# TITLE OF THE INVENTION

Skin Whitening Composition Containing Extracts Derived From Natural Rubber Latex

# FIELD OF THE INVENTION

This invention relates to compositions containing extract derived from natural rubber latex including protease inhibitor and optionally additional cosmetically active agents for skin depigmentation.

# BACKGROUND OF THE INVENTION

Different skin colors are simply due to the variations in concentration and distribution of melanin in keratinocytes which make up most of the skin epidermis. The higher the melanin content, the darker the skin color. The melanin is a mix of pigments synthesized in a melanosome inside the melanocytes which localized at the basal membrane of the epidermis. The melanosome contains the enzyme tyrosinase which is essential for the transformation of the propigmentary amino acid tyrosine into melanin. The melasome containing melanin is transferred to the dendrite tips and translocated into keratinocytes, forming caps over the keratinocyte nuclei. The mechanisms of melanosome transfer include melanosome release, keratinocyte-melanocyte membrane fusion and phagocytosis. Preotease-activated receptor-2 (PAR-2) was suggested to control melanosome ingestion and phagocytosis by keratinocytes and exerts a regulatory role in skin pigmentation (1-4). Hence, an effective skin whitening product should include

either tyrosinase inhibitor or PAR-2 inhibitor or both.

a) Tyrosinase inhibitor

In the past, hydroquinone had generally been been used topically to lighten skin. It depigments skin by inhibiting tyrosinase required for the conversion of tyrosine to melanin. However, due to its potential toxicity, it is regulated as a drug if used above 2 % concentration. It is toxic to melanocytes over prolonged usage and could exacerbate the melasma. The residual hydroquinone molecule reacts with molecular oxygen (autooxidation) in the presence of light and air and turns into dark brown color. Several natural tyrosinase inhibitors presently used in cosmetic industry are arbutin from bearberry, glabridin from licorice and kojic acid from fungus *Aspergilline oryzae*.

b) Protease-activated receptor-2 (PAR-2) inhibitor

A mechanism for regulation of pigmentation is mediated by the activation or inhibition of the keratinocyte receptor PAR-2. The proteolytic activity of trypsin was required to activate the PAR-2 receptor for an effective transfer of melanosome from melanocyte to keratinocyte. Inhibition of the keratinocyte PAR-2 pathway by soybean trypsin inhibitor has been shown to induce depigmentation by reducing the phagocytosis of melanosomes or melanin transfer (1-4).

It is an objective of the present invention to provide a novel composition for naturally affecting skin depigmentation derived the non-rubber component of locally abundant natural rubber latex.

## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Depigmentation experiments (A and B) of black swine skin by topical application of the extract derived from natural rubber latex. The extract (spot 1 and 2) and hydroquinone (spot 3) in 30 % glycerol solution was applied twice a day for 9 weeks.

Figure 2. Trypsin inhibitory activity of the extract derived from natural rubber latex.

## SUMMARY OF THE INVENTION

Latex tapped from the rubber tree (*Hevea brasiliensis*) consists of rubber and non-rubber particles suspended in aqueous cytoplasm. The non-rubber residuals are generally discarded as waste. In this invention, one of the non-rubber components was found to be a small protein that inhibits protease activity. Suitable methods for the extraction of the protease inhibitor and its application in skin depigmentation are disclosed.

## DETAILED DESCRIPTION OF THE INVENTION

Fresh latex is a milky fluid obtained by tapping of the rubber tree (Hevea brasiliensis). It contains in suspension not only rubber particles (30-35 gram %) but also other non-rubber particles. An active compound in the aqueous latex serum, obtained after rubber particle removal, was extracted and found to induce skin depigmentation.

**Extraction methods**

Fresh latex was coagulated by addition of formic acid. The latex serum was collected from the rubber coagulum by squeezing between the rollers. It was ultrafiltrated through a series of membrane including those with MWCO ≤ 10 kD and spray dried with temperature more than 100°C. The latex protease inhibitor was further purified from the spray dry powder by means of acetone fractionation at > 40% saturation. The active protease inhibitor fraction, serine protease inhibitor, composes of a combination small proteins or peptides which possesses identical amino acid sequences as follow: ASQCPVKDAWPELVGTNGDIAAGIIQTENANVKAIVVKEGLPITQDLNF NRVRVFVDENRVV TQVPAIG.

The protease activity can be inhibited by the extract which is effective in inducing depigmentation of mammalian skin. The protease inhibitory active agent is able to use in skin whitening products in an amount of from approximately 0.001% (w/v) to 30% (w/v).

**Efficacy of the extract derived from natural rubber latex on the *in vivo* skin depigmentation**

An *in vivo* depigmentation of native black skinned swine by topical application of the protease inhibitor, derived from natural rubber latex, was performed. The dorsum of the swine underwent twice daily spot application with 30% glycerol solution containing either the extract or 5% hydroquinone for 9 weeks. A similar marked depigmentation of black skin was revealed on the application sites of either the extract or hydroquinone (Fig.1).

**Protease inhibitory effect of the extract derived from natural rubber latex serum**

The extract derived from natural rubber latex serum was found to inhibit the activity of a serine protease, trypsin. The trypsin is required to activate PAR-2 for the uptake and distribution of melanosomes in the recipient keratinocytes. The extract could inhibit the trypsin activity in a dose dependent manner (Fig.2).

# CLAIMS

1. A composition for mammalian skin depigmentation comprises with the rubber latex extracts which are extracted from latex serum, roots, shoots, leaves or fruits of the rubber tree (*Hevea brasiliensis*) with molecular weight less than 10kD.

2. The composition according to claim 1, wherein the rubber latex extracts are prepared from rubber latex by using acid to coagulate the rubber latex and separate acidified serum from the coagulated latex by means of squeezing.

3. A method of the extraction of the composition in claim 1-2, comprising the steps:
   a. ultrafiltrating the said acidified serum obtained with various molecular weight cut-off membranes, including < 10 kD;
   b. subjecting the said ultrafiltrate to spray drying;
   c. subjecting the said ultrafiltrate to acetone fractionation at > 40% saturation.

4. The composition according to claim 1 or 2, comprising in a combination small proteins and peptides.

5. The composition according to claim 1 or 2, comprising native or fragmented protein with all or parts of the following amino acid sequence:
   ASQCPVK.DA WPEL
   VGTNGDIAAGIIQTENANVKAIVVKEGLPITQDLNFNR VR
   VFVDENR VV TQVP AIG.

6. The composition according to any of claims 1, 2, 4, or 5,

wherein said extract contains at least one protease inhibitory activity and protease inhibitory active agent is a serine protease inhibitor.

7. The composition claimed in any of claims 1, 2, 4, 5, or 6, wherein said at least one serine protease inhibitor is present in an amount, based upon the total volume of the topically active composition, of from about .001% (w/v) to 30 % (w/v).

8. The composition according to any of claims 1, 2, 4, 5, 6, or 7, wherein said composition is effective for whitening mammalian skin.

9. The composition according to any of claims 1, 2, 4, 5, 6, or 7, wherein said composition is used in skin whitening products.