

HUMAN SKINCARE COMPOSITIONS

Sequence Listing

[0001] This application contains a sequence listing, the copy of which is named Sequence listing.xml, created on January 13, 2025, and has a size of 2,483 bytes.

Field of the Invention

[0002] The present invention relates to the technology fields of cosmetics, and more specifically to human skincare compositions with anti-inflammatory effect, wrinkle reduction, and cell regeneration promotion.

Background of the Invention

[0003] Anti-aging peptides are widely used in cosmetics products, and the most used peptides in antiaging cosmetic formulations include palmitoyl tetrapeptide-7, palmitoyl oligopeptide and acetyl hexapeptide-8 (Ferreira et al. (2020) Trending Anti-Aging Peptides. *Cosmetics*. 7, 91; doi:10.3390/cosmetics7040091).

[0004] EP3233210 B1 discloses tripeptide having X-KGH-Z or X-KHG-Z formula for cosmetics use, where at the N-terminal end X is selected from H, -CO-R1 and -SO₂-R1, at the C-terminal end Z is selected from OH, OR1, NH₂, NHR1 or NR1R2, and R1 and R2 are, independently from each other, an alkyl chain of 1 to 24 carbon atoms, except tripeptides, where (X=H and Z=OH) or (X=H and Z=NH₂) and except the tripeptide KHG-N-octyl ester wherein X=H and Z=OR1 with R1 being an alkyl chain of 8 carbon atoms.

[0005] WO2012/164488 A2 discloses a mixture of A-GHK-B tripeptide and A-GQPR-B tetrapeptide for the therapeutic treatment of the papillary dermis, with A = H, CO-R1; B = OH, OR1, NH₂, NHR1, NR1R2; R1 and R2 being, independently of one another, selected from an alkyl, aryl, aralkyl, alkylaryl, alkoxy and aryloxy group, which may be linear, branched, cyclic, polycyclic, unsaturated, hydroxylated, carbonylated, phosphorylated and/or sulfured.

[0006] Exosome is a type of nano-sized extracellular vesicle surrounded by a lipid bilayer secreted from various cells. It has a size of about 50 to 200 nm, and contains biologically

active substances such as proteins, lipids, and nucleic acids. Plant-derived exosomes are nanosized (30-150 nm) membrane vesicles that contain biomolecules, and have been shown to be useful in cosmetics products (Cho et al. (2022) Confirmation of plant-derived exosomes as bioactive substances for skin application through comparative analysis of keratinocyte transcriptome. *Applied Biological Chemistry*. 65:8; doi: 10.1186/s13765-022-00676-z; Sarasati et al. (2023) Plant-derived exosome-like nanoparticles for biomedical applications and regenerative therapy. *Biomedicines*. 11: 1053; doi: 10.3390/biomedicines11041053).

[0007] While there are many cosmetics products available for anti-aging and anti-wrinkle treatments, human skincare complexes and compositions with improved/optimized efficacies are still needed.

Summary of the Invention

[0008] The present invention provides a human skincare composition with anti-inflammatory effect, wrinkle reduction, and cell regeneration promotion.

[0009] In certain embodiments, the human skincare composition comprises:

glycerin 8.80 – 17.50 w/v%,
 butylene glycol 3.20 – 5.30 w/v%,
 carbomer 0.15 – 0.25 w/v%,
 polysorbate 20 0.05 – 0.10 w/v%,
 1,2-hexanediol 2.30 – 3.20 w/v%,
 Centella asiatica exosomes 1×10^7 particles/ml or above,
 niacinamide 23.40 – 32.15 w/v%,
 Centella Asiatica leaf extract 2.30 -10.0 w/v%
 dipropylene glycol 1.15 – 2.30 w/v%,
 hydrogenated Lecithin 0.25 – 0.50 w/v%,
 panthenol 0.25 – 0.50 w/v%,
 asiaticoside 0.10 – 0.15 w/v%,
 madecassic acid 0.050 – 0.100 w/v%,
 asiatic acid 0.05 – 0.10 w/v%,
 beta-glucan 0.020 – 0.05 w/v%,
 ceramide NP 0.020 – 0.05 w/v%, and

water 40.90.00 – 49.70%.

[0010] In certain embodiments of the human skincare composition, wherein the concentration of *Centella asiatica* exosomes is in the range of 1×10^7 particles/ml to 1×10^8 particles/ml.

[0011] In certain embodiments of the human skincare composition, wherein the concentration of *Centella Asiatica* leaf extract in the range of 2.30 -3.20 w/v%.

[0012] In certain embodiments, the human skincare composition, further comprising:

hydrolyzed sponge 0.50 – 1.15 w/v%,

sodium lactate 0.15 – 0.25 w/v%, and

caprylyl glycol 0.020 – 0.05 w/v %.

[0013] In certain embodiments, the human skincare composition, further comprising:

palmitoyl tripeptide-1 0.0010 – 0.0020 w/v%,

palmitoyl tetrapeptide-7 0.00050 – 0.0010 w/v%, and

acetyl hexapeptide-8 0.00005 – 0.00050 w/v%.

[0014] The objectives and advantages of the invention will become apparent from the following detailed description of preferred embodiments thereof in connection with the accompanying drawings.

Brief Description of the Drawings

[0015] Preferred embodiments according to the present invention will now be described with reference to the Figures, in which like reference numerals denote like elements.

[0016] Fig. 1 shows an exemplary Cryo-TEM image of *Centella asiatica* exosomes.

[0017] Fig. 2 shows an exemplary oil-red staining image of *Centella asiatica* exosomes.

[0018] Fig. 3 shows an exemplary result of quantitative measurement of *Centella asiatica* exosomes through Nanoparticle Tracking Analysis (NTA) device.

[0019] Fig. 4 shows an exemplary result of quantitative measurement of miRNA content in *Centella asiatica* exosomes.

[0020] Fig. 5 shows an exemplary result of *in vitro* assay of NF- κ B expression in human epidermal keratinocytes treated with *Centella asiatica* extracts under UVB radiation.

[0021] Fig. 6 shows an exemplary result of *in vitro* assay of NF- κ B expression in human epidermal keratinocytes treated with *Centella asiatica* exosomes under UVB radiation.

[0022] Fig. 7 shows an exemplary result of *in vitro* assay of collagen type I alpha 1 expression in human dermal fibroblasts treated with Centella asiatica extracts.

[0023] Fig. 8 shows an exemplary result of *in vitro* assay of collagen type I alpha 1 expression in human dermal fibroblasts treated with Centella asiatica exosomes.

[0024] Fig. 9 shows an exemplary result of *in vitro* assay of matrix metalloproteinase-1 expression in human dermal fibroblasts treated with Centella asiatica extracts.

[0025] Fig. 10 shows an exemplary result of *in vitro* assay of matrix metalloproteinase-1 expression in human dermal fibroblasts treated with Centella asiatica exosomes.

[0026] Fig. 11 is a graph showing an exemplary result of *in vitro* assay of wound healing with HaCaT keratinocytes treated with Centella asiatica extracts and Centella asiatica exosomes.

[0027] Fig. 12 shows exemplary images of *in vitro* assay of wound healing with HaCaT keratinocytes treated with Centella asiatica extracts and Centella asiatica exosomes.

Detailed Description of the Invention

[0028] The present invention may be understood more readily by reference to the following detailed description of certain embodiments of the invention.

[0029] Throughout this application, where publications are referenced, the disclosures of these publications are hereby incorporated by reference, in their entireties, into this application in order to more fully describe the state of art to which this invention pertains.

[0030] The present invention provides human skincare compositions with anti-inflammatory effect, wrinkle reduction, and cell regeneration promotion.

[0031] In certain embodiments, the human skincare compositions comprise:

glycerin 8.80 – 17.50 w/v%,
 butylene glycol 3.20 – 5.30 w/v%,
 carbomer 0.15 – 0.25 w/v%,
 polysorbate 20 0.05 – 0.10 w/v%,
 1,2-hexanediol 2.30 – 3.20 w/v%,
 Centella asiatica exosomes 1×10^7 particles/ml or above,
 niacinamide 23.40 – 32.15 w/v%,
 Centella Asiatica leaf extract 2.30 -10.0 w/v%,

dipropylene glycol 1.15 – 2.30 w/v%,
hydrogenated Lecithin 0.25 – 0.50 w/v%,
panthenol 0.25 – 0.50 w/v%,
asiaticoside 0.10 – 0.15 w/v%,
madecassic acid 0.050 – 0.100 w/v%,
asiatic acid 0.05 – 0.10 w/v%,
beta-glucan 0.020 – 0.05 w/v%,
ceramide NP 0.020 – 0.05 w/v%, and
water 40.90.00 – 49.70%.

[0032] In certain embodiments, the concentration of *Centella asiatica* exosomes is in the range of 1×10^7 particles/ml to 1×10^8 particles/ml.

[0033] In certain embodiments, the concentration of *Centella asiatica* extract is in the range of 2.30-3.20 w/v%.

[0034] In certain embodiments, the human skincare compositions further comprise:

hydrolyzed sponge 0.50 – 1.15 w/v%,
sodium lactate 0.15 – 0.25 w/v%, and
caprylyl glycol 0.020 – 0.05 w/v%.

[0035] In certain embodiments, the human skincare compositions further comprise:

palmitoyl tripeptide-1 0.0010 – 0.0020 w/v%,
palmitoyl tetrapeptide-7 0.00050 – 0.0010 w/v%, and
acetyl hexapeptide-8 0.00005 – 0.00050 w/v%.

[0036] The palmitoyl tripeptide-1 contains Gly-His-Lys (GHK).

[0037] The palmitoyl tetrapeptide-7 contains Gly-Gln-Pro-Arg (GQPR, SEQ ID NO. 1).

[0038] The acetyl hexapeptide-8 contains Glu-Glu-Met-Glu-Arg-Arg (EEMERR, SEQ ID NO. 2).

[0039] The following examples are provided for the sole purpose of illustrating the principles of the inventions presented in the present application; they are, by no means, intended to limit the scope of the present inventions.

[0040] Examples

[0041] Preparation and characterization of *Centella asiatica* leaf extracts and *Centella asiatica* exosomes

[0042] Centella asiatica leaf extracts and Centella asiatica exosomes are prepared according substantially to the disclosures of Cho et al. (Cho et al. (2022) Confirmation of plant-derived exosomes as bioactive substances for skin application through comparative analysis of keratinocyte transcriptome. *Applied Biological Chemistry*. 65:8; doi: 10.1186/s13765-022-00676-z).

[0043] The obtained Centella asiatica exosomes are analysed for their sizes and concentrations, and contents of phospholipids and miRNAs.

[0044] Referring now to Fig. 1, there is provided an exemplary Cryo-TEM image of Centella asiatica exosomes showing that the sizes are less than 200 nm. Cryo-TEM can rapidly freeze-dry liquids at ultra-low temperatures to non-invasively preserve microscopic samples, including proteins or viruses, in their natural state as much as possible.

[0045] Referring now to Fig. 2, there is provided an exemplary oil-red staining image of Centella asiatica exosomes. The principle of staining phospholipids with oil-red O reagent is to identify phospholipids in stained Centella asiatica exosomes, showing the stained phospholipids in red.

[0046] Referring now to Fig. 3, there is provided an exemplary result of quantitative measurement of Centella asiatica exosomes through Nanoparticle Tracking Analysis (NTA) device. The diffusion coefficient is calculated by measuring the Brownian motion rate of nanoparticles.

[0047] Referring now to Fig. 4, there is provided an exemplary result of quantitative measurement of miRNA content in Centella asiatica exosomes. The small RNAs (miRNAs) in the Centella asiatica exosomes are separated, purified, and then quantified by measuring absorbance at A230, A260, and A280. miRNAs can suppress the expression of target genes through crosskingdom regulation.

[0048] Human skincare composition

[0049] Human skincare composition (I) comprises:

glycerin 11.54%,
butylene glycol 3.50%,
carbomer 0.175%,
polysorbate 20 0.088%,
palmitoyl tripeptide-1 0.00170%,
palmitoyl tetrapeptide-7 0.00087%,

1,2-hexanediol 2.92%,
 acetyl hexapeptide-8 0.00029%
 niacinamide 24.84%,
 Centella Asiatica leaf extract 1%, 2%, 5%, or 10%,
 dipropylene glycol 1.46%,
 hydrogenated Lecithin 0.29%
 panthenol 0.29%,
 asiaticoside 0.117%,
 madecassic acid 0.088%,
 asiatic acid 0.088%,
 beta-glucan 0.029%,
 ceramide NP 0.029%, and
 water 44.38%.

[0050] Human skincare compositions (II) comprise human skincare composition (I) and Centella asiatica exosomes 1×10^5 particles/ml, 1×10^6 particles/ml, 1×10^7 particles/ml, or 1×10^8 particles/ml into the compositions described above.

[0051] Human skincare compositions (IV) comprise human skincare composition (I), (II) or (III) and the following components:

hydrolyzed sponge 0.58%,
 sodium lactate 0.175%, and
 caprylyl glycol 0.029%.

[0052] Compositions (I)-(IV) are tested for their anti-inflammatory effects, wrinkle-improving efficacy, skin penetration efficacy, and glow, moisture, elasticity, and density *in vivo*. Human skincare compositions (III) and (IV) show the similar effects of Human skincare compositions (II), and their results will not be described in details hereinbelow.

[0053] Anti-inflammatory effects of human skincare compositions (I) and (II)

[0054] Human epidermal keratinocytes (HEKa) are cultured following the common procedures and conditions.

[0055] Referring now to Fig. 5, there is provided an exemplary graph showing *in vitro* assay results of NF- κ B expression in human epidermal keratinocytes (HEKa) treated with human skincare compositions (I) under UVB radiation with 20 mJ/cm^2 for 48 hours. No UVB radiation is included as a blank control (CTL(-)), the UVB radiation alone as model control

(CTL), and 0.01% allantoin as a positive control. The concentrations of *Centella asiatica* extract used in the assay are 1%, 2%, 5%, and 10%. The data demonstrate that inflammatory factors are significantly reduced within the treatment range of *Centella asiatica* extract.

[0056] Referring now to Fig. 6, there is provided an exemplary graph showing *in vitro* assay results of NF- κ B expression in cells treated with human skincare compositions (II) under UVB radiation. The control groups are set up as in Fig. 5. The concentrations of *Centella asiatica* exosomes used in the assay are 1×10^5 particles/ml, 1×10^6 particles/ml, 1×10^7 particles/ml, and 1×10^8 particles/ml. The results show that *Centella asiatica* exosomes can significantly reduce the NF- κ B expression in human epidermal keratinocytes (HEKa) under UVB radiation. Furthermore, *Centella asiatica* exosomes can also significantly reduce the NF- κ B expression in human epidermal keratinocytes (HEKa) treated by lipopolysaccharide (LPS). The data demonstrate that inflammatory factors are significantly reduced within the treatment range of *Centella asiatica* exosomes.

[0057] Wrinkle-improving efficacy of human skincare compositions (I) and (II)

[0058] Human dermal fibroblasts and HaCaT keratinocytes are cultured following the common procedures and conditions.

[0059] Referring now to Fig. 7, there is provided an exemplary graph showing *in vitro* assay results of collagen type I alpha 1 expression in human dermal fibroblasts treated with human skincare compositions (I). No treatment is included as a negative control (CTL), and 20nM TGF- β 1 treatment as positive control. The concentrations of *Centella asiatica* extract used in the assay are 1%, 2%, 5%, and 10%. The data demonstrate that collagen synthesis factors are significantly increased within the treatment range of *Centella asiatica* extracts.

[0060] Referring now to Fig. 8, there is provided an exemplary graph showing *in vitro* assay results of collagen type I alpha 1 expression in human dermal fibroblasts treated with human skincare compositions (II). The control groups are set up as in Fig. 7. The concentrations of *Centella asiatica* exosomes used in the assay are 1×10^5 particles/ml, 1×10^6 particles/ml, 1×10^7 particles/ml, and 1×10^8 particles/ml. The data demonstrate that collagen synthesis factors are significantly increased within the treatment range of *Centella asiatica* exosomes.

[0061] Referring now to Fig. 9, there is provided an exemplary graph showing *in vitro* assay results of matrix metalloproteinase-1 expression in human dermal fibroblasts treated with human skincare compositions (I) under UVB radiation with 20 mJ/cm^2 for 48 hours. No UVB radiation is included as a blank control (CTL(-)), the UVB radiation alone as model

control (CTL), and 20nM TGF- β 1 treatment as a positive control. The concentrations of *Centella asiatica* extract used in the assay are 1%, 2%, 5%, and 10%. The data demonstrate that collagen decomposition factors are significantly reduced within the treatment range of *Centella asiatica* extracts.

[0062] Referring now to Fig. 10, there is provided an exemplary graph showing *in vitro* assay results of matrix metalloproteinase-1 expression in human dermal fibroblasts treated with human skincare compositions (II) under UVB radiation with 20mJ/cm² for 48 hours. The control groups are set up as in Fig. 9. The concentrations of *Centella asiatica* exosomes used in the assay are 1x10⁵ particles/ml, 1x10⁶ particles/ml, 1x10⁷ particles/ml, and 1x10⁸ particles/ml. The data demonstrate that collagen decomposition factors are significantly reduced within the treatment range of *Centella asiatica* exosomes.

[0063] Referring now to Fig. 11, there is provided a graph showing an exemplary result of *in vitro* assay of wound healing with HaCaT keratinocytes treated with human skincare compositions (I) and human skincare compositions (II). Confluent HaCaT keratinocytes are scratched, and then treated with human skincare compositions (I) (*Centella asiatica* extract at 1%, 2%, 5%, or 10% respectively), or with human skincare compositions (II) (*Centella asiatica* exosomes at 1x10⁵ particles/ml, 1x10⁶ particles/ml, 1x10⁷ particles/ml, or 1x10⁸ particles/ml respectively). No treatment is included as a model control (CTL) and 0.1% madecassoside treatment as positive control. The width of the scratches is measured after 12 hours, and the data are shown in Fig. 11. Fig. 12 shows the exemplary images of wound healings with HaCaT keratinocytes treated with human skincare compositions (I) and human skincare compositions (II). The data demonstrate that cell growth and migration are significantly increased within the treatment range of *Centella asiatica* extract or *Centella asiatica* exosomes, showing that *Centella asiatica* extract and *Centella asiatica* exosomes are effective in cell regeneration.

[0064] While the present invention has been described with reference to particular embodiments, it will be understood that the embodiments are illustrative and that the invention scope is not so limited. Alternative embodiments of the present invention will become apparent to those having ordinary skill in the art to which the present invention pertains. Such alternate embodiments are considered to be encompassed within the scope of the present invention. Accordingly, the scope of the present invention is defined by the appended claims and is supported by the foregoing description.

CLAIMS

What is claimed is:

1. A human skincare composition comprise:

glycerin 8.80 – 17.50 w/v%,
butylene glycol 3.20 – 5.30 w/v%,
carbomer 0.15 – 0.25 w/v%,
polysorbate 20 0.05 – 0.10 w/v%,
1,2-hexanediol 2.30 – 3.20 w/v%,
Centella asiatica exosomes 1×10^7 particles/ml or above,
niacinamide 23.40 – 32.15 w/v%,
Centella Asiatica leaf extract 2.30 -3.20 w/v%
dipropylene glycol 1.15 – 2.30 w/v%,
hydrogenated Lecithin 0.25 – 0.50 w/v%,
panthenol 0.25 – 0.50 w/v%,
asiaticoside 0.10 – 0.15 w/v%,
madecassic acid 0.050 – 0.100 w/v%,
asiatic acid 0.05 – 0.10 w/v%,
beta-glucan 0.020 – 0.05 w/v%,
ceramide NP 0.020 – 0.05 w/v%, and
water 40.90.00 – 49.70%.

2. The human skincare composition of claim 1, wherein the concentration of Centella asiatica exosomes is in the range of 1×10^7 particles/ml to 1×10^8 particles/ml.
3. The human skincare composition of claim 1, wherein the concentration of Centella asiatica extract is in the range of 2.30-3.20 w/v%.
4. The human skincare composition of claim 1, further comprising:
hydrolyzed sponge 0.50 – 1.15 w/v%,
sodium lactate 0.15 – 0.25 w/v%, and

caprylyl glycol 0.020 – 0.05 w/v %.

5. The human skincare composition of claim 1, further comprising:
palmitoyl tripeptide-1 0.0010 – 0.0020 w/v %,
palmitoyl tetrapeptide-7 0.00050 – 0.0010 w/v %, and
acetyl hexapeptide-8 0.00005 – 0.00050 w/v %.