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SKIN WHITENING COMPOSITION FOR ORAL ADMINISTRATION

Field of the Invention

The present invention relates to a skin whitening composition suitable for oral administration such as in foods and beverages. In addition, the present invention relates to a skin whitener for oral administration containing said composition and to its use.

Background Art

White skin has conventionally been recognized as beautiful skin, and cosmetics for skin whitening have been proposed that contain substances having tyrosinase inhibitory activity, examples of which include hydroquinone and its derivatives (for example, arbutin), kojic acid and its derivatives, ascorbic acid and its derivatives, thiol compounds and various animal and plant extracts, for the purpose of preventing or treating mospatches and freckles to obtain white skin.

In addition, extracts of Pyracantha fortuneana and Pyracantha angusutifolia have tyrosinase inhibitory activity, and are known to be cosmetic compositions for skin whitening having excellent skin whitening effects (Japanese Patent No. 2749218).

In recent years, there has been a growing awareness with respect to skin whitening, and in addition to those using conventional cosmetics, there has come to be a need to provide skin whiteners that can easily be ingested orally such as in foods and beverages.

However, it is unclear as to whether substances conventionally used in skin whitening cosmetics have effects equivalent to cosmetics even when ingested orally, and the only substances that are known to have skin whitening effects when ingested orally are vitamin C preparations and thiol compounds. Moreover, since these

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substances have problems with respect to stability and absorptivity, they are not considered to demonstrate adequate effects.

Disclosure of the Invention

Therefore, the inventors of the present invention set out to provide a skin whitening composition that is effective and safe in oral administration under these circumstances.

As a result of conducting earnest research on plants that demonstrate skin whitening effects in oral administration among those which have a history of dietary consumption in consideration of safety, plants of the genus Rosaceae Pyracantha were found to be effective. More specifically, extracts in water, ethanol or their mixture of the fruit of Pyracantha fortuneana and Pyracantha angusutifolia as the genus Rosaceae Pyracantha were found to demonstrate skin whitening effects in oral administration, thereby leading to completion of the present invention.

Furthermore, although it is clear that there is a difference in efficacy according to differences in the administration form between applying directly to the skin in the form of a cosmetic and so forth of a substance having skin whitening effects, and oral administration in the form of a food or beverage, the following provides a brief description of that difference.

Cosmetics are applied directly to the skin surface, and the majority of cosmetic ingredients remain on the skin surface and demonstrate a function inherent to the cosmetic without impairing the physiological function of the skin.

On the other hand, in the case of oral administration, a substance having skin whitening effects first is dissolved in the juices inside the digestive tract and then absorbed from the stomach or small intestine. Substances absorbed from the small intestine

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mainly enter the liver from the portal vein, next enter the veins and finally arrive at various sites in the body by circulating throughout the body after passing through the heart, where they are then taken up into the cells of each tissue.

Thus, it is unclear as to whether substances that are effective in cosmetics demonstrate similar effects as cosmetics in oral administration as well.

In fact, although arbutin and ascorbic acid have tyrosinase inhibitory activity, and have been used in the past as cosmetics having excellent skin whitening effects, since their skin whitening effects are low in oral administration, there is clearly a difference by those effects between oral administration and in cosmetics.

In addition, in the present invention as well, it was found that a fraction of Pyracantha fortuneana having potent tyrosinase inhibitory activity in vitro has weak skin whitening activity by oral administration, while conversely, a fraction having weak tyrosinase inhibitory activity in vitro demonstrates skin whitening activity in oral administration, thereby leading to completion of the present invention.

Embodiment for Carrying Out the Invention

The fruit, known by its Chinese medicine name as "Sekiyoshi", of Pyracantha fortuneana, known by its Chinese name as "Kakyoku", used in the present invention is known to have effects that keep the spleen healthy and cure indigestion. In addition, Pyracantha angusutifolia is a shrub of Chinese origin the same as Pyracantha fortuneana, and is used in Japan as garden vegetation.

Although the solvent extracts of these plants are used in the present invention, it is preferable to use the solvent extracts of the fruits of these plants.

Although there are no particular restrictions on the solvent used during extraction of active ingredient from

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these plants provided it is a solvent by which active ingredient is effectively extracted, water, methanol, ethanol or other lower alcohols, or their mixtures are used preferably, and in terms of safety, water, ethanol or their mixture is used more preferably.

In the case of performing extraction by water, although water at normal temperature can be used, it is preferable to use hot water, for example hot water at 60-100°C. In addition, in the case of using an organic solvent such as methanol or ethanol, extraction may be performed at normal temperature or hot extraction may be performed at a temperature at or below the boiling point of said organic solvent. In addition, in the case of using a mixture of an organic solvent such as methanol or ethanol and water, extraction may be performed either at normal temperature or hot extraction less than boiling point of said solvent.

In addition, the extract can be used after purifying by a means such as concentration, liquid-liquid distribution or adsorption chromatography in order to blend active ingredient at a high concentration. For example, a synthetic adsorbent such as an aromatic synthetic adsorbent like styrene-divinylbenzene can be used as adsorbent in the case of performing adsorption chromatography on an extract of the present invention. A specific example of an aromatic synthetic adsorbent is Diaion HP-20 (trade name, Mitsubishi Kasei Kogyo). Moreover, Diaion HP-21 (trade name, Mitsubishi Kasei Kogyo), Amberlite XAD2 and XAD4 (trade names, Rohm and Hass) and so forth can also be used, the aromatic synthetic adsorbent is not limited to these.

In the case of using Diaion HP-20, it is preferable to apply the extract to a column filled with this adsorbent, and use the liquid that has flown through the column and the resulting fraction is obtained by washing the column with water. In addition, in the case of eluting using an organic solvent, the fraction can be

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used that is obtained by eluting with a, for example, 20 to 80% aqueous methanol or ethanol solution of a mixture of organic solvent and water.

The skin whitening composition for oral administration of the present invention obtained in this manner is a composition for oral administration that is effective for prevention or treatment of mospatches and freckles, etc. and has excellent skin whitening effects. It can be ingested as is in the form of tablets, capsules or granules or ingested by blending into a food or beverage such as candy, chewy candy or a beverage, and there are no restrictions on its form.

In addition, although the adult daily amount ingested of the extract used in the present invention varies according to age, state of health, body weight and so forth, it is desirable to ingest 0.01-20.0 g, and preferably 0.1-10.0 g, in terms of the amount of the solid component of the crude extract.

Examples

Although the following provides a more detailed explanation of the present invention through its examples, the scope of the present invention is not limited to these examples only.

Example 1 Hot Water Extract of Pyracantha fortuneana Fruit

5.00 g of the dried fruit of Pyracantha fortuneana was immersed in 50 ml of hot water at 90°C, filtered after boiling for 3 hours followed by filtration under reduced pressure of the resulting extract to obtain a crude extract (1.33 g as solid).

Example 2 Water/Ethanol Mixture Extract of Pyracantha fortuneana Fruit

5.00 g of the dried fruit of Pyracantha fortuneana was filtered after immersing for 1 week in 50 ml of a 50% aqueous ethanol solution followed by filtration under reduced pressure of the resulting extract to obtain a

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crude extract (1.08 g as solid).

Example 3 Hot Water Extract of Pyracantha angusutifolia Fruit

5.00 g of the dried fruit of Pyracantha angusutifolia was immersed in 50 ml of hot water at 90°C, filtered after boiling for 3 hours followed by filtration under reduced pressure of the resulting extract to obtain a crude extract (1.21 g as solid).

Example 4 Hot Water Extract Fractions of Pyracantha fortuneana Fruit

A crude extract of Pyracantha fortuneana fruit obtained according to the method of Example 1 (6.09 g as solid) was dissolved in 600 ml of water, and after loading onto a Diaion HP-20 column (3 cm in diameter x 11 cm, Vt = 80 ml), the column was washed with 800 ml of water. Following collection of the liquid that passed through the column and the washing and filtration under reduced pressure, the resulting product was freeze-dried to obtain 5.13 g of a solid (fraction 1).

Next, after eluting the column with 400 ml of a 20% aqueous ethanol solution and performing filtration under reduced pressure on the eluent, the resulting product was freeze-dried to obtain 0.22 g of a solid (fraction 2).

Next, after eluting the column with 400 ml of a 40% aqueous ethanol solution and performing filtration under reduced pressure on the eluent, the resulting product was freeze-dried to obtain 0.49 g of a solid (fraction 3).

Next, after eluting the column with 400 ml of an 80% aqueous ethanol solution and performing filtration under reduced pressure on the eluent, the resulting product was freeze-dried to obtain 4 mg of a solid (fraction 4).

Furthermore, the recovery rates of fractions 1 through 4 totaled 96%.

Example 5 Measurement of Tyrosinase Inhibitory Activity

Tyrosinase inhibitory activity was measured as described below.

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After thoroughly mixing 1.00 ml of 50 mM potassium-sodium phosphate buffer (pH 6.8), 0.25 ml of a 0.5 mg/ml aqueous solution of L-tyrosine (Nakarai Tesque) (1), and 1.00 ml of sample aqueous solution (2) (samples prepared to the various respective concentrations of the samples of Examples 1 through 4 and arbutin) and allowing to stand for 10 minutes at 30°C, 0.25 ml of a 0.1 mg/ml aqueous solution tyrosinase derived from mushroom (Sigma) were added. After allowing to react for about 12 minutes at 30°C (the absorbance of the control was about 0.5), absorbance A was measured at 475 nm. At the same time, absorbance B when water was used instead of aqueous sample solution (2), and absorbance C when water was used instead of aqueous L-tyrosine solution (1), were measured. The inhibition rates were calculated using the following equation from the absorbances of A, B and C.

Inhibition rate (%) = (B - (A - C))/B x 100

Tyrosinase inhibitory activity was measured for each of the samples with this measurement method, and the sample concentrations at which the inhibition rate was 50% (IC50 values) are shown in Table 1. As is clear from Table 1, with the exception of fraction 1 of Example 4, all of the samples exhibited tyrosinase inhibitory activity.

Table 1 Tyrosinase Inhibitory Activity of Extracts and Fractions

Sample	Sample concentration at 50% inhibition (IC50) (ppm)
Extract of Example 1	350
Extract of Example 2	320
Extract of Example 3	380
Fraction 1 of Example 4	No activity
Fraction 2 of Example 4	120
Fraction 3 of Example 4	70
Fraction 4 of Example 4	125
Arbutin	75

Example 6 Study of Skin Whitening Effect Using Mice (1)

0.04, 0.12, 0.40 or 10 mg of the crude extract

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obtained in Example 1 were dissolved in water (0.2 ml), and each was administered orally to mice (6 per group) once a day followed by exposure to a 180 mJ/cm^2 ultraviolet rays (UVB). Water was administered to mice of the control group instead of Pyracantha fortuneana extract. In addition, 0.67 mg of ascorbic acid was used as a comparative example. After continuing this administration for 10 days, a portion of the mice auricles were sampled, and slide specimens were prepared by dopa staining of the epidermis. The results of counting the number of dopa-positive melanocytes at five locations for each specimen are shown in Table 2.

As shown in Table 2, Pyracantha fortuneana extract caused a decrease in the number of dopa-positive melanocytes (significant difference of $P < 0.05$ relative to the control at 0.12 mg or more). On the basis of these results, oral ingestion of Pyracantha fortuneana extract clearly demonstrated a skin whitening effect. Furthermore, although ascorbic acid, which has been conventionally used as a cosmetic for skin whitening, was observed to exhibit a decrease in the number of dopa-positive melanocytes, there was no significant difference observed relative to the control.

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Table 2 Comparison of Number of Dopa-Positive Melanocytes After 10 Days of Exposure to UVB (180 mJ/cm^2) (judgment: significance level of 5%)

	Mean value	Standard deviation	Judgment
No UV exposure	254.2	32.1	--
Ascorbic acid	463.5	54.5	No significant difference
Pyracantha fortuneana at 10 mg/day	427.2	28.9	Significant difference
Pyracantha fortuneana at 0.40 mg/day	392.1	57.2	Significant difference
Pyracantha fortuneana at 0.12 mg/day	499.5	20.0	Significant difference
Pyracantha fortuneana at 0.04 mg/day	562.5	19.0	No significant difference
Control	612.4	44.2	--

Example 7 Study of Skin Whitening Effect Using Mice (2)

Fractions of Pyracantha fortuneana obtained in Example 4 were dissolved in water (0.2 ml) at the approximate ratios at which they are present in crude extract (10:1:1), namely 10 mg of fraction 1, 1 mg of fraction 2 and 1 mg of fraction 3, and each was administered orally to mice (n = 6 per group) once a day followed by exposure to a 180 mJ/cm^2 ultraviolet rays (UVB). Water was administered to mice of the control group instead of each of the fractions of Pyracantha fortuneana extract. In addition, 12 mg of arbutin was used as a comparative example. After continuing this administration for 10 days, a portion of the mice auricles were sampled, and slide specimens were prepared by dopa staining of the epidermis. The results of counting the number of dopa-positive melanocytes at five locations for each specimen are shown in Table 3.

Fraction 1 of Pyracantha fortuneana extract that did not exhibit tyrosinase inhibitory activity in Example 5

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significantly decreased the number of dopa-positive melanocytes (significant difference of $P < 0.05$). On the other hand, although fractions 2 and 3, which exhibited potent tyrosinase inhibitory activity, as well as arbutin tended to decrease the number of dopa-positive melanocytes, there were no significant differences observed.

On the basis of these results, it was clearly demonstrated that the main component of the skin whitening effect induced by oral ingestion of Pyracantha fortuneana extract is primarily contained in fraction 1. In addition, it was also clear that the degree of tyrosinase inhibitory activity does not coincide with the degree of skin whitening activity induced by oral ingestion.

In addition, since the Pyracantha fortuneana crude extract (10 mg/day) in Example 6 and fraction 1 of the Pyracantha fortuneana crude extract (10 mg/day) in Example 7 demonstrated nearly equivalent activity, it was thought that fractions other than fraction 1 do not impair skin whitening activity by oral ingestion. Therefore, in the following examples, it was decided to use the extract obtained in Example 1.

Table 3 Comparison of Number of Dopa-Positive Melanocytes After 10 Days of Exposure to UVB (180 mJ/cm^2) (judgment: significance level of 5%)

	Mean value	Standard deviation	Judgment
Fraction 1 at 10 mg/day	413.9	50.7	Significant difference
Fraction 2 at 1 mg/day	457.5	31.7	No significant difference
Fraction 3 at 1 mg/day	517.1	29.5	No significant difference
Arbutin at 12 mg/day	463.9	59.5	No significant difference
Control	563.7	34.6	--

Example 8 Formation of Pyracantha Fortuneana Extract Powder

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Two parts by weight of dextrin were added to 1 part by weight as solid of the extract obtained by filtration in accordance with the method of Example 1 followed by spray drying to obtain a powder of Pyracantha fortuneana. In addition, Pyracantha fortuneana powder was filled into No. 00 hard capsules so as to contain 0.5 g per capsule.

Example 9 Preparation of Tablets

Seventy parts by weight of the Pyracantha fortuneana powder obtained in Example 8, 26 parts by weight of crystalline cellulose and 4 parts by weight of sucrose fatty acid ester were mixed well to prepare tablets containing 250 mg per tablet by using a tablet making machine (Hata Seisakusho Ltd.).

Example 10 Preparation of candy

98 g of sugar, 90.7 g of thick malt syrup (75% solid) and 75 g of concentrate (40% solid) prepared from Pyracantha fortuneana were mixed well and boiled down to a moisture content of 2% to prepare candy weighing 2 g per piece (containing 0.3 g of Pyracantha fortuneana extract as solid).

The concentrate was obtained under reduced pressure from liquid extract prepared from the extract in Example 1.

Example 11 Study of Skin Whitening Effect Using Humans

Five healthy male adults were made to ingest two of the capsules obtained in Example 8 each in the morning, afternoon and at night daily, after which the lower left region of their backs were exposed to ultraviolet rays on day 8. Exposure to ultraviolet rays was performed by irradiating a circular area measuring 18 mm in diameter with a 168.6 mJ/cm^2 ultraviolet rays (UVB) using a therapeutic ultraviolet ray irradiation system (Dermaray). For the control, an equivalent dose of ultraviolet rays was irradiated onto the lower right region of the backs of the subjects during a time period when the same subjects did not ingest the test substance.

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After 7 days of irradiation, the melanin values of the irradiated sites were determined using a dermaspectrometer (Cortex Technology). Using the inhibitory effect on pigment deposition as an indicator, those subjects in which the melanin value during the Pyracantha fortuneana ingestion period was less than the melanin value during the control period by 2 or more were evaluated with a ○, and those in which there was no difference with the melanin value during the control period were evaluated with a △. Those results are shown in Table 4.

As shown in Table 4, the composition of the present invention was clearly effective during oral administration in improving skin color.

Table 4 Comparison of Melanin Values After 7 Days of UVB (168.6 mJ/cm^2) Irradiation

	Melanin values		Judgment
Subject	Control period	Pyracantha fortuneana ingestion period	
1	34	31	○
2	33.5	30	○
3	34.5	31	○
4	33.5	31.5	○
5	28	28	△

Effect of the Invention

According to the present invention, a skin whitening composition for oral administration is provided that is able to be easily ingested orally in the form of a food, beverage and so forth and has excellent skin whitening effects by inhibiting the deposition of pigment for prevention or treatment of mospatches, mospatches and so forth.

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CLAIMS

1. The use of a solvent extract of a plant of the genus Rosaceae Pyracantha for the manufacture of a skin whitening composition for oral administration.

2. The use according to claim 1 wherein the extract is of the fruit of a plant of the genus Rosaceae Pyracantha.

3. The use according to claims 1 or 2 wherein said plant of the genus Rosaceae Pyracantha is Pyracantha fortuneana or Pyracantha angusutifolia.

4. The use according to any one of claims 1 to 3 wherein said solvent is water, methanol, ethanol or a mixture thereof.

5. The use according to any one of claims 1 to 3, wherein the extract contains a hot water extract of Pyracantha fortuneana.

6. The use according to any one of claims 1 to 3 wherein the extract contains the fraction that passes through an adsorption chromatography column and the fraction obtained by washing the column with water after loading said hot water extract of Pyracantha fortuneana onto said adsorption chromatography column.

7. The use according to claim 6 wherein the adsorbent of said adsorption chromatography column is synthetic adsorbent.

8. The use according to claim 7 wherein said synthetic adsorbent is an aromatic synthetic adsorbent.