

【DESCRIPTION】

【Invention Title】

COMPOSITION FOR PROMOTING HAIR SPROUTING AND HAIR
GROWTH

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【Technical Field】

The present disclosure relates to a composition for promoting hair sprouting and hair growth, which contains a ginseng extract with increased ginsenoside contents and promotes the growth of hair by accelerating transition from telogen to anagen.

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【Background Art】

Recently, due to increased social stress, environmental pollution, Westernized eating habits such as convenience food, frequent hair perm and hair dyeing, etc., those suffering from hair loss are increasing. Hair grows in cycles of various phases: anagen is a hair growth phase; catagen is a phase where the growth stops and the hair bulb withdraws; telogen is a phase where the dermal papilla stops functioning and the hair remains on the scalp; and a morphogenesis phase is a phase where the dermal papilla begins functioning or forms new hair and sheds old hair.

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The anagen phase (2-7 years) where the hair grows is divided again into a stage where the hair grows from a hair bulb to a hair follicle and a stage where hard keratin is formed inside the hair follicle. The hair continues to grow until the catagen. The catagen phase (2-3 weeks) is a phase where the anagen ends and the shape of the hair is maintained as the metabolic process slows down. Keratin is not produced

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in this phase. About 1% of the entire hair is in the catagen. During this phase, the hair bulb withdraws and is divided to form a dermal papilla. It is shifted upward being surrounded by the hair follicle and cell division is stopped. In the telogen phase (3 months), the dermal papilla withdraws and the hair follicle shrinks gradually. The hair falls as the hair root is shifted upward. It takes about 3-4 months until the next anagen phase begins.

Whereas normal people have much of their hair in the anagen phase, those who suffer from hair loss (alopecia) have much of their hair in the telogen phase. As hair loss proceeds, the anagen phase shortens and the hair becomes shorter. Accordingly, to treat hair loss, it is important to accelerate transition of the hair follicle in telogen phase to anagen and lengthen the anagen phase.

Male pattern hair loss occurs due to the male hormone called testosterone. Testosterone is changed to dihydrotestosterone (DHT), which is a more potent hormone, by the enzyme called 5 α -reductase. This hormone acts on the hair follicle, causing the hair follicle to transit from the anagen phase to the catagen phase, thereby leading to hair loss. Therefore, inhibition of DHT synthesis by 5 α -reductase is a major target in the treatment of male pattern hair loss.

Female pattern hair loss occurs mainly after menopause due to decrease in estrogen. For treatment of female pattern hair loss, minoxidil or estrogen is used frequently.

Alopecia areata is an autoimmune disease caused by psychological stress or genetic factors. Alopecia areata is fundamentally different from androgenic hair loss in its cause and treatment method is also different. Adrenocortical hormone therapy, application of minoxidil at the affected region or artificial stimulation of the affected region is used.

Drugs that promote blood circulation, inhibit the action of male hormone, strengthen hair root, etc. are commercially available for the purpose of relieving hair loss. However, no one exhibits a clear effect and some have undesired side effects. For example, minoxidil is sticky and is reported to cause skin irritation. Finasteride, which is currently available for oral administration, is reported to have side effects such as sexual dysfunction. In addition, it is inconvenient to use because it has to be taken orally.

[References of Related Art]

[Patent Documents]

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【Disclosure】

【Technical Problem】

The present disclosure is directed to providing a ginseng extract with increased ginsenoside contents and a composition for promoting hair sprouting and hair growth with proven safety, which contains the same and promotes the growth of hair by accelerating transition from telogen to anagen.

【Technical Solution】

In an aspect, the present disclosure provides a ginseng extract containing 2.5 wt% or more of ginsenoside Rb2, 3 wt% or more of ginsenoside Rc and 2 wt% or more of ginsenoside Rg1 based on the total weight of the ginseng extract.

In another aspect, the present disclosure provides a ginseng extract which

further contains 6 wt% or more of ginsenoside Re.

In another aspect, the present disclosure provides a method for preparing the ginseng extract, which includes:

5 a step of extracting ginseng by adding water, an organic solvent or a mixture of water and an organic solvent and repeatedly applying and then reducing pressure; and

a step of preparing a ginseng extract by dissolving the resulting ginseng extract in water, extracting with an organic solvent, removing the organic solvent layer and then extracting the aqueous layer again with an organic solvent.

10 In another aspect, the present disclosure provides a composition for promoting hair sprouting and hair growth, which contains the ginseng extract with increased ginsenoside contents.

【Advantageous Effects】

15 A composition for promoting hair sprouting and hair growth according to the present disclosure, which contains ginsenosides Rb2, Rc and Rg1 or Rb2, Rc, Rg1 and Re at increased concentrations, can exhibit a better effect of promoting hair sprouting and hair growth than that of the existing hair growth stimulant.

20 Also, it exhibits superior safety because it uses a plant-derived natural product and thus can be used as a composition for external application to skin for promoting hair sprouting and hair growth. In addition, it can be widely used as a cosmetic composition, a pharmaceutical composition, a food composition, etc.

【Brief Description of Drawings】

25 Fig. 1 shows the chemical structure of ginsenosides according to an

exemplary embodiment of the present disclosure.

Fig. 2 shows an HPLC analysis of ginsenosides contained in a ginseng extract according to an exemplary embodiment of the present disclosure.

Fig. 3 shows an HPLC analysis of ginsenosides contained in a general
5 ginseng extract as a comparative example.

Fig. 4 shows increased expression of VEGF as a hair growth factor in dermal papilla cells by a ginseng extract according to an exemplary embodiment of the present disclosure (Example 1) and a general ginseng extract as a comparative example (Comparative Example 1).

10 Fig. 5 shows the effect of a ginseng extract according to an exemplary embodiment of the present disclosure (Example 1) and a general ginseng extract as a comparative example (Comparative Example 1) on proliferation of dermal papilla cells (hDPc).

Fig. 6 shows the effect of ginsenosides contained in a ginseng extract
15 according to an exemplary embodiment of the present disclosure on proliferation of dermal papilla cells (hDPc).

Fig. 7 shows the effect of a ginseng extract according to an exemplary embodiment of the present disclosure (Example 1) and a general ginseng extract as a comparative example (Comparative Example 1) on promotion of hair growth in human
20 hair follicle.

Fig. 8 shows the effect of a ginseng extract according to an exemplary embodiment of the present disclosure (Example 1), a general ginseng extract as a comparative example (Comparative Example 1) and ginsenosides Rb1, Rb2, Rc, Rd, Re and Rg1 on promotion of hair growth in human hair follicle.

【Best Mode】

Hereinafter, the present disclosure is described in detail.

In an exemplary embodiment, the present disclosure provides a ginseng extract containing 2.5 wt% or more of ginsenoside Rb2, 3 wt% or more of ginsenoside Rc and 2 wt% or more of ginsenoside Rg1 based on the total weight of the ginseng extract.

In an exemplary embodiment, the present disclosure provides a ginseng extract which further contains 6 wt% or more of ginsenoside Re.

Ginsenosides are a class of glycosides called saponins and are derived from the root, stem, leaf, pericarp, seed, etc. of ginseng. There are various kinds of ginsenosides, e.g., ginsenosides Rb1, Rb2, Rc, Rd, Re, Rg1, etc. The kinds and contents of the ginsenoside existing in plants are different depending on the kind of ginseng, cultivation condition, processing condition, extraction method, the part of ginseng, etc. The chemical structure of different ginsenosides is shown in Fig. 1.

Ginsenosides can be classified into PPT (Re, Rg1) and PPD (Rb1, Rb2, Rc, Rd) based on their structure. Ginsenosides Rb1, Rb2, Rc, Rd, Re and Rg1 are ginsenosides existing in raw ginseng, without being processes to red ginseng, and are water-soluble polar substances.

In another exemplary embodiment, the present disclosure provides a ginseng extract obtained from the root of ginseng. Specifically, the present disclosure provides a ginseng extract obtained from the rootlet of ginseng, which contains the aforementioned ginsenosides at high concentrations.

In an exemplary embodiment, the present disclosure provides a ginseng extract, which contains 2.5 wt% or more of ginsenoside Rb2, 3 wt% or more of ginsenoside Rc, 6 wt% or more of ginsenoside Re and 2 wt% or more of ginsenoside

Rg1 based on the total weight of the ginseng extract. More specifically, the ginseng extract may contain 2.5-4.5 wt% of ginsenoside Rb2, 3-7.5 wt% of ginsenoside Rc, 6-20 wt% of ginsenoside Re and 2-5 wt% of ginsenoside Rg1.

In an exemplary embodiment of the present disclosure, the ginseng extract
5 containing 2.5 wt% or more of ginsenoside Rb2, 3 wt% or more of ginsenoside Rc, 6 wt% or more of ginsenoside Re and 2 wt% or more of ginsenoside Rg1 based on the total weight of the ginseng extract may be prepared by any method that enables extraction of ginsenosides or other ingredients from ginseng. For example, it may be prepared by pressure swing extraction of alternately applying and then reducing
10 pressure, solvent fractionation, etc. or by a combination of them in sequence. Specifically, the pressure swing extraction may be performed by adding water, an organic solvent or a mixture thereof to ginseng and applying and then reducing pressure at given time intervals.

More specifically, in an exemplary embodiment of the present disclosure, the
15 ginseng extract containing ginsenosides Rb2, Rc and Rg1 at high concentrations can be prepared as follows.

The ginseng extract may be prepared by a method including:

a step of extracting ginseng by adding water, an organic solvent or a mixture of water and an organic solvent and repeatedly applying and then reducing pressure;
20 and

a step of preparing a ginseng extract by dissolving the resulting ginseng extract in water, extracting with an organic solvent, removing the organic solvent layer and then extracting the aqueous layer again with an organic solvent

The solvent that can be used in an exemplary embodiment of the present
25 disclosure is not particularly limited. For example, it may be water, an organic

solvent or a mixture of water and an organic solvent. For example, the organic solvent may be one or more selected from a group consisting of ethanol, methanol, butanol, ether, ethyl acetate and chloroform. For example, in the mixture solvent of water and an organic solvent, the ratio of the organic solvent may be 10-90% (v/v).

5 More specifically, the extract may be obtained using 10-90% (v/v) ethanol, more specifically 70% (v/v) ethanol, as a solvent.

In an exemplary embodiment of the present disclosure, the pressure swing extraction may be performed by repeatedly applying and then reducing pressure using a pressure swing extraction apparatus for 20-40 minutes each, over a total of
10 1.5-2.5 hours. In an exemplary embodiment of the present disclosure, the pressure when applying the pressure may be 1-3 kgf/cm² and the pressure when reducing the pressure may be 550-650 mmHg. Extraction temperature may be 65-85 °C.

When ginseng is extracted by repeatedly applying and then reducing pressure as described above, thermal denaturation of ginseng can be minimized and, therefore,
15 denaturation of ginsenosides, e.g., glycolysis of ginsenoside Rb1 to Rg3, can be minimized. As a result, extraction yield can be improved by 25-30% as compared to when the extraction is performed using the existing simple extraction apparatus without repetition. In addition, by removing nonpolar substances using an organic solvent such as ethyl acetate and then extracting the aqueous layer using an organic
20 solvent such as butanol, the contents of ginsenosides can be maximized. Specifically, according to this solvent fractionation method, the total contents of ginsenosides in the ginseng extract can be improved from 1.5-2 wt% to 10 times or more, specifically to 15-25 wt%.

In an exemplary embodiment, the present disclosure provides a composition
25 for promoting hair sprouting and hair growth, which contains the ginseng extract.

The composition exhibits remarkably superior effect of promoting the growth of hair than one containing a general ginseng extract by accelerating transition from telogen to anagen in the hair growth cycle. Accordingly, an unprecedented remarkable effect of promoting hair sprouting and hair growth can be achieved.

5 In an exemplary embodiment, the present disclosure provides a use of the ginseng extract according to the present disclosure for promoting hair sprouting and hair growth or a use of the composition containing the ginseng extract for promoting hair sprouting and hair growth.

10 In another exemplary embodiment, the present disclosure provides a method for promoting hair sprouting and hair growth, which includes administering an effective amount of the ginseng extract according to the present disclosure as an active ingredient to a subject. The ginseng extract may be administered being contained in a composition.

15 In another exemplary embodiment, the present disclosure provides the ginseng extract according to the present disclosure for use in promoting hair sprouting and hair growth. The ginseng extract may be used being contained in a composition.

20 In an exemplary embodiment of the present disclosure, the amount of the ginseng extract containing ginsenosides Rb2, Rc and Rg1 contained in the composition is not particularly limited. For example, it may be contained in an amount of 2 wt% or more, for example, 2-20 wt%, based on the total weight of the composition. When the ginseng extract is contained in an amount less than 2 wt%, a sufficient effect of promoting hair growth cannot be expected. And, when it is contained in an amount exceeding 20 wt%, there may be difficulties in terms of safety or preparation.

For example, the composition according to an exemplary embodiment of the present disclosure may be a pharmaceutical composition, a cosmetic composition or a health food composition.

The pharmaceutical composition for promoting hair sprouting and hair growth
5 an exemplary embodiment of the present disclosure may further contain a pharmaceutical adjuvant such as an antiseptic, a stabilizer, a wetting agent, an emulsifier, a salt and/or a buffer for control of osmotic pressure, etc. or other therapeutically useful substance and may be prepared into various formulations for oral or parenteral administration.

10 The formulation for oral administration includes, for example, a tablet, a pill, a hard or soft capsule, a liquid, a suspension, an emulsifier, a syrup, a powder, a dust, a fine granule, a granule, a pellet, etc. and may further contain, in addition to the active ingredient, a surfactant, a diluent (e.g., lactose, dextrose, sucrose, mannitol, sorbitol, cellulose and glycine) or a lubricant (e.g., silica, talc, stearic acid and its magnesium
15 or calcium salt and polyethylene glycol). The tablet may further contain a binder such as magnesium aluminum silicate, starch paste, gelatin, tragacanth, methyl cellulose, sodium carboxymethyl cellulose and polyvinylpyrrolidone and may further contain, if necessary, a pharmaceutical additive such as a disintegrant, e.g., starch, agar, alginic acid or its sodium salt, a humectant, a colorant, a flavor, a sweetener, etc.
20 The tablet may be prepared by a commonly employed mixing, granulation or coating method. And, the formulation for parenteral administration may be a formulation for transdermal administration. For example, it may be an injection, a drop, an ointment, a lotion, a gel, a cream, a spray, a suspension, an emulsion, a suppository, a patch, etc., although not being limited thereto.

The pharmaceutical composition according to an exemplary embodiment of the present disclosure may be administered parenterally, e.g., rectally, topically, transdermally, subcutaneously, etc. For example, the pharmaceutical composition according to an exemplary embodiment of the present disclosure may be administered topically to the scalp.

Determination of the administration dosage of the active ingredient is within the level of those skilled in the art. A daily administration dosage varies depending on various factors such as severity of related condition, progress of the condition, age, physical condition, presence of complications(s), etc. For an adult, the composition may be administered at a dosage of 1 µg/kg to 200 mg/kg, specifically 50 µg/kg to 50 mg/kg, 1-3 time(s) a day. However, the administration dosage does not limit the scope of the present disclosure by any means.

The composition for promoting hair sprouting and hair growth according to an exemplary embodiment of the present disclosure may be a cosmetic composition. The cosmetic composition contains a cosmetically or dermatologically acceptable medium or base. The composition may be provided in any topically applicable formulation, e.g., a solution, a gel, a solid, an anhydrous paste, an oil-in-water emulsion, a suspension, a microemulsion, a microcapsule, a microgranule, an ionic (liposome) or nonionic vesicular dispersion, a cream, a skin lotion, a powder, an ointment, a spray or a conceal stick. The composition may be prepared to according a method commonly employed in the art. The composition according to the present disclosure may be used in the form of a foam composition or an aerosol composition further containing a compressed propellant.

The cosmetic composition according to an exemplary embodiment of the present disclosure is not particularly limited in in formulation. For example, it may be

prepared into a cosmetic formulation such as a softening lotion, an astringent lotion, a nourishing lotion, a nourishing cream, a massage cream, an essence, an eye cream, an eye essence, a cleansing cream, a cleansing foam, a cleansing water, a pack, a powder, a body lotion, a body cream, a body oil, a body essence, etc.

5 When the formulation of the cosmetic composition according to the present disclosure is a paste, a cream or a gel, an animal oil, a plant oil, a wax, a paraffin, a starch, tragacanth, a cellulose derivative, polyethylene glycol, silicone, bentonite, silica, talc, zinc oxide, etc. may be used as a carrier.

10 When the formulation of the cosmetic composition according to the present disclosure is a powder or a spray, lactose, talc, silica, aluminum hydroxide, calcium silicate or polyamide powder may be used as a carrier. Especially, the spray may further contain a propellant such as a chlorofluorohydrocarbon, propane/butane or dimethyl ether.

15 When the formulation of the cosmetic composition according to the present disclosure is a solution or an emulsion, a solvent, a solubilizer or an emulsifier may be used as a carrier. Examples include water, ethanol, isopropanol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, a glycerol aliphatic ester, polyethylene glycol or a fatty acid ester of sorbitan.

20 When the formulation of the cosmetic composition according to the present disclosure is a suspension, a liquid diluent such as water, ethanol or propylene glycol, a suspending agent such as ethoxylated isostearyl alcohol, polyoxyethylene sorbitol ester and polyoxyethylene sorbitan ester, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar, tragacanth, etc. may be used as a carrier.

25 When the formulation of the cosmetic composition according to the present disclosure is a surfactant-containing cleanser, an aliphatic alcohol sulfate, an aliphatic

alcohol ether sulfate, sulfosuccinic acid monoester, an isethionate, an imidazolinium derivative, methyl taurate, a sarcosinate, a fatty acid amide ether sulfate, an alkyl amidobetaine, an aliphatic alcohol, a fatty acid glyceride, a fatty acid diethanolamide, a vegetable oil, a lanolin derivative, an ethoxylated glycerol fatty acid ester, etc. may
5 be used as a carrier.

In an exemplary embodiment of the present disclosure, the content of the active ingredient is not particularly limited. The composition may contain 0.001-20 wt% of the extract as the active ingredient based on the total weight of the composition. A superior effect may be achieved without side effects when the above
10 content is satisfied.

The cosmetic composition according to an exemplary embodiment of the present disclosure may further include, in addition to the extract as the active ingredient, a functional additive and an ingredient usually contained in a cosmetic composition. The functional additive may include an ingredient selected from a
15 group consisting of a water-soluble vitamin, an oil-soluble vitamin, a polypeptide, a polysaccharide, a sphingolipid and a seaweed extract.

If necessary, the cosmetic composition according to an aspect of the present disclosure may further contain an ingredient usually contained in a cosmetic composition together with the functional additive. Such an ingredient may include an
20 oil, a fat, a humectant, an emollient, a surfactant, an organic or inorganic pigment, an organic powder, a UV absorbent, an antiseptic, a sterilizer, an antioxidant, a plant extract, a pH control agent, an alcohol, a colorant, a fragrance, a blood circulation stimulant, a cooling agent, an antiperspirant, purified water, etc.

In addition, the composition according to an exemplary embodiment of the
25 present disclosure for promoting hair sprouting and hair growth may be a composition

for external application to skin. The composition for external application to skin is a collective term including anything that can be applied on the skin from outside. Cosmetics and drugs in various formulations may be included therein.

Also, the composition for promoting hair sprouting and hair growth according
5 to an exemplary embodiment of the present disclosure may be a health food composition. Specifically, the health food composition of the present disclosure may contain 0.001-20 wt% of a ginseng extract containing ginsenosides Rb2, Rc, Re and Rg1 as active ingredients based on the total weight of the composition. When the content of the extract is less than 0.001 wt%, the desired effect may not achieved
10 sufficiently. And, when it exceeds 20 wt%, the efficiency of the addition of the active ingredient may decrease.

The health food composition according to an exemplary embodiment of the present disclosure may be prepared into a liquid or solid formulation such as a tablet, a capsule, a soft capsule, a pill, a granule, a drink, a diet bar, a chocolate, a caramel,
15 confectionery, etc., but is not particularly limited in formulation. The health food composition of the present disclosure may further contain, in addition to the active ingredient, an excipient, a sugar, a fragrance, a colorant, an oil, a fat, a protein, etc., if necessary.

20 **【Mode for Invention】**

Hereinafter, the present disclosure will be described in detail through examples and test examples. However, the following examples and test examples are for illustrative purposes only and the scope of the present disclosure is not limited by them.

Also, it will be obvious to those of ordinary skill in the art that various changes and modifications can be made without departing from the scope of the present disclosure defined in the appended claims.

5 [Example 1] Preparation of ginseng extract containing ginsenosides Rb2, Rc, Rg1 and Re at increased concentrations

Ginseng (Geumsan ginseng, acquired from Geumsaninsam Nonghyup) was washed with purified water, dried and then pulverized into fine powder. 2 g of the obtained ginseng powder was added to 20 mL of 50% ethanol and then extracted for
10 a total of 2 hours by repeatedly applying and then reducing pressure using a pressure swing extraction apparatus for 30 minutes each using a pressure swing extraction apparatus. The pressure when applying the pressure was 2 kgf/cm² and the pressure when reducing the pressure was 600 ± 50 mmHg. Extraction temperature was set to 75 °C. The ginseng extract obtained from the pressure swing extraction
15 process was filtered and a supernatant was dried under reduced pressure. The resulting dried product (dry weight = 0.57 g) was dissolved in water and extracted with ethyl acetate. After removing the ethyl acetate layer, the aqueous layer was extracted with butanol and then dried under reduced pressure (dry weight = 2.7 g).

Ginsenosides contained in the ginseng extract of Example 1 were analyzed by
20 HPLC using the following analysis instrument and analysis condition. Based on the result, the content of each ginsenoside ingredient contained in the extract was calculated in wt% unit. The result is shown in Table 2 and Fig. 2.

HPLC analysis instrument and analysis condition

Instrument: Waters 2998 PDA detector, 1525 pump, 2707 autosampler.

25 Column: Sun fire C18 305 μm, 4.6 x 150 mm.

Detector: UV 203 nm.

Flow rate: 1 mL/min.

Injection volume: 20 µL.

Condition: gradient (A: water, B: acetonitrile).

5 **【Table 1】**

Time (min)	A (water)	B (acetonitrile)
0	82	18
22	82	18
32	70	30
60	50	50

【Table 2】

Ginseng extract (Example 1)	Content (%)					
	Rg1	Re	Rb1	Rc	Rb2	Rd
	2.1	6.23	3.52	3.14	2.91	1.58

As seen from Table 2 and Fig. 2, the ginseng extract of Example 1 according to the present disclosure contained 2.91 wt% (i.e., 2.5 wt% or more) of ginsenoside Rb2, 3.14 wt% (i.e., 3 wt% or more) of ginsenoside Rc, 6.23 wt% (i.e., 6 wt% or more) of ginsenoside Re and 2.1 wt% (i.e., 2.0 wt% or more) of ginsenoside Rg1.

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[Comparative Example 1] Preparation of general ginseng extract

Ginseng (Geumsan ginseng, acquired from Geumsaninsam Nonghyup) was washed with purified water, dried and then pulverized into fine powder. 2 g of the obtained ginseng powder was extracted with 100 mL of 50% ethanol at 75 °C for 2

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hours. After filtering, a supernatant was dried under reduced pressure (dry weight = 0.30 g).

Ginsenosides contained in the ginseng extract of Comparative Example 1 were analyzed by HPLC using the same analysis instrument and analysis condition as in Example 1. Based on the result, the content of each ginsenoside ingredient contained in the extract was calculated in wt% unit. The result is shown in Table 3 and Fig. 3.

【Table 3】

Ginseng extract (Comparative Example 1)	Content (%)					
	Rg1	Re	Rb1	Rc	Rb2	Rd
	0.14	0.16	0.67	0.55	0.33	0.13

As seen from Table 3 and Fig. 3, the ginseng extract of Comparative Example 1 contained 0.33 wt% of ginsenoside Rb2, 0.55 wt% of ginsenoside Rc, 0.16 wt% of ginsenoside Re and 0.14 wt% of ginsenoside Rg1. Therefore, it was confirmed that the ginseng extract of Example 1 according to the present disclosure contains 5-15 times more of each ginsenoside than the general ginseng extract. Also, it was confirmed that the total weight of the ginsenosides contained in the ginseng extract is much greater for Example 1 according to the present disclosure than for Comparative Example 1.

[Test Example 1] Evaluation of expression of hair growth factor VEGF in dermal papilla cells

The expression of the vascular endothelial growth factor (VEGF) which is a growth factor necessary for blood vessel formation for transition to the anagen phase

of the hair cycle was evaluated after application of the ginseng extract using an in-vitro system.

4x10⁵ dermal papilla cells (DPC) (P.11) of a 47-year-old male adult were seeded onto a 12-well plate and cultured overnight in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). Then, the cells were treated with 20 ppm of the ginseng extract of Example 1 or Comparative Example 1. DMSO was used for a negative control group. 24 hours later, the cell culture treated with the extract of Example 1 or Comparative Example 1 was soup collected and the expression level of VEGF was investigated using the VEGF ELISA kit (vascular endothelial growth factor enzyme-linked immunosorbent assay; R&D Biosystems). The result is shown in Fig. 4.

As seen from Fig. 4, the general ginseng extract of Comparative Example 1 showed no significant difference from the control group not treated with a ginseng extract. In contrast, the ginseng extract of Example 1 according to the present disclosure increased the expression of the hair growth factor VEGF in the dermal papilla cells about 3 times at the same concentration of the ginseng extract of Comparative Example 1. That is to say, the ginseng extract of Example 1 which contains ginsenosides Rb2, Rc, Rg1, etc. in larger quantities than the general ginseng extract of Comparative Example 1 effectively increased the expression of the hair growth factor VEGF in the dermal papilla cells. Accordingly, it was confirmed that the ginseng extract according to the present disclosure has a superior effect of promoting hair sprouting.

[Test Example 2] Evaluation of proliferation of dermal papilla cells

The protein keratin which constitutes hair is produced by keratinocytes at the

hair root and the keratinocytes are differentiated from dermal papilla cells. Accordingly, if that the ginseng extract according to the present disclosure can promote the activity of dermal papilla cells, it will be able to promote the activity of the keratinocytes differentiated therefrom and can promote hair sprouting.

5 Therefore, the effect of the ginseng extract on the promotion of the activity of the dermal papilla cells was evaluated as follows.

A human dermal papilla cell line was used for the experiment. The cell line was cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Gaithersburg, MD, USA) containing 10% fetal bovine serum (FBS) for 24 hours in an incubator maintained at 5% CO₂ and 37 °C and then treated with the ginseng extract of Example 1 at 10 ppm or 20 ppm or with the ginseng extract of Comparative Example 1 at 20 ppm. DMSO was used for a negative control group. 24 hours after the treatment with the test substance, cell proliferation (%) was measured using the WST-1 kit (Roche). The result is shown in Fig. 5.

15 As seen from Fig. 5, the ginseng extract of Example 1 according to the present disclosure significantly increased the proliferation of the cells at 10 ppm and 20 ppm. In particular, the proliferation of the dermal papilla cells was increased by about 1.5 times when they were treated with the ginseng extract of Example 1 at 10 ppm than when the cells were treated with the general ginseng extract of Comparative Example 1 at 20 ppm. The fact that the ginseng extract according to the present disclosure can promote the proliferation of the dermal papilla cells better than the existing general ginseng extract means that it can more effectively promote the activity of keratinocytes and hair sprouting.

[Test Example 3] Evaluation of hair growth promoting effect using human hair follicle

The hair growth promoting effect of the ginseng extract of the present disclosure was evaluated using the human hair follicle.

5 First, dermal papilla cells were treated with each of ginsenosides Rb1, Rb2, Rc, Rd, Re and Rg1, which are major ingredients of the ginseng extract, at 0.1 μ M or 1 μ M in the same manner as in Test Example 2. 24 hours later, cell proliferation (%) was measured using the WST-1 kit (Roche). The result is shown in Fig. 6.

10 Then, hair follicles were separated one by one from the scalp tissue at the occipital region of a 55-year-old male adult and cultured in William's medium (2 mM L-glutamine, 10 μ g/mL insulin, 40 ng/mL hydrocortisone, 1% antibiotic, 1% antifungal agent). 3 days later, the hair follicle tissues that had grown were cut to a size of 3 mm and then treated with nothing (control), with the ginseng extract of Example 1 at 2 ppm or 5 ppm or with the ginseng extract of Comparative Example 1 at 5 ppm. 8
15 days after the treatment, the hair follicle tissues were measured and photographed. Also, in the same manner as described above, hair follicle tissues were treated with each of ginsenosides Rb1, Rb2, Rc, Rd, Re and Rg1, the ginseng extract of Comparative Example 1 and the ginseng extract of Example 1 at 5 ppm. The result is shown in Fig. 7 and Fig. 8.

20 As seen from Fig. 6, ginsenosides Rb1, Rc and Rg1 showed distinct and statistically significant hair growth promoting effect among the ginsenosides.

Also, as seen from Fig. 7, the hair follicle tissues treated with the ginseng extract of Example 1 at 5 ppm showed superior hair growth promoting effect in terms of increased hair length than the ginseng extract of Comparative Example 1 or the
25 control. In particular, Comparative Example 1 showed smaller average growth

length than the control group. As seen from Fig. 8, although the ginsenosides Rb1, Rb2, Rc, Rd, Re and Rg1 also led to larger average growth length than Comparative Example 1, Example 1 showed the longest average growth length.

Accordingly, from the results of Fig. 6, Fig. 7 and Fig. 8, it can be seen that the ginseng extract according to the present disclosure provides superior effect of promoting hair growth in hair follicles because it contains the ginsenosides Rb1, Rc and Rg1 having hair growth promoting effect at increased concentrations as compared to the existing general ginseng extract.

[Test Example 4] Clinical test on patients with hair loss

Female adults aged between 20 and 60 with hair loss were divided into a test group (Example 1) and a control group, with 23 people each. Each group was asked to apply a scalp essence containing 2% of the ginseng extract of Example 1 or a control scalp essence not containing the extract on the depilated area once a day in the evening. For 16 weeks, the effect on prevention of hair loss and promotion of hair growth was evaluated subjectively by the test subjects, objectively by a researcher based on photographs and using phototrichograms as a non-invasive hair evaluation method with hair density, hair thickness, hair growth speed and number of shedding hairs as biological variables. The test was designed as a randomized, double-blind, comparative clinical study. The result is shown in Tables 4-8.

The hair density of Example 1 and the control group was compared in Table 4. For Example 1, the hair density increased significantly by 5.485% and 7.409% at 8 and 16 weeks, respectively. The ratio of subjects who experienced improvement was 86.364% and 86.364% at 8 and 16 weeks, respectively. In contrast, for the control group, the hair density increased by 1.615% and 1.334% at 8 and 16 weeks,

respectively.

The hair growth speed of Example 1 and the control group was compared in Table 4. For Example 1, the hair growth speed increased significantly by 3.218% and 8.783% at 8 and 16 weeks, respectively. The ratio of subjects who experienced improvement was 68.182% and 81.818% at 8 and 16 weeks, respectively. In contrast, for the control group, the hair growth speed increased by 2.480% and decreased by 1.142% at 8 and 16 weeks, respectively.

【Table 4】

	Mean ± STDEV			Improvement (%)		Ratio of subjects who experienced improvement (%)		Significant difference vs. before use (p value)		Significant difference vs. control (p value)	
Example 1	Before	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks
Density (n/cm ²)	111.045 ± 20.285	117.136 ± 19.833	119.273 ± 19.452	5.485	7.409	86.364	86.364	0.000*	0.000*	0.008#	0.001#
Growth speed (μm/day)	0.292 ± 0.056	0.301 ± 0.080	0.318 ± 0.044	3.218	8.783	68.182	81.818	0.098	0.004*	0.496	0.025**
Control	Before	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks
Density (n/cm ²)	123.826 ± 18.968	125.826 ± 23.796	125.478 ± 25.293	1.615	1.334	47.826	43.478	0.267	0.444	N.A	N.A

Growth speed ($\mu\text{m/day}$)	0.289 $\pm$ 0.047	0.297 $\pm$ 0.042	0.286 $\pm$ 0.046	2.480	-1.142	50.000	45.455	0.471	0.732	N.A	N.A
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*: Paired samples t-test, $p < 0.05$.

**: Independent samples t-test, $p < 0.05$.

#: Mann-Whitney U test, $p < 0.05$.

The hair thickness of Example 1 and the control group was compared in Table

5. For Example 1, the hair thickness increased significantly by 1.526%, 2.611%, 4.782% and 7.178 % at 2, 4, 8 and 16 weeks, respectively. The ratio of subjects who experienced improvement was 72.727%, 81.818%, 95.455% and 95.455% at 2, 4, 8 and 16 weeks, respectively. In contrast, for the control group, the hair thickness decreased by 0.903%, 0.765%, 0.595% and 1.084% at 2, 4, 8 and 16 weeks, respectively.

【Table 5】

	Mean $\pm$ STDEV		Improvement (%)		Ratio of subjects who experienced improvement (%)		Significant difference vs. before use (p value)		Significant difference vs. control (p value)
Thickness (μm)	Example 1	Control	Example 1	Control	Example 1	Control	Example 1	Control	
Before	0.080 $\pm$ 0.010	0.082 $\pm$ 0.011	N.A	N.A	N.A	N.A	N.A	N.A	N.A
2 weeks	0.082 $\pm$ 0.011	0.081 $\pm$ 0.011	1.526	-0.903	72.727	34.783	0.021†	0.084	0.002 #
4 weeks	0.083 $\pm$ 0.011	0.081 $\pm$ 0.010	2.611	-0.765	81.818	52.174	0.001†	0.235	0.001 #
8 weeks	0.084 $\pm$ 0.012	0.081 $\pm$ 0.011	4.782	-0.595	95.455	43.478	0.000†	0.134	0.000 #
16 weeks	0.086 $\pm$ 0.012	0.081 $\pm$ 0.010	7.178	-1.084	95.455	52.174	0.000*	0.218	0.000 #

*: Paired samples t-test, $p < 0.05$.

†: Wilcoxon signed rank test, $p < 0.05$.

#: Mann-Whitney U test, $p < 0.05$.

The number of shedding hairs of Example 1 and the control group was compared in Table 6. For Example 1, the number of shedding hairs increased by

1.589% at 4 weeks, decreased by 4.849% at 8 weeks and decreased by 33.278% at 16 weeks. The decrease at 16 weeks was significant. The ratio of subjects who experienced improvement was 50.000%, 54.545% and 72.727% at 4, 8 and 16 weeks, respectively. In contrast, for the control group, the number of shedding hairs increased by 26.721% at 4 weeks, decreased by 1.953% at 8 weeks and increased by 2.364% at 16 weeks.

【Table 6】

	Mean ± STDEV		Improvement (%)		Ratio of subjects who experienced improvement (%)		Significant difference vs. before use (p value)		Significant difference vs. control (p value)
Number of shedding hairs (ea)	Example 1	Control	Example 1	Control	Example 1	Control	Example 1	Control	
Before	54.364 ± 29.589	42.304 ± 20.328	N.A	N.A	N.A	N.A	N.A	N.A	N.A
4 weeks	55.227 ± 28.846	53.609 ± 38.577	1.589	26.721	50.000	43.478	0.903	0.102	0.452
8 weeks	51.727 ± 36.482	41.478 ± 25.472	-4.849	-1.953	54.545	43.478	0.733	0.870	0.776

16 weeks	36.273 ± 23.511	43.304 ± 34.189	-33.278	2.364	72.727	52.174	0.006*	0.833	0.047**
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*: Paired samples t-test, p < 0.05.

** : Independent samples t-test, p < 0.05.

The result of visual evaluation of hair loss improvement of Example 1 and the control group was compared in Table 7. For Example 1, the hair loss improvement score increased by 0.045, 0.318 and 0.364 at 4, 8 and 16 weeks, respectively. The improvement at 8 and 16 weeks was significant. The ratio of subjects who experienced improvement was 9.091%, 22.727% and 36.364% at 4, 8 and 16 weeks, respectively. In contrast, for the control group, the hair loss improvement score decreased by 0.130, increased by 0.087 and decreased by 0.087 at 4, 8 and 16 weeks, respectively. The ratio of subjects who experienced improvement was 0.000%, 17.391% and 17.39% at 4, 8 and 16 weeks, respectively.

【Table 7】

	Mean ± STDEV		Ratio of subjects who experienced improvement (%)		Significant difference vs. before use (p value)		Significant difference vs. control (p value)
	Example 1	Control	Example 1	Control	Example 1	Control	
Hair loss improvement (score)							
Before	0.000 ± 0.000	0.000 ± 0.000	N.A	N.A	N.A	N.A	N.A
4 weeks	0.045 ± 0.375	-0.130 ± 0.344	9.091	0.000	0.564	0.083	0.110

8 weeks	0.318 ± 0.646	0.087 ± 0.515	22.727	17.391	0.038†	0.414	0.297
16 weeks	0.364 ± 0.790	-0.087 ± 0.848	36.364	17.391	0.046†	0.627	0.070

†: Wilcoxon signed rank test, $p < 0.05$.

※ Visual evaluation of hair loss improvement: improved greatly (3), improved (2), improved slightly (1), no difference (0), worsened slightly (-1), worsened (-2), worsened greatly (-3).

※ Improvement rate cannot be calculated because score of hair loss improvement before use is 0.

- 5 Thus, ratio of subjects who experienced improvement is evaluated rather than improvement rate.

The result of subjective evaluation of improvement by the subjects of Example 1 and the control group was compared in Table 8. For Example 1, 40.9% and 63.6% of the subjects responded positively at 8 and 16 weeks, respectively, and only 0.0% and 4.4% responded negatively at 8 and 16 weeks, respectively. For the control group, 52.1% and 39.1% of the subjects responded positively at 8 and 16 weeks, respectively, and 0.0% and 8.7% responded negatively at 8 and 16 weeks, respectively.

【Table 8】

	Mean ± STDEV		Positive response (%)		Negative response (%)		Significant difference vs. control (p value)	
	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks
Hair loss improvement (score)	2.455 ± 0.596	2.727 ± 0.767	40.909	63.636	0.000	4.545	0.486	0.087

Control	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks	8 weeks	16 weeks
Hair loss improvement (score)	2.565 ± 0.590	2.348 ± 0.714	52.174	39.130	0.000	8.696	N.A	N.A

※ Subjective evaluation of improvement by subjects: very good (4), good (3), moderate (2), poor (1), very poor (0).

※ Positive response (%): ratio of subjects who responded very good (4) or good (3).

※ Negative response (%): ratio of subjects who responded poor (1) or very poor (0).

5 To conclude, the ginseng extract of Example 1 according to the present disclosure exhibited excellent effect of preventing hair loss and promoting hair growth with application only once a day.

Hereinafter, the present disclosure will be described in detail through
10 formulation examples. However, the following examples are for illustrative purposes only and the scope of the present disclosure is not limited by them.

[Formulation Example 1] Shampoo as formulation for external application to skin

15 A shampoo was prepared according to a commonly employed method with the composition described in Table 9.

【Table 9】

Ingredients	Contents (wt%)
Extract of Example 1	2.00
Sodium lauryl sulfate	10.00

Ingredients	Contents (wt%)
Cocamidopropyl betaine	3.00
Carboxyvinyl polymer	0.30
Polyquaternium-10	0.20
Cetyl trimethyl ammonium chloride	0.10
Purified water	balance
Total	100.00

[Formulation Example 2] Rinse as formulation for external application to skin

A rinse was prepared according to a commonly employed method with the composition described in Table 10.

5 【Table 10】

Ingredients	Contents (wt%)
Extract of Example 1	2.00
Cetyl alcohol	2.00
Stearyl alcohol	2.50
Behenyl alcohol	0.50
Silicone emulsion	0.40
Cyclomethicone	1.00
Dimethyl stearyl ammonium chloride	0.10
Purified water	balance
Total	100.00

[Formulation Example 3] Ointment as formulation for external application to skin

An ointment was prepared according to a commonly employed method with the composition described in Table 11.

5 【Table 11】

Ingredients	Contents (wt%)
Extract of Example 1	2.00
Glycerin	8.00
Butylene glycol	4.00
Liquid paraffin	15.00
β -Glucan	7.00
Carbomer	0.10
Caprylic/capric triglyceride	3.00
Squalane	1.00
Cetearyl glucoside	1.50
Sorbitan stearate	0.40
Cetearyl alcohol	1.00
Beeswax	4.00
Purified water	balance
Total	100.00

[Formulation Example 4] Lotion

A lotion was prepared according to a commonly employed method with the composition described in Table 12.

【Table 12】

Ingredients	Contents (wt%)
Extract of Example 1	2.00
L-Ascorbic acid 2-phosphate magnesium salt	1.00
Water-soluble collagen (1% aqueous solution)	1.00
Sodium citrate	0.10
Citric acid	0.05
Licorice extract	0.20
1,3-Butylene glycol	3.00
Purified water	balance
Total	100.00

5

[Formulation Example 5] Cream

A cream was prepared according to a commonly employed method with the composition described in Table 13.

【Table 13】

Ingredients	Contents (wt%)
Extract of Example 1	2.00

Ingredients	Contents (wt%)
Polyethylene glycol monostearate	2.00
Self-emulsifying glyceryl monostearate	5.00
Cetyl alcohol	4.00
Squalane	6.00
Glyceryl tri(2-ethylhexanoate)	6.00
Sphingoglycolipid	1.00
1,3-Butylene glycol	7.00
Purified water	balance
Total	100.00

[Formulation Example 6] Pack

A pack was prepared according to a commonly employed method with the composition described in Table 14.

5 【Table 14】

Ingredients	Contents (wt%)
Extract of Example 1	2.00
Polyvinyl alcohol	13.00
L-Ascorbic acid 2-phosphate magnesium salt	1.00
Lauroyl hydroxyproline	1.00
Water-soluble collagen (1% aqueous solution)	2.00

Ingredients	Contents (wt%)
1,3-Butylene glycol	3.00
Ethanol	5.00
Purified water	balance
Total	100.00

[Formulation Example 7] Beauty care solution

A beauty care solution was prepared according to a commonly employed method with the composition described in Table 15.

5 【Table 15】

Ingredients	Contents (wt%)
Extract of Example 1	2.00
Hydroxyethylene cellulose (2% aqueous solution)	12.00
Xanthan gum (2% aqueous solution)	2.00
1,3-Butylene glycol	6.00
Thick glycerin	4.00
Sodium hyaluronate (1% aqueous solution) 5.00	5.00
Purified water	balance
Total	100.00

[Formulation Example 8] Soft capsule

A soft capsule was prepared according to a commonly employed method by mixing 50 mg of the extract of Example 1, 80-140 mg of L-carnitine, 180 mg of soybean oil, 2 mg of palm oil, 8 mg of hydrogenated vegetable oil, 4 mg of yellow
5 beeswax and 6 mg of lecithin and filling 400 mg of the mixture in a capsule.

[Formulation Example 9] Tablet

50 mg of the extract of Example 1, 200 mg of galactooligosaccharide, 60 mg of lactose and 140 mg of maltose were mixed and granulated using a fluidized-bed
10 drier. After adding 6 mg of sugar ester, the granule was prepared into a table using a tablet making machine.

[Formulation Example 10] Granule

50 mg of the extract of Example 1, 250 mg of anhydrous crystalline glucose
15 and 550 mg of starch were mixed and granulated using a fluidized-bed drier. The resulting granule was filled in a pouch.

[Formulation Example 11] Drink

50 mg of the extract of Example 1, 10 g of glucose, 0.6 g of citric acid and 25 g
20 of oligosaccharide syrup were mixed. After adding 300 mL of purified water, 200 mL of the mixture was filled in a bottle. The resulting drink was sterilized at 130 °C for 4-5 seconds.

【CLAIMS】

【Claim 1】

A ginseng extract comprising 2.5 wt% or more of ginsenoside Rb2, 3 wt% or more of ginsenoside Rc and 2 wt% or more of ginsenoside Rg1 based on the total
5 weight of the ginseng extract.

【Claim 2】

The ginseng extract according to claim 1, wherein the ginseng extract further comprises 6 wt% or more of ginsenoside Re based on the total weight of the ginseng
10 extract.

【Claim 3】

The ginseng extract according to claim 1, wherein the ginseng extract is one obtained from the root of ginseng.
15

【Claim 4】

The ginseng extract according to claim 1, wherein the ginseng extract is one obtained from the rootlet of ginseng.

【Claim 5】

A method for preparing the ginseng extract according to claim 1, which comprises:

a step of extracting ginseng by adding water, an organic solvent or a mixture of water and an organic solvent and repeatedly applying and then reducing pressure;
25 and

a step of preparing a ginseng extract by dissolving the resulting ginseng extract in water, extracting with an organic solvent, removing the organic solvent layer and then extracting the aqueous layer again with an organic solvent.

5 **【Claim 6】**

A ginseng extract prepared by the method according to claim 5.

【Claim 7】

A composition for promoting hair sprouting and hair growth, which comprises
10 the ginseng extract according to any of claims 1 to 4 as an active ingredient.

【Claim 8】

The composition for promoting hair sprouting and hair growth according to
claim 7, which comprises 2 wt% or more of the ginseng extract based on the total
15 weight of the composition.

【Claim 9】

The composition for promoting hair sprouting and hair growth according to
claim 7, wherein the ginsenosides contained in the ginseng extract accelerates
20 transition from telogen to anagen in the hair growth cycle.

【Claim 10】

The composition for promoting hair sprouting and hair growth according to
claim 7, wherein the composition is a pharmaceutical composition.

【Claim 11】

The composition for promoting hair sprouting and hair growth according to claim 7, wherein the composition is a cosmetic composition.

5 **【Claim 12】**

The composition for promoting hair sprouting and hair growth according to claim 7, wherein the composition is a composition for external application to skin.

【Claim 13】

10 The composition for promoting hair sprouting and hair growth according to claim 7, wherein the composition is a food composition.