* 131: 470, 2001). Except these results, no other reports have been made on *Pentaphylloides fruticosa*. Examination on the components of the plant has not been made yet. There are no patents on *Pentaphylloides fruticosa* and studies on skin aging using the plant have not been reported yet.

Thus, the present inventors studied and completed this invention by confirming that *Pentaphylloides fruticosa*

has excellent inhibition effect on AP-1 (activator protein-1), reduces type I collagenase biosynthesis, increases collagen biosynthesis, has radical scavenging activity, but has no side effects, so that it can be effectively used as a skin-care agent for preventing and improving skin aging, as an antioxidative pharmaceutical composition or as a health improving functional food.

【Disclosure】

【Technical Problem】

It is an object of the present invention to provide a pharmaceutical composition for anti-oxidation, preventing skin aging and improving wrinkles or a health improving functional food containing *Pentaphylloides fruticosa* extract as an active ingredient.

【Technical Solution】

To achieve the above object, the present invention provides an antioxidative agent containing *Pentaphylloides fruticosa* extract extracted by using water, alcohol or a mixture thereof as a solvent as an active ingredient.

The present invention also provides a preventive and therapeutic agent for a disease caused by the accumulation of reactive oxygen species containing *Pentaphylloides fruticosa* extract as an active ingredient.

The present invention further provides a composition for preventing skin aging and improving wrinkles containing *Pentaphylloides fruticosa* extract as an active ingredient.

5 The present invention also provides a health improving functional food for preventing skin aging and improving wrinkles containing *Pentaphylloides fruticosa* extract as an active ingredient.

10 In addition, the present invention provides a method for preventing and treating a disease caused by the accumulation of reactive oxygen species.

Hereinafter, the present invention is described in detail.

15 *Pentaphylloides fruticosa* extract of the present invention is preferably prepared by the method comprising the following steps, but not always limited thereto.

- 1) extracting dried *Pentaphylloides fruticosa* using an extraction solvent;
- 20 2) filtering the extract of step 1) after cooling; and
- 3) concentrating the filtered extract of step 2) under reduced pressure, followed by drying thereof.

25 In the above method, *Pentaphylloides fruticosa* of step 1) can be purchased or cultivated. Aerial part or

roots of *Pentaphylloides fruticosa* can be used, but aerial part is preferred.

In the above method, the extraction solvent is water, alcohol or a mixture thereof. Herein, the alcohol is preferably C₁ - C₄ lower alcohol and the lower alcohol is exemplified by ethanol or methanol. The preferable amount of the solvent is 5 - 15 times the amount of dried *Pentaphylloides fruticosa*, and more preferably 10 times the amount, but not always limited thereto. The temperature of the extraction is 80 - 120°C and preferably 100°C, but not always limited thereto. The extraction time is preferably 2 - 24 hours and more preferably 3 hours, but not always limited thereto. The extraction is preferably repeated 1 - 5 times and more preferably 3 times, but not always limited thereto.

In this method, the concentration under reduced pressure of step 3) is preferably performed by using rotary vacuum evaporator, but not always limited thereto. The extract is preferably dried by freeze-drying, but not always limited thereto.

The present invention provides an antioxidative agent containing *Pentaphylloides fruticosa* extract as an active ingredient.

The antioxidative agent herein is characteristically

designed to prevent and treat a disease caused by the accumulation of reactive oxygen species, and the disease herein is selected from the group consisting of liver disease, stroke, myocardial infraction, diabetic vascular disorder, hyperlipidemia, acute inflammation, rheumatism and cancer.

The present invention also provides a composition for the prevention of skin aging and the improvement of wrinkles containing *Pentaphylloides fruticosa* extract as an active ingredient.

The prevention of skin aging and the improvement of wrinkles herein are characteristically achieved by the improvement of elasticity.

The present inventors measured antioxidative activity of *Pentaphylloides fruticosa* extract by DPPH (1,1-diphenyl-2-picryl-hydrazyl) method (Leu SJ, et. al., *Biol Pharm Bull.* 29: 740, 2006). DPPH is a comparatively stable free radical and exhibits the maximum absorbance at 517 nm as a radical. But, when it is eliminated, it loses absorbance. Based on this, the antioxidative activity can be measured. As a result, *Pentaphylloides fruticosa* extract of the present invention demonstrated high DPPH radical scavenging activity. Therefore, *Pentaphylloides fruticosa* extract was confirmed to have antioxidative

effect. Active oxygen generated during metabolism is a cause of aging (Muller FL, et. al., *Free Radic Biol Med.* 43: 477, 2007). *Pentaphylloides fruticosa* extract of the present invention has antioxidative activity, so that it
5 can be effectively used as a skin aging inhibitor.

To confirm whether *Pentaphylloides fruticosa* extract could inhibit AP-1 (activator protein-1), which is a major transcription factor of MMP-1, the present inventors measured AP-1 inhibition by reporter gene assay using a
10 luciferase conjugated plasmid. As a result, *Pentaphylloides fruticosa* extract of the present invention was confirmed to inhibit AP-1 dose-dependently, indicating that *Pentaphylloides fruticosa* extract of the present invention has skin aging preventive effect and anti-
15 wrinkle and elasticity improving effect.

The present inventors further investigated if *Pentaphylloides fruticosa* extract could inhibit type I collagenase (MMP-1) biosynthesis mediated by tumor necrosis factor α (TNF α) or UV. Human fibroblasts were
20 treated with TNF α or irradiated by UV A, and then treated with *Pentaphylloides fruticosa* extract of the present invention, followed by ELISA to quantify type I collagenase. As a result, *Pentaphylloides fruticosa* extract was confirmed to inhibit type I collagenase
25 biosynthesis dose-dependently. Therefore, it was confirmed

that *Pentaphylloides fruticosa* extract of the present invention has preventive and improving effect on the reduction of skin matrix material, loss of elasticity and wrinkles.

5 The present inventors also investigated if *Pentaphylloides fruticosa* extract could accelerate collagen biosynthesis and inhibit collagen decomposition. Human fibroblasts were treated with TNF α and then treated with *Pentaphylloides fruticosa* extract of the present
10 invention, followed by ELISA to quantify procollagen. As a result, *Pentaphylloides fruticosa* extract of the present invention was confirmed to accelerate collagen biosynthesis dose-dependently and to inhibit collagen decomposition mediated by TNF α . Therefore, it was
15 confirmed that *Pentaphylloides fruticosa* extract of the present invention has improving effect on skin wrinkles and elasticity.

 The composition of the present invention can include,
20 in addition to *Pentaphylloides fruticosa* extract, one or more effective ingredients having the same or similar function to *Pentaphylloides fruticosa* extract.

Pentaphylloides fruticosa extract can be administered orally or parenterally and be used in general forms of
25 pharmaceutical formulation.

Pentaphylloides fruticosa extract of the present invention can be prepared for oral or parenteral administration by mixing with generally used diluents or excipients such as fillers, extenders, binders, wetting agents, disintegrating agents and surfactant. Solid formulations for oral administration are tablets, pills, powders, granules and capsules. These solid formulations are prepared by mixing *Pentaphylloides fruticosa* extract of the present invention with one or more suitable excipients such as starch, calcium carbonate, sucrose or lactose, gelatin, etc. Except for the simple excipients, lubricants, for example magnesium stearate, talc, etc, can be used. Liquid formulations for oral administrations are suspensions, solutions, emulsions and syrups, and the above-mentioned formulations can contain various excipients such as wetting agents, sweeteners, aromatics and preservatives in addition to generally used simple diluents such as water and liquid paraffin. Formulations for parenteral administration are sterilized aqueous solutions, water-insoluble excipients, suspensions, emulsions, lyophilized preparations and suppositories. Water insoluble excipients and suspensions can contain, in addition to the active compound or compounds, propylene glycol, polyethylene glycol, vegetable oil like olive oil, injectable ester like ethylolate, etc. Suppositories can

contain, in addition to the active compound or compounds, witepsol, macrogol, tween 61, cacao butter, laurin butter, glycerogelatin, etc.

The effective dosage of *Pentaphylloides fruticosa* extract of the present invention can be determined according to weight, age, gender, health condition, diet, administration frequency, administration method, excretion and severity of a disease. The dosage is 0.1~100 mg/kg per day, preferably 30~80 mg/kg per day, and more preferably 50~60 mg/kg per day, and administration frequency is preferably 1~6 times a day.

Pentaphylloides fruticosa extract of the present invention can be administered alone or treated together with surgical operation, hormone therapy, chemo-therapy and biological regulators.

The present invention also provides a composition for the prevention of skin aging and the improvement of wrinkles containing *Pentaphylloides fruticosa* extract as an active ingredient as a preparation for skin external application.

The preparation for skin external application containing *Pentaphylloides fruticosa* extract of the present invention can additionally include a supplement generally used in the field of skin science such as fatty

substance, organic solvent, resolvent, concentrate, gelling agent, softener, antioxidant, suspending agent, stabilizer, foaming agent, odorant, surfactant, water, ionic or non-ionic emulsifying agent, filler, sequestering agent, 5 chelating agent, preserving agent, vitamin, blocker, moisturizing agent, essential oil, dye, pigment, hydrophilic or hydrophobic activator, lipid vesicle or other components generally used in a preparation for skin external application. The amount of the above supplement can be 10 determined as generally accepted in the field of skin science.

The present invention also provides a composition for the prevention of skin aging and the improvement of wrinkles containing *Pentaphylloides fruticosa* extract of 15 the present invention as an active ingredient as cosmetics.

The cosmetics containing *Pentaphylloides fruticosa* extract of the present invention as an active ingredient can be formulated as general emulsified cosmetics and 20 solubilized cosmetics. The emulsified cosmetics are exemplified by lotion, cream and essence, and the solubilized cosmetics are exemplified by skin.

The proper formulation for the cosmetics can be exemplified by solution, gel, solid or dough anhydride, 25 emulsion prepared by dispersing oil phase on water phase,

suspension, microemulsion, microcapsule, microgranule, ionic (liposome) or non-ionic vesicle, cream, skin, lotion powder, spray, and conceal stick. In addition, the cosmetics can be formulated as an aerosol composition containing an foam or compressed propellant.

The cosmetics of the present invention can include, in addition to *Pentaphylloides fruticosa* extract of the present invention, a supplement generally used in the field of cosmetics such as fatty substance, organic solvent, resolvent, concentrate, gelling agent, softener, antioxidant, suspending agent, stabilizer, foaming agent, odorant, surfactant, water, ionic or non-ionic emulsifying agent, filler, sequestering agent, chelating agent, preserving agent, vitamin, blocker, moisturizing agent, essential oil, dye, pigment, hydrophilic or hydrophobic activator, lipid vesicle or other components generally used in cosmetics.

The present invention also provides a health improving functional food for the prevention of skin aging and the improvement of wrinkles containing *Pentaphylloides fruticosa* extract as an active ingredient.

The prevention of skin aging and the improvement of wrinkles herein are characteristically achieved by the improvement of elasticity.

Pentaphylloides fruticosa extract of the present invention can be used as a food additive. In that case, *Pentaphylloides fruticosa* extract can be added as it is or as mixed with other food components according to the conventional method. The mixing ratio of active ingredients can be regulated according to the purpose of use (prevention, health enhancement or treatment). In general, to produce health food or beverages, *Pentaphylloides fruticosa* extract is added preferably by up to 15 weight part and more preferably by up to 10 weight part. However, if long term administration is required for health and hygiene or regulating health condition, the content can be lower than the above but higher content can be accepted as well since *Pentaphylloides fruticosa* extract has been proved to be very safe.

The food herein is not limited. For example, *Pentaphylloides fruticosa* extract can be added to meats, sausages, breads, chocolates, candies, snacks, cookies, pizza, ramyuns, flour products, gums, dairy products including ice cream, soups, beverages, tea, drinks, alcohol drinks and vitamin complex, etc, and in wide sense, almost every food applicable in the production of health food can be included.

The composition for health beverages of the present invention can additionally include various flavors or

natural carbohydrates, etc, like other beverages. The natural carbohydrates above can be one of monosaccharides such as glucose and fructose, disaccharides such as maltose and sucrose, polysaccharides such as dextrin and cyclodextrin, and glucose alcohols such as xilytole, sorbitol and erythritol. Besides, natural sweetening agents such as thaumatin and stevia extract, and synthetic sweetening agents such as saccharin and aspartame can be included as a sweetening agent. The content of the natural carbohydrate is preferably 0.01-0.04 g and more preferably 0.02-0.03 g in 100 ml of the composition.

In addition to the ingredients mentioned above, *Pentaphylloides fruticosa* extract of the present invention can include in a variety of nutrients, vitamins, minerals, flavors, coloring agents, pectic acid and its salts, alginic acid and its salts, organic acid, protective colloidal viscosifiers, pH regulators, stabilizers, antiseptics, glycerin, alcohols, carbonators which used to be added to soda, etc. *Pentaphylloides fruticosa* extract of the present invention can also include natural fruit juice, fruit beverages and/or fruit flesh addable to vegetable beverages. All the mentioned ingredients can be added singly or together. The mixing ratio of those ingredients does not matter in fact, but in general, each can be added by 0.01-0.1 weight part per 100 weight part of

Pentaphylloides fruticosa extract of the present invention.

In addition, the present invention provides a method for the prevention and treatment of a disease caused by the
5 excessive accumulation of active oxygen.

The method for the prevention and treatment above includes the step of administering an effective dose of the said *Pentaphylloides fruticosa* extract extracted by using water, ethanol or a mixture thereof as a solvent to a
10 subject with a disease caused by the excessive accumulation of active oxygen.

In this method, the disease caused by the excessive accumulation of active oxygen is selected from the group consisting of liver disease, stroke, myocardial infraction, diabetic vascular disorder, hyperlipidemia, acute
15 inflammation, rheumatism and cancer, but not always limited thereto.

Pentaphylloides fruticosa extract of the present invention can be administered orally or parenterally and be
20 used in general forms of pharmaceutical formulation. The formulation method for *Pentaphylloides fruticosa* extract of the present invention is described hereinbefore.

The effective dosage of *Pentaphylloides fruticosa* extract of the present invention can be determined
25 according to weight, age, gender, health condition, diet,

administration frequency, administration method, excretion and severity of a disease. The dosage is 0.1~100 mg/kg per day, preferably 30~80 mg/kg per day, and more preferably 50~60 mg/kg per day, and administration frequency is preferably 1~6 times a day.

Pentaphylloides fruticosa extract of the present invention can be administered alone or treated together with surgical operation, hormone therapy, chemo-therapy and biological regulators.

【Advantageous Effect】

Pentaphylloides fruticosa extract of the present invention extracted by using water, alcohol or a mixture thereof as a solvent has radical scavenging activity, inhibits the activity of AP-1 (activator protein-1), the MMP-1 transcription factor, inhibits type I collagenase (MMP-1), the skin matrix decomposing enzyme, but increases collagen, one of major components of skin, so that it can be effectively used as a preparation for skin external application including cosmetics owing to its wrinkle and elasticity improvement effects. In addition, *Pentaphylloides fruticosa* extract of the present invention has no side effects, so that it can also be used as a pharmaceutical composition or health improving functional food.

【Description of Drawings】

The application of the preferred embodiments of the present invention is best understood with reference to
5 the accompanying drawings, wherein:

Figure 1 is a graph illustrating the inhibition effect of *Pentaphylloides fruticosa* methanol extract of the present invention on AP-1, the transcription factor.

10 Figure 2 is a graph illustrating the inhibition effect of *Pentaphylloides fruticosa* methanol extract of the present invention on MMP-1 biosynthesis mediated by TNF α .

15 Figure 3 is a graph illustrating the inhibition effect of *Pentaphylloides fruticosa* methanol extract of the present invention on MMP-1 biosynthesis mediated by UV.

Figure 4 is a graph illustrating the accelerating effect of *Pentaphylloides fruticosa* methanol extract of the present invention on collagen biosynthesis.

20 Figure 5 is a graph illustrating the inhibition effect of *Pentaphylloides fruticosa* methanol extract of the present invention on collagen decomposition mediated by TNF α .

25 **【Mode for Invention】**

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled
5 in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1: Preparation of *Pentaphylloides fruticosa*
10 extract

<1-1> Preparation of *Pentaphylloides fruticosa* methanol
extract

100 g of the dried *Pentaphylloides fruticosa* aerial
part (provided by NIOC, Novosibirsk Institute of Organic
15 Chemistry, Russia) was pulverized, which was put in an
extraction sack. The sack was dipped in 10 times the
volume of 100% methanol, followed by reflux-extraction at
100°C for 3 hours. The extraction was repeated three times.
The extract was cooled down to room temperature and
20 filtered with a filter paper. The extract was concentrated
under reduced pressure in a concentrator until the solution
was completely evaporated to give crystalline. As a result,
7.3 g of extract was obtained from 100 g of the dried
Pentaphylloides fruticosa aerial part.

<1-2> Preparation of *Pentaphylloides fruticosa* water extract

Extraction was performed by the same manner as described in Example <1-1> except that water was used instead of methanol as an extraction solvent. As a result,
5 12.3 g of extract was obtained.

<1-3> Preparation of *Pentaphylloides fruticosa* ethanol extract

10 Extraction was performed by the same manner as described in Example <1-1> except that 100% ethanol was used instead of methanol as an extraction solvent. As a result, 7.2 g of extract was obtained.

15 Experimental Example 1: Investigation of radical scavenging activity

The antioxidative activity of *Pentaphylloides fruticosa* extract was measured by DPPH (1,1-diphenyl-2-picryl-hydrazyl) method (Leu SJ, et. al., *Biol Pharm Bull.*
20 29: 740, 2006).

Pentaphylloides fruticosa extract obtained in Example 1 was serial-diluted (1/2, 10 steps) in 70% ethanol from the concentration of 100 µg/ml in a 96-well plate. DPPH (1,1-diphenyl-2-picryl-hydrazyl) prepared in 100 µM
25 ethanol was added thereto to make total volume 200 µl/well.

Reaction was induced at 37°C for 30 minutes, followed by measuring OD₅₂₀ with ELISA reader, which was calculated by the following mathematical formula 1. The control group was not treated with samples. Vitamin C and Trolox which have been known to have radical scavenging effect were used as positive controls. IC₅₀ (inhibitory concentration) was determined by measuring the concentration that could be able to scavenge 50% of DPPH radical.

10 Mathematical Formula 1

DPPH radical scavenging activity (%) = (OD of control - OD of sample)/OD of control X 100

【Table 1】

15 Radical scavenging activity of *Pentaphylloides fruticosa* extract (IC₅₀, unit: µg/ml)

	IC ₅₀ (µg/ml)
Example <1-1>	9.9
Example <1-2>	10.0
Example <1-3>	10.2
Vitamin C	2.6
Trolox	3.1

As a result, as shown in Table 1, *Pentaphylloides fruticosa* extract of the present invention has high radical scavenging activity (see Table 1).

Experimental Example 2: Inhibition effect on AP-1
(activator protein-1)

To investigate the inhibition effect of *Pentaphylloides fruticosa* extract of Example 1 on the major
5 MMP-1 transcription factor AP-1 (activator protein-1),
reporter gene assay was performed using a luciferase
conjugated plasmid. HeLa cells (ATCC, CCL-2) were
inoculated in a 24-well plate, followed by culture in DMEM
supplemented with 10% FBS and 1% penicillin/streptomycin
10 for 24 hours. When the transcription factor was activated,
transfection was performed with AP-1-luciferase reporter
plasmid DNA (Stratagene) designed to report the activation
by using lipofectamin transfection reagent (Invitrogen).
24 hours after the transfection, *Pentaphylloides fruticosa*
15 extract was added to the medium at different concentrations,
which was stimulated by PMA (Phobol ester, Sigma). 24
hours later, the cells were lysed and the luciferase
activity was measured by luminometer to investigate the
inhibition effect. AP-1 inhibition, that is the inhibition
20 of transactivation of AP-1 binding element, was measured.
The measured level in the control group treated with PMA
alone was regarded as 100 for comparison.

【Table 2】

Inhibition effect of *Pentaphylloides fruticosa*

extract on AP-1

	Concentration	AP-1
Example <1-1>	10 $\mu\text{g/ml}$	76 %
Example <1-1>	1 $\mu\text{g/ml}$	53 %
Example <1-1>	0.1 $\mu\text{g/ml}$	30 %
Example <1-2>	10 $\mu\text{g/ml}$	75 %
Example <1-2>	1 $\mu\text{g/ml}$	50 %
Example <1-2>	0.1 $\mu\text{g/ml}$	32 %
Example <1-3>	10 $\mu\text{g/ml}$	74 %
Example <1-3>	1 $\mu\text{g/ml}$	52 %
Example <1-3>	0.1 $\mu\text{g/ml}$	30 %
Control		100 %

As a result, as shown in Table 2, *Pentaphylloides fruticosa* extract of the present invention inhibited AP-1
 5 dose-dependently (see Table 2).

Experimental Example 3: Inhibition effect on TNF α mediated
 type I collagenase (MMP-1) biosynthesis

Human fibroblasts directly separated from neonatal
 10 foreskin were inoculated in a 48-well plate at the
 concentration of 10^4 cells/well, followed by culture. 24
 hours later, the concentration of TNF α (tumor necrosis
 factor α) was adjusted to 20 ng/ml and the medium was
 replaced with fresh ones containing different
 15 concentrations of *Pentaphylloides fruticosa* extract of
 Example 1. On the 2nd day of culture, the supernatant was
 harvested, followed by ELISA (AP biotech RPN2610) to

quantify type I collagenase. The measured level in the control group treated with TNF α alone was regarded as 100 for comparison.

5 **【Table 3】**

Inhibition effect of *Pentaphylloides fruticosa* extract on TNF α mediated type I collagenase biosynthesis

	Concentration	MMP-1 biosynthesis (%)
Example <1-1>	10 μ g/ml	17 %
Example <1-1>	1 μ g/ml	35 %
Example <1-1>	0.1 μ g/ml	58 %
Example <1-2>	10 μ g/ml	16 %
Example <1-2>	1 μ g/ml	32 %
Example <1-2>	0.1 μ g/ml	55 %
Example <1-3>	10 μ g/ml	15 %
Example <1-3>	1 μ g/ml	36 %
Example <1-3>	0.1 μ g/ml	54 %
Control		100 %

10 As a result, as shown in Table 3, *Pentaphylloides fruticosa* extract of the present invention reduced TNF α mediated type I collagenase biosynthesis dose-dependently (see Table 3).

15 Experimental Example 4: Inhibition effect on UV mediated type I collagenase (MMP-1) biosynthesis

Human fibroblasts directly separated from neonatal foreskin were inoculated in a 48-well plate at the

concentration of 10^4 cells/well, followed by culture. 24 hours later, the cells were irradiated with UV A by 15 J/cm². The medium was replaced with fresh ones containing different concentrations of *Pentaphylloides fruticosa* extract of Example 1. On the 2nd day of culture, the supernatant was harvested, followed by ELISA (AP biotech RPN2610) to quantify type I collagenase. The measured level in the control group treated with UV A alone was regarded as 100 for comparison.

【Table 4】

Inhibition effect of *Pentaphylloides fruticosa* extract on UV mediated type I collagenase biosynthesis

	Concentration	MMP-1 biosynthesis (%)
Example <1-1>	10 µg/ml	25 %
Example <1-1>	1 µg/ml	42 %
Example <1-1>	0.1 µg/ml	70 %
Example <1-2>	10 µg/ml	28 %
Example <1-2>	1 µg/ml	45 %
Example <1-2>	0.1 µg/ml	72 %
Example <1-3>	10 µg/ml	26 %
Example <1-3>	1 µg/ml	43 %
Example <1-3>	0.1 µg/ml	70 %
Control		100 %

As a result, as shown in Table 4, *Pentaphylloides fruticosa* extract of the present invention reduced UV A mediated type I collagenase biosynthesis dose-dependently

(see Table 4).

Experimental Example 5: Acceleration of collagen biosynthesis in skin cells

Human fibroblasts directly separated from neonatal foreskin were inoculated in a 48-well plate at the concentration of 10^4 cells/well, followed by culture. 24 hours later, the medium was replaced with fresh ones containing different concentrations of *Pentaphylloides fruticosa* extract of Example 1. On the 2nd day of culture, the supernatant was harvested, followed by ELISA (Takara MK101) to quantify procollagen. The measured level in the non-treated control group was regarded as 100 for comparison.

【Table 5】

Accelerating effect of *Pentaphylloides fruticosa* extract on collagen biosynthesis

	Concentration	Procollagen biosynthesis (%)
Example <1-1>	10 $\mu\text{g/ml}$	212 %
Example <1-1>	1 $\mu\text{g/ml}$	175 %
Example <1-1>	0.1 $\mu\text{g/ml}$	141 %
Example <1-2>	10 $\mu\text{g/ml}$	202 %
Example <1-2>	1 $\mu\text{g/ml}$	168 %
Example <1-2>	0.1 $\mu\text{g/ml}$	135 %
Example <1-3>	10 $\mu\text{g/ml}$	208 %
Example <1-3>	1 $\mu\text{g/ml}$	170 %

Example <1-3>	0.1 μ g/ml	139 %
Control		100 %

As a result, as shown in Table 5, *Pentaphylloides fruticosa* extract of the present invention accelerated collagen biosynthesis dose-dependently (see Table 5).

5

Experimental Example 6: Inhibition of collagen decomposition

Human fibroblasts directly separated from neonatal foreskin were inoculated in a 48-well plate at the concentration of 10^4 cells/well, followed by culture. 24 hours later, the concentration of TNF α (tumor necrosis factor α) was adjusted to 20 ng/ml and the medium was replaced with fresh ones containing different concentrations of *Pentaphylloides fruticosa* extract of Example 1. On the 2nd day of culture, the supernatant was harvested, followed by ELISA (Takara MK101) to quantify procollagen remaining as not-decomposed. The measured level in the control group treated with TNF α alone was regarded as 100 for comparison. The level of the non-treated group was also counted for comparison.

【Table 6】

Inhibition effect of *Pentaphylloides fruticosa*

extract on collagen decomposition

	Concentration	Procollagen biosynthesis (%)
Example <1-1>	10 $\mu\text{g/ml}$	238 %
Example <1-1>	1 $\mu\text{g/ml}$	189 %
Example <1-1>	0.1 $\mu\text{g/ml}$	154 %
Example <1-2>	10 $\mu\text{g/ml}$	188 %
Example <1-2>	1 $\mu\text{g/ml}$	228 %
Example <1-2>	0.1 $\mu\text{g/ml}$	178 %
Example <1-3>	10 $\mu\text{g/ml}$	150 %
Example <1-3>	1 $\mu\text{g/ml}$	225 %
Example <1-3>	0.1 $\mu\text{g/ml}$	188 %
Control		100 %
Non-treated group		200 %

As a result, as shown in Table 6, *Pentaphylloides fruticosa* extract of the present invention inhibited TNF α mediated collagen decomposition dose-dependently (see Table 6).

Experimental Example 7: Acute toxicity test of *Pentaphylloides fruticosa* extract

Acute toxicity test was performed in mice via oral administration as follows to confirm the stability of *Pentaphylloides fruticosa* extract. 6-week old SPF mice were used in the test for acute toxicity. *Pentaphylloides fruticosa* extract of the present invention was dissolved in 0.9% NaCl and orally administered once to 10 mice per group fasted for 17 hours at the concentrations of 2 g/kg

and 5 g/kg. Death, clinical symptoms, and weight changes in mice were observed, hematological tests and biochemical tests of blood were performed, and any abnormal signs in the gastrointestinal organs of chest and abdomen were checked with eyes during autopsy.

The results showed that the extract did not cause any specific clinical symptoms, weight change, or death in mice. No change was observed in hematological tests, biochemical tests of blood, and autopsy.

Pentaphylloides fruticosa extract of the present invention is evaluated to be a safe substance since it does not cause any toxic change in mice up to the level of 5 g/kg and its estimated LD₅₀ value is much greater than 5 g/kg in mice.

The Manufacturing Examples of the composition for the present invention are described hereinafter.

Manufacturing Example 1: Preparation of pharmaceutical formulations

<1-1> Preparation of powders

Extract of Example <1-1>	2 g
Lactose	1 g

Powders were prepared by mixing all the above components, which were filled in airtight packs according

to the conventional method for preparing powders.

<1-2> preparation of tablets

	Extract of Example <1-1>	100 mg
5	Corn starch	100 mg
	Lactose	100 mg
	Magnesium stearate	2 mg

Tablets were prepared by mixing all the above components by the conventional method for preparing tablets.

10

<1-3> Preparation of capsules

	Extract of Example <1-1>	100 mg
	Corn starch	100 mg
	Lactose	100 mg
15	Magnesium stearate	2 mg

Capsules were prepared by mixing all the above components, which were filled in gelatin capsules according to the conventional method for preparing capsules.

20

<1-4> Preparation of pills

	Extract of Example <1-1>	1 g
	Lactose	1.5 g
	Glycerin	1 g
	Xylitol	0.5 g

25

Pills were prepared by mixing all the above

components according to the conventional method for preparing pills. Each pill contained 4 g of the mixture.

<1-5> Preparation of granules

5	Extract of Example <1-1>	150 mg
	Soybean extract	50 mg
	Glucose	200 mg
	Starch	600 mg

All the above components were mixed, to which 100 mg
10 of 30% ethanol was added. The mixture was dried at 60°C
and the prepared granules were filled in packs.

Manufacturing Example 2: Preparation of foods

Foods containing *Pentaphylloides fruticosa* extract of
15 the present invention were prepared as follows.

<2-1> Preparation of flour food

0.5 ~ 5.0 weight% of the extract of Example <1-2> was
added to the flour. Health enhancing foods such as bread,
20 cake, cookies, crackers and noodles were prepared with the
flour mixture according to the conventional method.

<2-2> Preparation of dairy products

5 ~ 10 weight% of the extract of Example <1-2> was
25 added to milk. Health enhancing dairy products such as

butter and ice cream were prepared with the milk mixture according to the conventional method.

<2-3> Preparation of Sun-Sik

5 Brown rice, barley, glutinous rice and Yulmu (Job's tears) were gelatinized according to the conventional method, dried and pulverized to obtain 60-mesh powders.

 Black soybean, black sesame and wild sesame were steamed and dried according to the conventional method and
10 pulverized to obtain 60-mesh powders.

 The extract of Example <1-2> was concentrated under reduced pressure, spray-dried and pulverized to obtain 60-mesh dry powders.

 Sun-Sik was prepared by mixing the dry powders of the
15 grains, seeds and the extract of Example <1-2> according to the below ratio.

 Grains (brown rice: 30 weight%, Yulmu: 15 weight%, barley: 20 weight%),

 Seeds (wild sesame: 7 weight%, black soybean: 8
20 weight%, black sesame: 7 weight%),

 Dry powders of the extract of Example <1-2> (3 weight%),

Ganoderma lucidum (0.5 weight%),

Rehmannia glutinosa (0.5 weight%)

Manufacturing Example 3: Preparation of beverages<3-1> Preparation of health beverages

	Extract of Example <1-2>	1000 mg
	Citric acid	1000 mg
5	Oligosaccharide	100 g
	Maesil (Prunus mume) Extract	2 g
	Taurine	1 g
	Purified water	up to 900 ml

10 The above constituents were mixed according to the conventional method for preparing health beverages. The mixture was heated at 85°C for 1 hour with stirring and then filtered. The filtrate was loaded in 2 liter sterilized containers, which were sealed and sterilized again, stored in a refrigerator until they would be used
15 for the preparation of a composition for health beverages.

The constituents appropriate for favorite beverages were mixed according to the preferred mixing ratio but the composition ratio can be adjusted according to regional and national preferences, etc.

20

<3-2> Preparation of vegetable juice

Health enhancing vegetable juice was prepared by adding 5 g of the extract of Example <1-2> of the present invention to 1,000 ml of tomato or carrot juice according
25 to the conventional method.

<3-3> Preparation of fruit juice

Health enhancing vegetable juice was prepared by adding 1 g of the extract of Example <1-2> of the present invention to 1,000 ml of apple or grape juice according to the conventional method.

Manufacturing Example 4: Preparation of cosmetics

Functional cosmetics containing *Pentaphylloides fruticosa* extract of the present invention as an active ingredient can be prepared. The present inventors prepared functional cosmetics, which are exemplified by such emulsified cosmetics as lotion, cream and essence; and such solubilized cosmetics as skin, etc.

<4-1> Preparation of emulsified cosmetics

Emulsified cosmetics were prepared according to the composition shown in Table 7. The method for the preparation is as follows.

1) heating the mixture comprising the raw materials 1-9 at 65-70°C;

2) adding the raw material 10 to the mixture of step 1);

3) dissolving the mixture comprising the raw materials 11-13 by heating at 65-70°C;

4) slowly adding the mixture of step 2) during performing step 3), followed by emulsification at 8,000 rpm for 2-3 minutes;

5) dissolving the raw material 14 in water and then adding the solution to the mixture of step 4), followed by emulsification for 2 minutes;

6) weighing the raw materials 15-17, which were added to the mixture of step 5), followed by emulsification for 30 seconds; and

7) degassing the mixture of step 6) finished with the emulsification process and then cooling thereof at 25-35°C to give emulsified cosmetics.

【Table 7】

Compositions of emulsified formulations 1, 2 and 3

Composition		Emulsified formulation 1	Emulsified formulation 2	Emulsified formulation 3
1	Stearic acid	0.3	0.3	0.3
2	Stearyl alcohol	0.2	0.2	0.2
3	Glyceryl monostearate	1.2	1.2	1.2
4	Wax	0.4	0.4	0.4
5	Polyoxyethylenesorbitan monolauric acid ester	2.2	2.2	2.2
6	Paraoxybenzoic acid methyl	0.1	0.1	0.1
7	Paraoxybenzoic acid propyl	0.05	0.05	0.05
8	Cetyl ethyl hexanoate	5	5	5
9	Triglyceride	2	2	2
10	Cyclomethicone	3	3	3

11	Distilled water	to 100	to 100	to 100
12	Concentrated glycerin	5	5	5
13	Triethanolamine	0.15	0.15	0.15
14	Polyacrylic acid polymer	0.12	0.12	0.12
15	Pigment	0.0001	0.0001	0.0001
16	Flavor	0.10	0.10	0.10
17	Extract of Example <1-1>	0.0001	1	10

<4-2> Preparation of solubilized cosmetics

Solubilized cosmetics were prepared according to the composition shown in Table 8. The method for the preparation is as follows.

1) adding the raw materials 2-6 to the raw material 1 (purified water), which were dissolved by using Agi-mixer;

2) adding the raw materials 8-11 to the raw material 7 (alcohol) and completely dissolved; and

3) slowly adding the mixture of step 2) to the mixture of step 1), followed by solubilization.

【Table 8】

Compositions of solubilized formulations 1, 2 and 3

Composition		solubilized formulation 1	solubilized formulation 2	solubilized formulation 3
1	Purified water	to 100	to 100	to 100
2	Concentrated glycerin	3	3	3
3	1,3-butyleneglycol	2	2	2

4	EDTA-2Na	0.01	0.01	0.01
5	Pigment	0.0001	0.0002	0.0002
6	Extract of Example <1-1>	0.1	5	5
7	Alcohol (95%)	8	8	8
8	Paraoxybenzoic acid methyl	0.1	0.1	0.1
9	Polyoxyethylene hydrogenated ester	0.3	0.3	0.3
10	Flavor	0.15	0.15	0.15
11	Cyclomethicone	-	-	0.2

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

【CLAIMS】**【Claim 1】**

An antioxidative agent containing *Pentaphylloides fruticosa* extract extracted by using water, alcohol or a mixture thereof as a solvent as an active ingredient.

【Claim 2】

The antioxidative agent according to claim 1, wherein the alcohol is C₁ - C₄ lower alcohol.

【Claim 3】

The antioxidative agent according to claim 2, wherein the lower alcohol is ethanol or methanol.

【Claim 4】

The antioxidative agent according to claim 1, wherein the antioxidative agent has radical scavenging activity.

【Claim 5】

The antioxidative agent according to claim 1, wherein the antioxidative agent inhibits the activity of the transcription factor AP-1 (activator protein-1).

【Claim 6】

A preventive and therapeutic agent for a disease

caused by the excessive accumulation of active oxygen containing *Pentaphylloides fruticosa* extract extracted by using water, alcohol or a mixture thereof as an active ingredient.

5

【Claim 7】

The preventive and therapeutic agent according to claim 6, wherein the disease caused by the excessive accumulation of active oxygen is selected from the group consisting of liver disease, stroke, myocardial infraction,
10 diabetic vascular disorder, hyperlipidemia, acute inflammation, rheumatism and cancer.

【Claim 8】

15 A composition for the prevention of skin aging and the improvement of wrinkles containing *Pentaphylloides fruticosa* extract extracted by using water, alcohol or a mixture thereof as a solvent as an active ingredient.

20 **【Claim 9】**

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein the alcohol is C₁ - C₄ lower alcohol.

25 **【Claim 10】**

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein the lower alcohol is ethanol or methanol.

5 **【Claim 11】**

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein the composition has radical scavenging activity.

10 **【Claim 12】**

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein the composition inhibits the activity of the transcription factor AP-1 (activator protein-1).

15

【Claim 13】

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein the composition inhibits type I collagenase (matrix metalloproteinase-1, MMP-1) biosynthesis.

20

【Claim 14】

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein the composition promotes collagen biosynthesis.

25

【Claim 15】

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein
5 the composition inhibits collagen decomposition.

【Claim 16】

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein
10 the composition is formulated as a preparation for skin external application.

【Claim 17】

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein
15 the composition is provided as cosmetics.

【Claim 18】

The composition for the prevention of skin aging and the improvement of wrinkles according to claim 8, wherein
20 the composition improves skin elasticity.

【Claim 19】

A health improving functional food for the prevention
25 of skin aging and the improvement of wrinkles containing

Pentaphylloides fruticosa extract extracted by using water, ethanol or a mixture thereof as a solvent as an active ingredient.

5 **【Claim 20】**

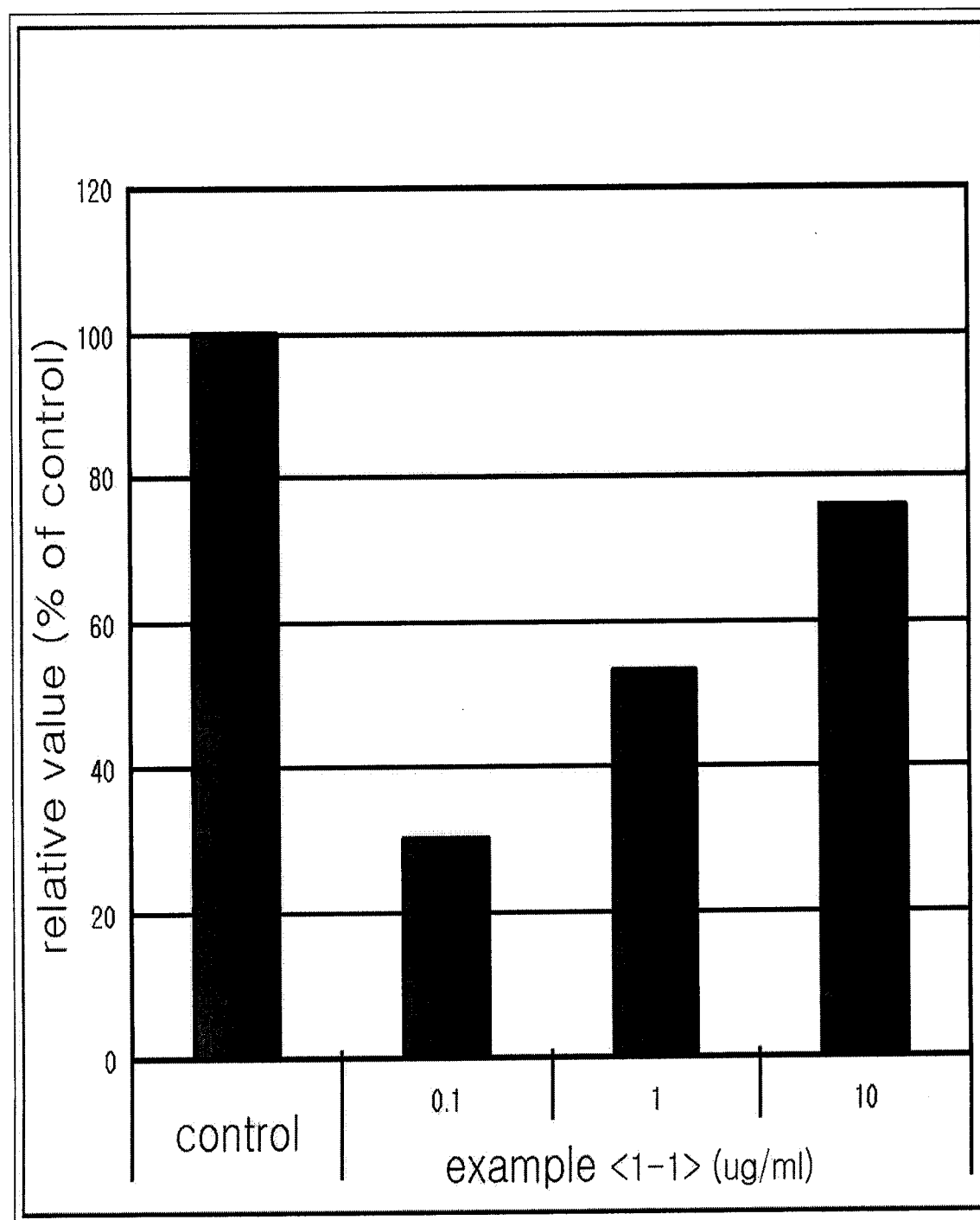
A method for the prevention and treatment of a disease caused by the excessive accumulation of active oxygen containing the step of administering an effective dose of *Pentaphylloides fruticosa* extract extracted by
10 using water, ethanol or a mixture thereof as a solvent to a subject with a disease caused by the excessive accumulation of active oxygen.

【Claim 21】

15 The method for the prevention and treatment of a disease caused by excessive accumulation of active oxygen according to claim 20, wherein the disease caused by the excessive accumulation of active oxygen is selected from the group consisting of liver disease, stroke, myocardial
20 infraction, diabetic vascular disorder, hyperlipidemia, acute inflammation, rheumatism and cancer

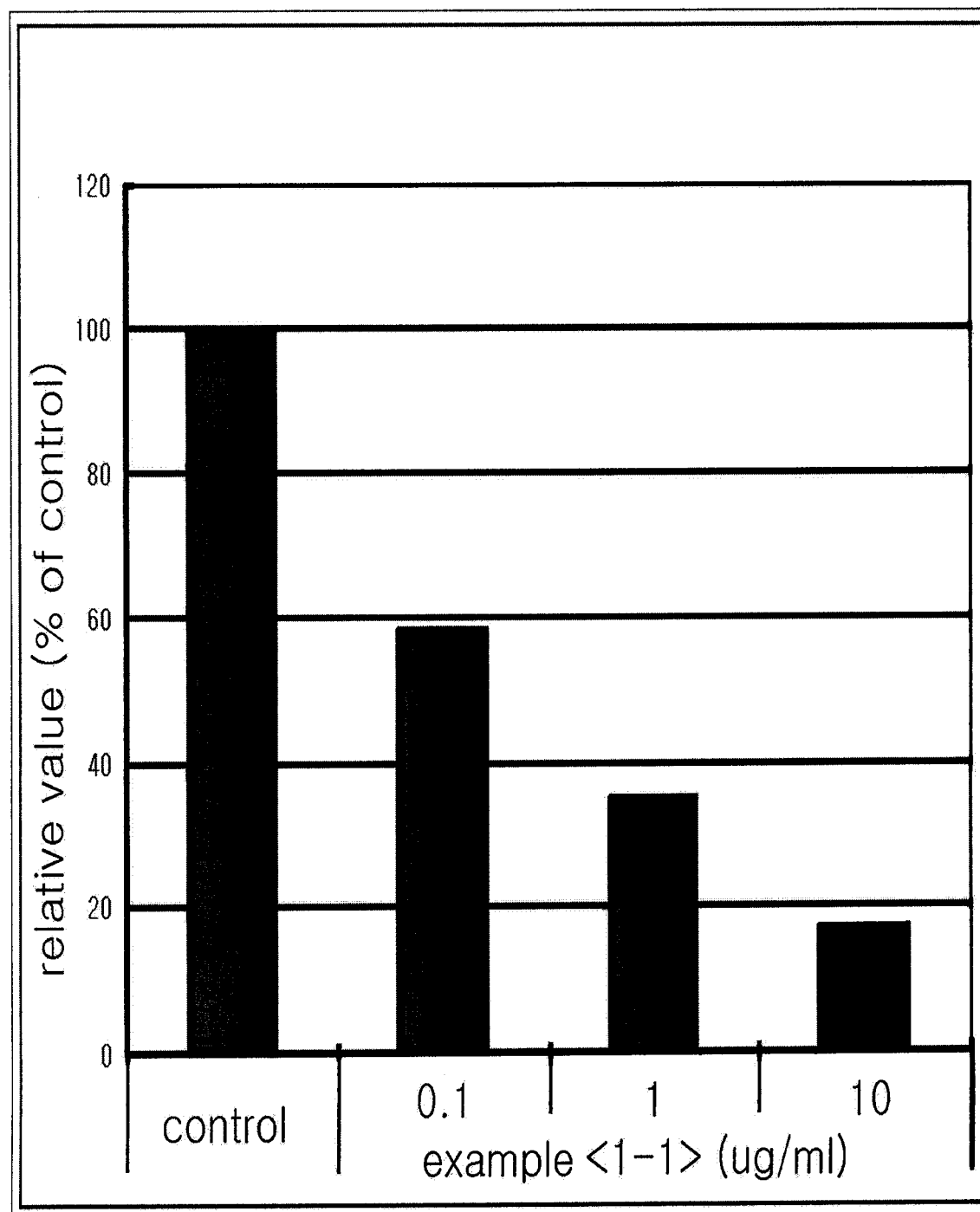
1/5

[Fig. 1]



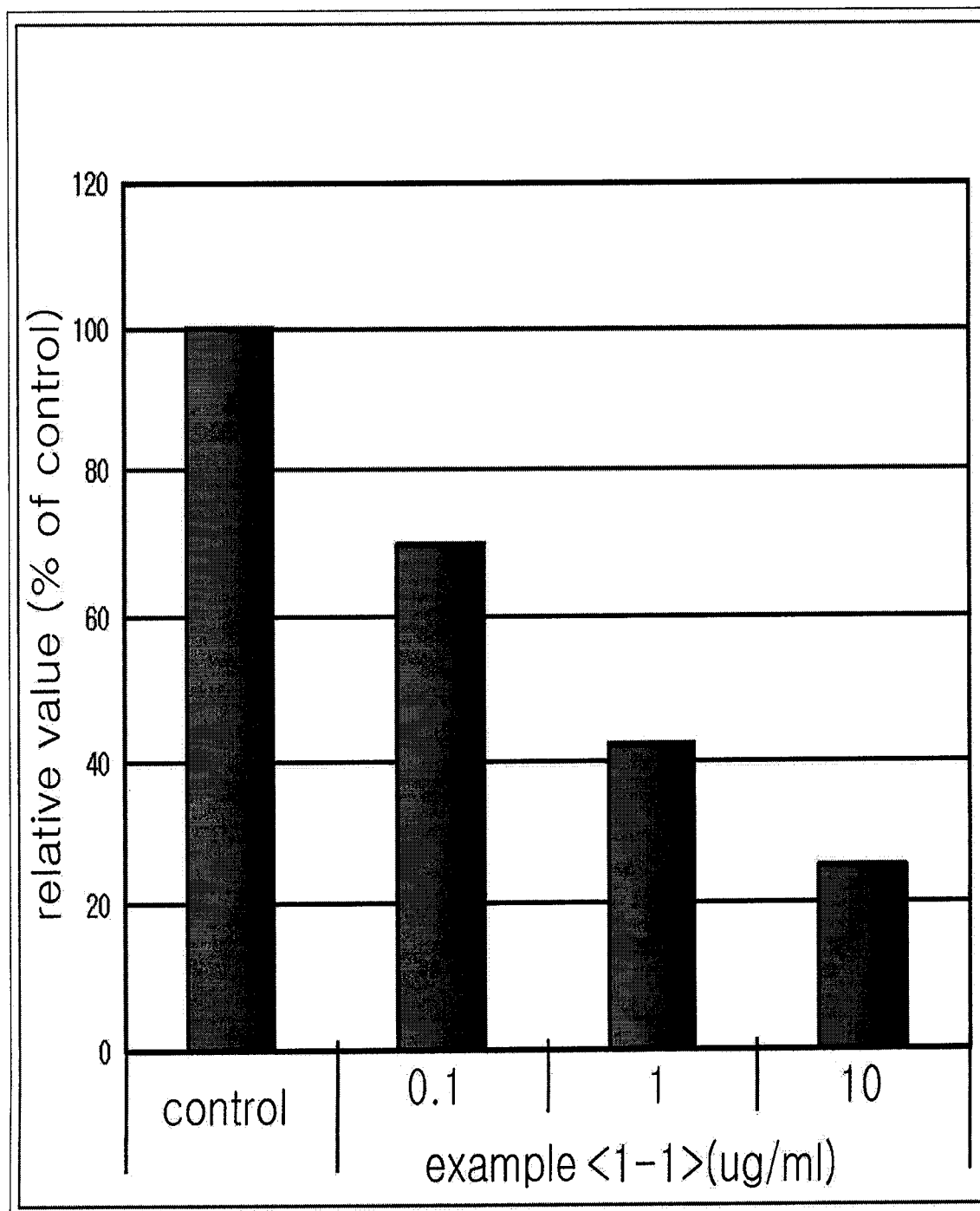
2/5

[Fig. 2]



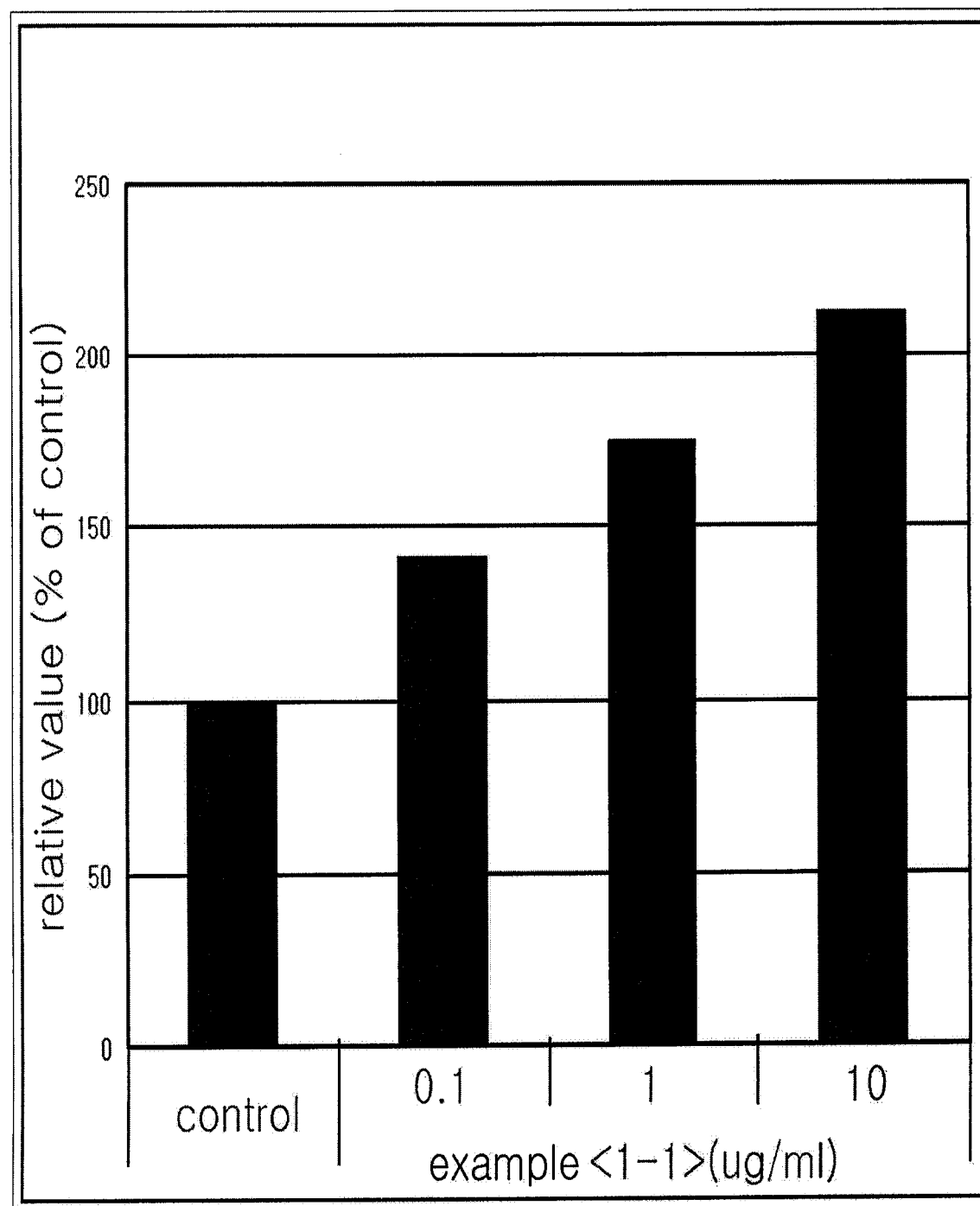
3/5

[Fig. 3]



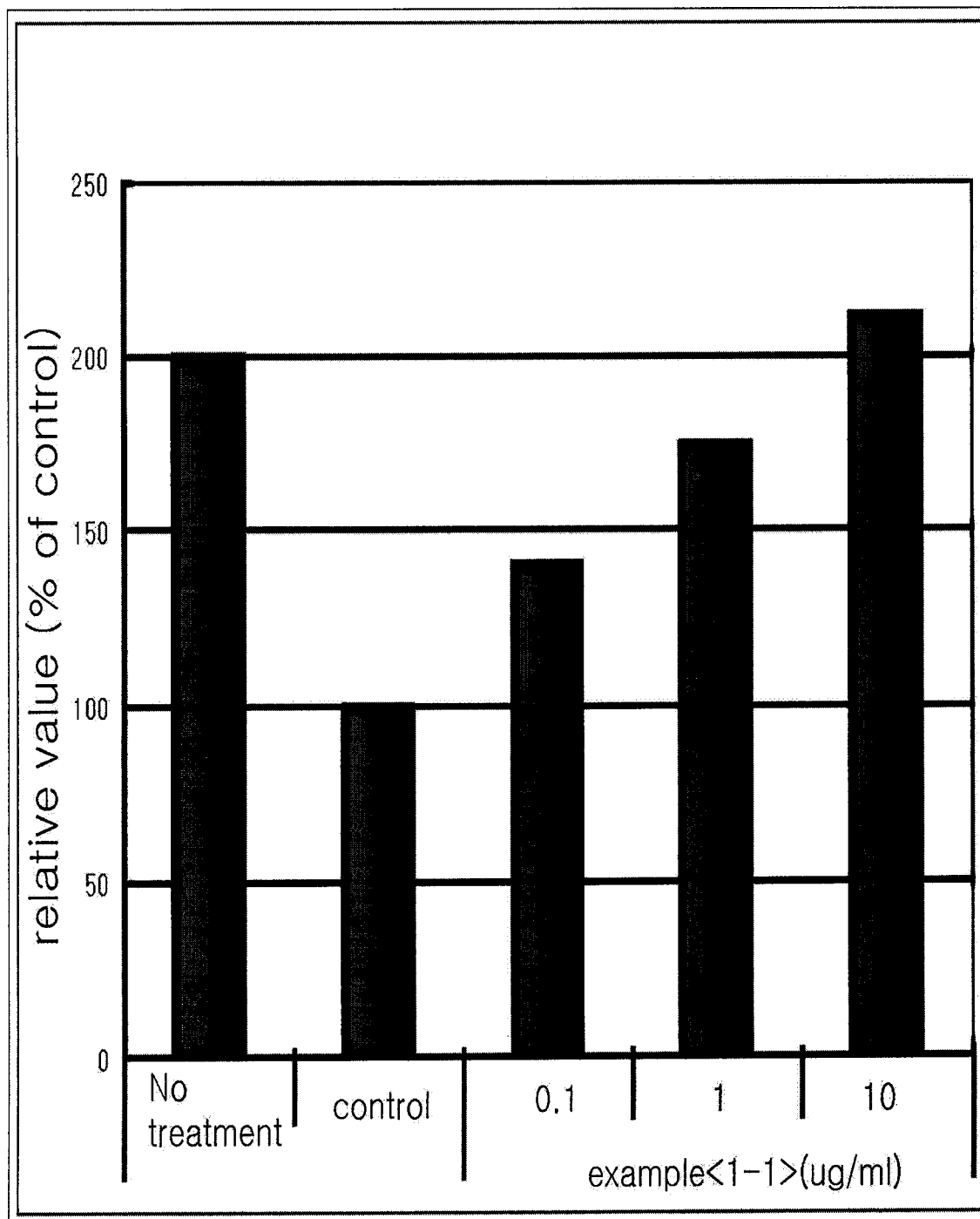
4/5

[Fig. 4]



5/5

[Fig. 5]



INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR2008/004842**A. CLASSIFICATION OF SUBJECT MATTER****A61K 36/73(2006.01)i**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 8 as above

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

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

eKIPASS(KIPO internal), PubMed, NCBI, Esp@snet, PAJ, USPTO, Google

keywords: pentaphylloides fruticosa & anti-oxidative & anti-skin aging

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	KOLPAKOV MA et al., Hepatoprotective properties of aqueous extract from Pentaphylloides fruticosa during chronic toxic hepatitis, 1: Bull Exp Biol Med. 2001 May; 131(5): 470-2 See the page 472	1-7 8-19
X A	US 2007-0122492 A1 (STEPHEN BEHR et al.) 2007.05.31 See the abstract and page 21	8-19 1-7
A	US 2006-0228426 A1 (BENOIT CYR) 2006.10.12 See the abstract	1-19
A	US 2004-0175439 A1 (BENOIT CYR) 2004.09.09 See the abstract	1-19

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

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

23 FEBRUARY 2009 (23.02.2009)

Date of mailing of the international search report

23 FEBRUARY 2009 (23.02.2009)

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Telephone No. 82-42-481-5049



INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2008/004842**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.: 20-21
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 20-21 pertain to methods for treatment of the human or animal body by therapy, and thus relate to a subject matter which this International Searching Authority is not required to search under PCT Article 17(2)(a)(i) and Rule 39.1(iv).
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

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

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2008/004842

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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