

Composition and method for treating skin pigmentation disorders

Field

The present disclosure relates to compositions for preventing and/or treating a skin pigmentation disorder, by degrading melanin in dermal fibroblasts. The present disclosure also relates to methods and uses for treating skin pigmentation disorders, and for degrading melanin in dermal fibroblasts, and non-therapeutic methods for lightening skin.

Background

Melanogenesis is a process that occurs in melanosomes by melanocytes involving a series of enzymatic and chemical reactions to produce the pigment melanin, which predominantly occurs in the epidermal layer of the skin. Skin hyperpigmentation is a common condition in humans that is primarily caused by excessive melanin synthesis and storage through uncontrolled melanogenesis. Increased melanin production can be triggered by numerous factors including excessive sun exposure, hormonal changes, skin inflammation and age, as well as many other biological processes (Nieuweboer-Krobotova, *et al.*, 2013).

Oxidative stress caused by the sun's UVA and UVB rays, together with high-energy visible light, is major cause of sun-induced skin hyperpigmentation issues which results in small, darkened patches of skin, known as sun spots. Larger patches of hyperpigmentation, mainly on the face, called melasma, which are particularly common among women, are thought to occur when sex hormones such as estrogen and progesterone stimulate the overproduction of melanin, which can also be exacerbated through sun exposure. Post-inflammatory hyperpigmentation refers to the darkening of skin that occurs due to uncontrolled melanogenic processes resulting from an inflammatory eruption or cutaneous injury. These and other hyperpigmentary skin disorders can significantly impact an individual's appearance and may cause emotional and psychological distress and reduced quality of life.

Because of the cosmetically important issue of hyperpigmentation, there is an ever-growing demand for melanogenesis inhibitors. Existing topical hyperpigmentation treatments are based on accelerated epidermal turnover which rely on the removal of melanin from the outer layers of the skin. Such treatments include exfoliating agents (e.g. AHA or BHA), anti-melanogenic agents including kojic acid and hydroquinone, that act to

inhibit melanin synthesis, melanosome blockers (e.g. niacinamide) which act to retard melanosome transfer from melanocytes to keratinocytes, and anti-inflammatory agents (e.g. stearyl glycyrrhetinate) which act to retard skin inflammation, as well as combinations of any of above.

5 Although the above-mentioned topical treatments are effective in lightening skin tone, they are less effective in treating hyperpigmentation disorders. This is due to their mode of action primarily acting upon epidermal melanosis. In addition, many products currently available on the market for treating hyperpigmentation may contain ingredients that have been associated with possible adverse effects, including hydroquinone and kojic
10 acid. As an alternative to the above topical treatments, facial laser treatments including Q-switched with nano-second pulse-width technology are considered effective methods for lightening or eliminating dermal melanosis, but these treatments are costly and invasive (Watanabe, 2014).

 Therefore, there remains a need for highly safe and effective anti-hyperpigmentation
15 compositions to overcome the limitations and drawbacks of the prior art.

Summary of the Invention

 It has now been discovered that dermal fibroblast cells are involved in uptake of melanosomes, and that melanin can be retained in fibroblast cells and may be degraded at a
20 slow rate contributing to the persistent darker skin tone. It has also been identified that 3-O-ethyl ascorbic acid, an ascorbic acid derivative, is particularly effective in reducing the melanin content in fibroblast cells in a biomimetic skin melanosis model, and potent anti-hyperpigmentation efficacy in diminishing dermal melanin. It has further been found that the combination of 3-O-ethyl ascorbic acid together with certain anti-melanogenic agents
25 provides superior anti-hyperpigmentation properties, providing superior results in diminishing melanin content in fibroblast cells.

 Accordingly, in one aspect there is provided a composition for preventing and/or treating a skin pigmentation disorder, the composition including:

- a) ascorbic acid or a derivative and/or salt thereof; and
 - 30 b) an anti-melanogenic agent selected from the group consisting of 4-n-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof;
- in an amount effective to degrade melanin in dermal fibroblasts.

In some embodiments, component a) is selected from the group consisting of 3-*O*-ethyl ascorbic acid, L-ascorbic acid, 2-*O*-ethyl ascorbic acid, ascorbyl glucoside, ascorbyl tetraispalmitate, magnesium ascorbyl phosphate, sodium ascorbic phosphate, 3-glyceryl ascorbate, bis-glyceryl ascorbate, hexyl 3-glyceryl ascorbate, myristyl 3-glyceryl ascorbate, and lauryl glyceryl ascorbate. In some embodiments, component a) is selected from the group consisting of 3-*O*-ethyl ascorbic acid, L-ascorbic acid, 2-*O*-ethyl ascorbic acid, ascorbyl tetraispalmitate, sodium ascorbic phosphate, caprylyl-2-glyceryl ascorbate, bis-glyceryl ascorbate, hexyl 3-glyceryl ascorbate, myristyl 3-glyceryl ascorbate, and lauryl glyceryl ascorbate. In some embodiments, component a) is 3-*O*-ethyl ascorbic acid.

10 In some embodiments, component b) is resveratrol. In some embodiments, component b) is pterostilbene. In some embodiments, component b) is sclareolide. In some embodiments, component b) is 4-*n*-butylresorcinol.

In some embodiments, the amount of component a) is in the range of from 0.01% to 50% by weight of the composition, optionally from 0.5% to 5% by weight of the composition, optionally from 0.5% to 1.5% by weight of the composition.

In some embodiments, the amount of component b) is in the range of from 0.01% to 5% by weight of the composition, optionally from 0.1% to 0.5% by weight of the composition, or optionally from 0.2% to 0.35% by weight of the composition.

In some embodiments, the weight ratio of component a) to component b) is in the range of from 2:1 to 200:1, optionally from 2:1 to 50:1, or optionally from 3:1 to 5:1.

In some embodiments, the composition does not contain any anti-melanogenic agent other than a) and b).

In some embodiments, the composition comprises 3-*O*-ethyl ascorbic acid as component a) and resveratrol or 4-*n*-butylresorcinol as component b), and wherein the weight ratio of component a) to component b) is in the range of from 5:1 to 50:1.

In some embodiments, the composition is for topical application.

In some embodiments, the composition comprises one or more of the following: an anti-inflammatory agent, a sunscreen agent, an antioxidant, an anti-aging peptide, a desquamation agent, and an exfoliating agent.

30 In some embodiments, the composition comprises one or more of the following: water, an alkyl benzoate, PPG-3 myristyl ether, hydroxyacrylate/sodium acryloyldimethyl taurate copolymer, ethylhexylglycerin, phenoxythanol, methylpropanediol, betaine, sclerotium gum, sodium polyacryloyldimethyl taurate, disodium EDTA, sodium

hyaluronate, bis-PEG-18 methyl ether dimethyl silane, PEG-12 dimethicone, glycerine, chlorphenesin, sodium metabisulfite, lactic acid, dicaprylyl carbonate, and polyacrylate crosspolymer-6.

In some embodiments, the composition is in the form of a cream, lotion, paste, wax,
5 liquid, semisolid composition or a sprayable composition.

In another aspect, there is provided a method of preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder in a subject, or a method of degrading melanin in dermal fibroblasts of a subject, comprising administering an effective amount of a composition as defined herein to the subject.

10 In another aspect, there is provided use of a composition as defined herein for the manufacture of a medicament for the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder in a subject, or for degrading melanin in dermal fibroblasts of a subject.

In another aspect, there is provided a composition as defined herein for use in the
15 prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder.

In another aspect, there is provided a composition as defined herein for use in degrading melanin in dermal fibroblasts.

In some embodiments, the skin pigmentation disorder is selected from the group
20 consisting of skin hyperpigmentation, melasma, solar lentigo, nevus of Ota, nevus of Ito, and Mongolian spots.

In some embodiments, the composition is applied topically to the skin of the subject.

In another aspect, there is provided a method of preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder in a subject, or of degrading
25 melanin in dermal fibroblasts of a subject, comprising administering an effective amount of

a) ascorbic acid or a derivative and/or salt thereof,
in an amount effective to degrade melanin in dermal fibroblasts.

In some embodiments, the ascorbic acid or a derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-
30 butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

In another aspect, there is provided ascorbic acid or a derivative and/or salt thereof, for use in the prevention, treatment, and/or reduction of a symptom associated with, a skin

pigmentation disorder, wherein the ascorbic acid or derivative and/or salt thereof is administered in an amount effective to degrade melanin in dermal fibroblasts.

In some embodiments, the ascorbic acid or derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

In another aspect, there is provided ascorbic acid or a derivative and/or salt thereof, for use in degrading melanin in dermal fibroblasts.

In some embodiments, the ascorbic acid or derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

In another aspect, there is provided an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for use in the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

In another aspect, there is provided an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for use in degrading melanin in dermal fibroblasts, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

In another aspect, there is provided use of ascorbic acid or a derivative and/or salt thereof, for the manufacture of a medicament, for preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder, or for degrading melanin in dermal fibroblasts, wherein the ascorbic acid or derivative and/or salt thereof is administered in an amount effective to degrade melanin in dermal fibroblasts.

In some embodiments, the ascorbic acid or derivative and/or salt thereof is administered in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

In another aspect, there is provided use of an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for the manufacture of a medicament, for preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder, or for degrading melanin in dermal fibroblasts, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

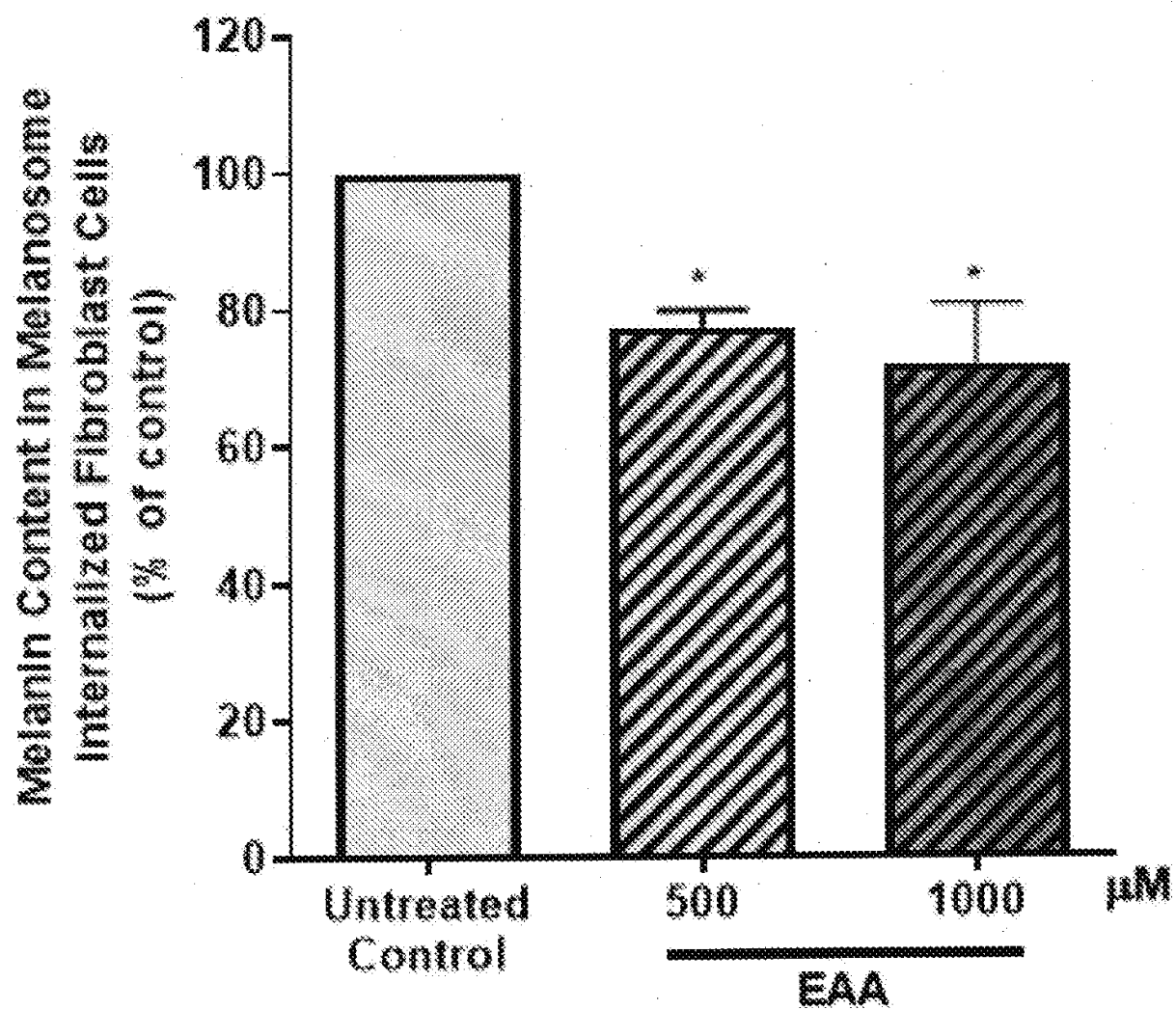


Figure 4

Figure 3 shows an illustration of the biomimetic dermal melanosis skin model.

Figure 4 provides a graphical representation of the effect of 3-*O*-ethyl ascorbic acid on inhibition of melanin content in melanosome internalized fibroblast cells. Data are expressed in mean $\pm$ S.E.M of two independent experiments (n=2). *p<0.05 when compared to untreated control by one-way ANOVA.

Detailed Description

Definitions

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., chemistry, biology, and the like).

The present disclosure refers to the entire contents of certain documents being incorporated herein by reference. In the event of any inconsistent teaching between the teaching of the present disclosure and the contents of those documents, the teaching of the present disclosure takes precedence.

It is to be understood that if any prior art publication is referred to herein, such reference does not constitute an admission that the publication forms a part of the common general knowledge in the art.

As used herein, the term "and/or", e.g., "X and/or Y" shall be understood to mean either "X and Y" or "X or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

As used herein, the term about, unless stated to the contrary, refers to $\pm$ 10%, of the designated value.

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter. Thus, as used herein, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. For example, reference to "a" includes a single as well as two or more; reference to "an" includes a single as well as two or more; reference to "the" includes a single as well as two or more and so forth.

Unless otherwise indicated, terms such as "first," "second," etc. are used herein merely as labels, and are not intended to impose ordinal, positional, or hierarchical requirements on the items to which these terms refer. Moreover, reference to a "second" item does not require or preclude the existence of lower-numbered item (e.g., a "first" item) and/or a higher-numbered item (e.g., a "third" item).

As used herein, the phrase "at least one of", when used with a list of items, means different combinations of one or more of the listed items may be used and only one of the items in the list may be needed. The item may be a particular object, thing, or category. In other words, "at least one of" means any combination of items or number of items may be used from the list, but not all of the items in the list may be required. For example, "at least one of item A, item B, and item C" may mean item A; item A and item B; item B; item A, item B, and item C; or item B and item C. In some cases, "at least one of item A, item B, and item C" may mean, for example and without limitation, two of item A, one of item B, and ten of item C; four of item B and seven of item C; or some other suitable combination.

As used herein, the word "comprise" and other forms of the word, such as "comprising" and "comprises," means including but not limited to, and is not intended to exclude, for example, other additives, components, integers, or steps.

As used herein, the term "subject" refers to any organism that is susceptible to a disease or condition. For example, the subject can be an animal, a mammal, a primate, a livestock animal (e.g., sheep, cow, horse, pig), a companion animal (e.g., dog, cat), or a laboratory animal (e.g., mouse, rabbit, rat, guinea pig, hamster). In one embodiment, the subject is a mammal. In one embodiment, the subject is human. In one embodiment, the subject is a non-human animal.

As used herein, the term "treating" includes alleviation of symptoms associated with a specific disorder or condition.

As used herein, the term "prevention" includes prophylaxis of the specific disorder or condition.

The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

Each embodiment of the present disclosure described herein is to be applied mutatis mutandis to each and every other embodiment unless specifically stated otherwise, or required otherwise by context.

Compositions

As discussed above, the present disclosure relates to compositions and methods for preventing and/or treating a skin pigmentation disorder, by degrading melanin in dermal fibroblasts. The composition has an anti-melanogenic agent which, when used in combination with ascorbic acid or a derivative and/or salt thereof, provides for effective degradation of dermal melanin.

Accordingly, in one aspect, there is provided a composition for preventing and/or treating a skin pigmentation disorder, the composition including: (a) ascorbic acid or a derivative and/or salt thereof; and (b) an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof; in an amount effective to degrade melanin in dermal fibroblasts.

As used herein, the term "anti-melanogenic agent", refers to an agent that possesses melanogenic inhibitory activity. Without wishing to be bound by theory, the inhibitory activity may in some embodiments act through inhibition of tyrosinase activity. Tyrosinase is a copper-containing enzyme present in plant and animal tissues that catalyses the production of melanin and other pigments from tyrosine by oxidation.

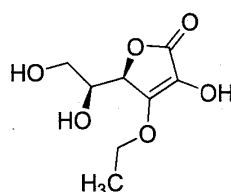
The ascorbic acid derivatives and/or salt forms thereof encompassed by the present invention may be ascorbic acid or any known derivative of ascorbic acid and/or salt form thereof. The ascorbic acid derivatives and/or salt forms thereof can be chemically synthesized or extracted from any natural resource known to persons skilled in art of synthetic organic chemistry, medicinal chemistry, and phytochemistry. Those skilled in the art will appreciate that there are many processes, both known in the art and described in various patents and publications that can be used to obtain the ascorbic acid derivatives and/or salt forms thereof to be used in practicing the present invention.

In some embodiments, the ascorbic acid derivative and/or salt form thereof is selected from the group consisting of 3-*O*-ethyl ascorbic acid (3-EAA), L-ascorbic acid, 2-*O*-ethyl ascorbic acid, ascorbyl glucoside, ascorbyl tetraisopalmitate, magnesium ascorbyl phosphate, sodium ascorbic phosphate, caprylyl-2-glyceryl ascorbate, bis-glyceryl ascorbate, 3-glyceryl ascorbate, hexyl 3-glyceryl ascorbate, myristyl 3-glyceryl ascorbate, laurylglyceryl ascorbate.

In some embodiments, the ascorbic acid derivative and/or salt form thereof is selected from the group consisting of 3-*O*-ethyl ascorbic acid, L-ascorbic acid, 2-*O*-ethyl ascorbic acid, ascorbyl tetraisopalmitate, sodium ascorbic phosphate, caprylyl-2-glyceryl

ascorbate, 3-glyceryl ascorbate, bis-glyceryl ascorbate, hexyl 3-glyceryl ascorbate, myristyl 3-glyceryl ascorbate, and lauryl glyceryl ascorbate.

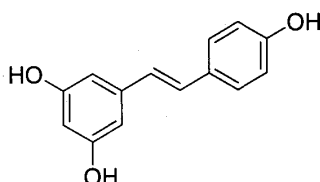
In some embodiments, the ascorbic acid derivative and/or salt forms thereof is 3-*O*-ethyl ascorbic acid (3-EAA). The chemical structure of 3-*O*-ethyl ascorbic acid is depicted below:



3-*O*-ethyl ascorbic acid can be chemically synthesized or extracted from any natural resource known to persons skilled in art in synthetic organic chemistry, medicinal chemistry, and phytochemistry or obtained from any commercial sources. Commercial sources for obtaining 3-*O*-ethyl ascorbic acid include, but are not limited to, Nippon Fine Chemical Co. Ltd. (Trade name: Ethyl ascorbic acid), Corum Inc. (Trade name: Et-VCTM), Kimika, LLC (Trade name: Neosome OL EAA), and Naturalis SRL (Trade name: Nio-VCS).

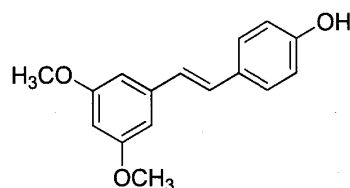
The aforementioned anti-melanogenic agents can also be chemically synthesized or extracted from any natural resource known to persons skilled in art of synthetic organic or medicinal chemistry, and phytochemistry or obtained from any commercial sources.

In some embodiments, the anti-melanogenic agent is resveratrol. The chemical structure of resveratrol is depicted below:



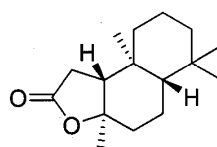
Commercial sources for obtaining resveratrol include, but are not limited to, SpecChem Industry (Trade name: SpecKare ® Resveratrol), DSM Nutritional Products LLC (Trade name: Regu®-Fade), Salvona Technologies Inc. (Trade name: Salphere Resveratrol), Biogenics Inc. (Trade name: BioGenic Resveratrol-200), and BCR Bio Component Research (Trade name: Resveratrol BT).

In some embodiments, the anti-melanogenic agent is pterostilbene. The chemical structure of pterostilbene is depicted below:



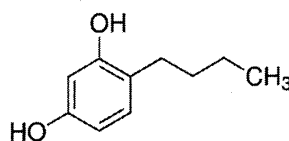
Commercial sources for obtaining pterostilbene include, but are not limited to, Glanbia Nutritional (Trade name: pTeroPure), Sabinsa Cosmetics (Trade name: PteroWhite® 90%), and Spec-Chem Industry (Trade name: SpecPure® PTB).

- 5 In some embodiments, the anti-melanogenic agent is sclareolide. The chemical structure of sclareolide is depicted below:



Commercial sources for obtaining sclareolide include, but are not limited to, Symrise (Trade name: Symbright ® 2036), and MMP International (Trade name: Clary Sage FE).

- 10 In some embodiments, the anti-melanogenic agent is 4-*n*-butylresorcinol. The chemical structure of 4-*n*-butylresorcinol is depicted below:



- Commercial sources for obtaining 4-*n*-butylresorcinol include, but are not limited to, Kumar Organic Product Limited (Trade name: Kopcino), and Biochempro Incorporation (Trade name: Actosome Whitenol).
- 15

- The ascorbic acid or a derivative and/or salt thereof and anti-melanogenic agent may be provided in any suitable form. For example, in some embodiments, one or more of the substances may be provided in an encapsulated format. Encapsulation can be used to improve water-dispersibility, chemical stability, and bioavailability. In some other
- 20
- embodiments, some or all of the substances are not provided in an encapsulated format.

The compositions of the present disclosure may provide component a) and an anti-melanogenic agent, component b) in any combination that is effective to degrade melanin in dermal fibroblasts. Advantageously, combinations of a) and b) have been found to work synergistically in providing enhanced melanin degradation relative to the components alone.

In some embodiments, component a) is 3-*O*-ethyl ascorbic acid and component b) is resveratrol. Advantageously, the present inventors have shown that 3-*O*-ethyl ascorbic acid and resveratrol work synergistically to inhibit melanogenesis activity in the epidermis to diminish melanin content in melanosome internalized fibroblasts in a skin model of dermal melanosis. In some other embodiments, component a) is 3-*O*-ethyl ascorbic acid and component b) is pterostilbene. In other embodiments, component a) is 3-*O*-ethyl ascorbic acid and component b) is sclareolide. In some other embodiments, component a) is 3-*O*-ethyl ascorbic acid and component b) is 4-*n*-butylresorcinol.

The composition of the present disclosure comprises an amount of component a) and component b) effective to degrade melanin in dermal fibroblasts. For example, the composition of the present disclosure may comprise component a) in a range from 0.01% to 50% by weight of the composition, or from 0.01% to 40%, or from 0.01% to 30%, or from 0.01% to 20%, or from 0.01% to 10%, or from 0.01% to 5%, or from 0.05% to 10%, or from 0.15 to 10%, or from 0.25 to 10%, or from 0.50 to 10%, or from 0.50 to 7.5%, or from 0.5 to 5%, or from 0.5 to 4%, or from 0.5 to 3%, or from 0.5 to 2%, or from 1 to 2%, or from 0.5 to 1.5%.

In some embodiments, the composition comprises an amount of component a) in the range of from 0.01% to 50% by weight of the composition, optionally from 0.5% to 5% by weight of the composition, optionally from 0.5% to 1.5% by weight of the composition.

In some embodiments, the composition of the present disclosure comprises 3-*O*-ethyl ascorbic acid in an amount within the range of from 0.01% to 50% by weight of the composition, or from 0.01% to 40%, or from 0.01% to 30%, or from 0.01% to 20%, or from 0.01% to 10%, or from 0.01% to 5%, or from 0.05% to 10%, or from 0.15 to 10%, or from 0.25 to 10%, or from 0.50 to 10%, or from 0.50 to 7.5%, or from 0.5 to 5%, or from 0.5 to 4%, or from 0.5 to 3%, or from 0.5 to 2%, or from 1-2%, or from 0.5 to 1.5%.

The composition of the present disclosure may comprise component b) in a range from 0.01 to 5%, or from 0.01 to 4.5%, or from 0.01 to 4%, or from 0.01 to 3.5%, or from 0.02 to 5%, or from 0.03 to 5%, or from 0.05 to 5%, or from 0.05 to 4.5%, or from 0.05 to 4%, or from 0.05 to 3.5%, or from 0.05 to 3%, or from 0.05 to 2.5%, or from 0.05 to 2.0%, or from 0.05 to 1%, or from 0.05 to 0.3%, or from 0.1 to 2%, or from 0.1 to 1%, or from 0.1 to 0.5%, or from 0.1 to 0.4%, or from 0.1 to 0.3%.

In some embodiments, the composition comprises an amount of component b) in the range of from 0.01% to 5% by weight of the composition, optionally from 0.1% to 0.5% by weight of the composition, or optionally from 0.2% to 0.35% by weight of the composition.

In some embodiments, the composition of the present disclosure comprises
5 resveratrol in an amount within the range of from 0.01 to 5%, or from 0.01 to 4.5%, or from 0.01 to 5%, or from 0.01 to 5%, or from 0.02 to 5%, or from 0.03 to 4%, or from 0.05 to 3.5%, or from 0.05 to 4.5%, or from 0.05 to 4%, or from 0.05 to 3.5%, or from 0.05 to 3%, or from 0.05 to 2.5%, or from 0.05 to 2.0%, or from 0.05 to 1%, or from 0.05 to 0.3%, or from 0.1 to 2%, or from 0.1 to 1%, or from 0.1 to 0.5%, or from 0.1 to 0.4%, or from 0.1 to
10 0.3%, or about 0.05%, or about 1%. In some embodiments, the resveratrol is encapsulated resveratrol.

In some embodiments, the composition of the present disclosure comprises 4-n-butylresorcinol in an amount within the range of from 0.01 to 5%, or from 0.01 to 4.5%, or from 0.01 to 4%, or from 0.01 to 3.5%, or from 0.02 to 5%, or from 0.03 to 5%, or from
15 0.05 to 5%, or from 0.05 to 4.5%, or from 0.05 to 4%, or from 0.05 to 3.5%, or from 0.05 to 3%, or from 0.05 to 2.5%, or from 0.05 to 2.0%, or from 0.05 to 1%, or from 0.05 to 0.3%, or from 0.1 to 2%, or from 0.1 to 1%, or from 0.1 to 0.5%, or from 0.1 to 0.4%, or from 0.1 to 0.3%, or about 0.3%. In some embodiments, the 4-n-butylresorcinol is encapsulated 4-n-butylresorcinol.

In some embodiments, the composition of the present disclosure comprises
20 pterostilbene in an amount within the range of from 0.01 to 5%, or from 0.01 to 4.5%, or from 0.01 to 4%, or from 0.01 to 3.5%, or from 0.02 to 5%, or from 0.03 to 5%, or from 0.05 to 5%, or from 0.05 to 4.5%, or from 0.05 to 4%, or from 0.05 to 3.5%, or from 0.05 to 3%, or from 0.05 to 2.5%, or from 0.05 to 2.0%, or from 0.05 to 1%, or from 0.05 to 0.3%,
25 or from 0.1 to 2%, or from 0.1 to 1%, or from 0.1 to 0.5%, or from 0.1 to 0.4%, or from 0.1 to 0.3%. In some embodiments, the pterostilbene is encapsulated pterostilbene.

In some embodiments, the composition of the present disclosure comprises
30 sclareolide in an amount within the range of from 0.01 to 5%, or from 0.01 to 4.5%, or from 0.01 to 4%, or from 0.01 to 3.5%, or from 0.02 to 5%, or from 0.03 to 5%, or from 0.05 to 5%, or from 0.05 to 4.5%, or from 0.05 to 4%, or from 0.05 to 3.5%, or from 0.05 to 3%, or from 0.05 to 2.5%, or from 0.05 to 2.0%, or from 0.05 to 1%, or from 0.05 to 0.3%, or from 0.1 to 2%, or from 0.1 to 1%, or from 0.1 to 0.5%, or from 0.1 to 0.4%, or from 0.1 to 0.3%.
In some embodiments, the sclareolide is encapsulated sclareolide.

The compositions of the present disclosure may comprise an amount of component a) and an amount of component b) in a weight ratio effective to degrade melanin in dermal fibroblasts. For example, the composition of the present disclosure may comprise an amount of component a) in greater amounts relative to component b), such as in weight ratios from
5 200:1, or from 180:1, or from 160:1, or from 140:1, or from 120:1, or from 100:1, or from 80:1, or from 60:1, or from 50:1, or from 40:1, or from 30:1, or from 20:1, or from 10:1, or from 8:1, or from 6:1, or from 5:1, or from 4:1, or from 3:1, or from 2:1, or more than 1:1.

In some embodiments, the composition comprises a weight ratio of component a) to component b) in the range of from 2:1 to 200:1, from 2:1 to 100:1, from 2:1 to 50:1, from
10 2:1 to 30:1, from 3:1 to 25:1, from 10:1 to 25:1, from 3:1 to 10:1, or from 3:1 to 5:1.

In some embodiments, the composition comprises a weight ratio of component a) to component b) in the range of from 2:1 to 200:1, optionally from 2:1 to 50:1, or optionally from 3:1 to 5:1.

In some embodiments, the composition comprises from 0.5 to 5% by weight of 3-*O*-ethyl ascorbic acid as component a) and comprises from 0.01 to 0.5% by weight of resveratrol as component b), and optionally wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 25:1, or from 4:1 to 25:1, or from 10:1 to 25:1, or about 20:1. In some embodiments, the resveratrol is encapsulated resveratrol.

In some embodiments, the composition comprises from 1 to 2% by weight of 3-*O*-ethyl ascorbic acid as component a) and comprises from 0.1 to 0.2% by weight of resveratrol as component b), and optionally wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 25:1, or from 4:1 to 25:1, or from 10:1 to 25:1, or about 20:1. In some embodiments, the resveratrol is encapsulated resveratrol.

In some embodiments, the composition comprises from 0.5 to 5% by weight of 3-*O*-ethyl ascorbic acid as component a) and comprises from 0.1 to 0.5% by weight of 4-*n*-butylresorcinol as component b), and optionally wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 10:1, from 4:1 to 10:1, or about 6.7:1. In some embodiments, the 4-*n*-butylresorcinol is encapsulated 4-*n*-butylresorcinol.

In some embodiments, the composition comprises from 1 to 2% by weight of 3-*O*-ethyl ascorbic acid as component a) and comprises about 0.3% by weight of 4-*n*-butylresorcinol as component b), and optionally wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 10:1, or from 4:1 to 10:1, or about 6.7:1. In some embodiments, the 4-*n*-butylresorcinol is encapsulated 4-*n*-butylresorcinol.

In some embodiments, the composition comprises from 0.5 to 5% by weight of 3-*O*-ethyl ascorbic acid as component a) and comprises from 0.01 to 0.5% by weight of sclareolide as component b), and optionally wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 25:1, or from 4:1 to 25:1, or from 10:1 to 25:1, or about 20:1. In some embodiments, the sclareolide is encapsulated sclareolide.

In some embodiments, the composition comprises from 1 to 2% by weight of 3-*O*-ethyl ascorbic acid as component a) and comprises from 0.1 to 0.2% by weight of sclareolide as component b), and optionally wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 25:1, or from 4:1 to 25:1, or from 10:1 to 25:1, or about 20:1. In some embodiments, the sclareolide is encapsulated sclareolide.

In some embodiments, the composition comprises from 0.5 to 5% by weight of 3-*O*-ethyl ascorbic acid as component a) and comprises from 0.01 to 0.5% by weight of pterostilbene as component b), and optionally wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 25:1, or from 4:1 to 25:1, or from 10:1 to 25:1, or about 20:1. In some embodiments, the pterostilbene is encapsulated pterostilbene.

In some embodiments, the composition comprises from 1 to 2% by weight of 3-*O*-ethyl ascorbic acid as component a) and comprises from 0.1 to 0.2% by weight of pterostilbene as component b), and optionally wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 25:1, or from 4:1 to 25:1, or from 10:1 to 25:1, or about 20:1. In some embodiments, the pterostilbene is encapsulated pterostilbene.

In some embodiments, the composition comprises 3-*O*-ethyl ascorbic acid as component a) and resveratrol or 4-*n*-butylresorcinol as component b), and wherein the weight ratio of component a) to component b) is in the range of from 5:1 to 50:1.

The synthesis and the degradation of tyrosinase is tightly coupled to its function which are important parameters in the regulation of melanin synthesis. Accordingly, in some embodiments the compositions of the present disclosure may also include additional agents from any natural or synthetic source that act to inhibit or decrease melanogenesis via any mechanism including the down-regulation of tyrosinase and its activity including, but not limited to, *p*-Coumaric Acid; Biochanin A; Calycosin; TMBC; Taxifolin; Luteolin; 2,5-DHAP; Linderanolide B; and Subamolide A.

However, it has also been found that a comparatively simple formulation containing a) and b) provides unexpectedly effective results. Thus, in some other embodiments, the composition does not contain any anti-melanogenic agent other than a) and b). In some

embodiments, the composition consists of or consists essentially of components a) and b), and one or more carriers and/or excipients.

The composition may be formulated as appropriate for any desired method of administration. For example, the composition of the present disclosure may be formulated
5 as a topical composition, suitable to be applied topically to the skin.

The compositions of the present disclosure additionally may also contain various other additives. For example, the compositions of the present disclosure may include any number of known additional cosmetic or pharmaceutical agents. In some embodiments, the composition consists of or consists essentially of components a) and b), one or more carriers
10 and/or excipients, and optionally one or more additional cosmetic or pharmaceutical agents which are not anti-melanogenic agents.

The CTFA Cosmetic Ingredient Handbook, Second Edition, 1992 describes a wide variety of non-limiting cosmetic and pharmaceutical ingredients commonly used in the skin care industry, which may be suitable for use in the compositions of the present invention.
15 Examples of suitable formulation ingredients include: antioxidants, binders, biological additives, buffering agents, colorants, thickeners, pearlescent agents, polymers, astringents, silicones, odor absorbers, gelling agents (e.g. hydrophilic or lipophilic gelling agents), fragrance, humectants, fillers, solvents, opacifying agents, skin-lightening agents, skin tanning agents, perfumes, conditioners, exfoliating agents, solubilisers (e.g.
20 methylpropanediol), sunscreens, vitamins, coloring agents, pH adjusters (e.g. sodium hydroxide), preservatives (e.g. ethylhexylglycerin, phenoxyethanol, or hydroxyacetophenone), rheological modifiers, foaming agents, surfactants, emollients, natural extracts, essential oils, skin sensates, scalp soothing agents, and scalp healing agents.

The composition of the present disclosure may also comprise other skin conditioning
25 agents, including, but not limited to, vitamins or vitamin derivatives and macro or micro capsules containing vitamins or their derivatives, cosmetic peptides, plant extracts, polyphenol compounds, sunscreen additives, sunscreen agents, sensorial additives or texturisers, natural extracts and essential oils.

In some embodiments, the composition of the present disclosure comprises one or
30 more of the following: an anti-inflammatory agent, a sunscreen agent, an antioxidant, an anti-aging peptide, a desquamation agent, and an exfoliating agent. The compositions may be formulated with one or more other components that are advantageous for treatment of skin conditions, for example, hyaluronic acid and/or aloe vera gel.

In some embodiments, the composition comprises a pharmaceutically or cosmetically acceptable carrier or vehicle, and/or comprises one or more excipients.

The pharmaceutically or cosmetically acceptable vehicle may be any pharmaceutically or cosmetically acceptable vehicle, including pharmaceutically and/or dermatologically acceptable carriers, including surfactants. Pharmaceutically and/or dermatologically acceptable carriers are preferably compatible with skin, nails, mucous membranes, tissues and/or hair, and may include any conventionally used pharmaceutical or dermatological carriers meeting these requirements. The carriers and/or vehicles may aid in formulating the ascorbic acid or a derivative and/or a salt thereof, or anti-melanogenic agent, into suitable pharmaceutical or cosmetic formulations. Such carriers may be readily selected by one of ordinary skill in the art. Carriers include, but are not limited to, emollients, emulsifiers, and rheological modifiers.

Emollients suitable for use in the vehicle or carrier for the compositions of the invention include, for example, C₁₂₋₁₅ alkyl benzoates, PPG-3 myristyl ether, paraffinum liquidum and dimethicone. Emulsifiers suitable for use in the vehicle or carrier for the compositions of the invention include, for example, hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, Cetareth-20 (cetostearyl alcohol, which is polyethylene glycol ether of cetearyl alcohol). Rheological modifiers suitable for use in the vehicle or carrier for the compositions of the invention include, for example, carbomer, glyceryl polyacrylate, and acrylates/C₁₀₋₃₀ alkyl acrylate crosspolymer. Other suitable carriers include, but are not limited to, mineral oil, liquid petroleum, white petroleum, propylene glycol, polyoxyethylene and/or polyoxypropylene compounds, emulsifying wax, sorbitan monostearate, polysorbate 60 (polyoxyethylene(20) sorbitan monostearate), cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, sodium hyaluronate, hyaluronic acid, and water.

For topical administration, the compositions of the present invention may for example be formulated as ointments, liquids, lotions, pastes, foams, sprays, serums, masks, gels, hydrogels or transdermal patches, leave-on and rinse-off lotions or toners, skin ampoules, skin cleansers, facial and eye make-up removers, leave on hair conditioners or hair vitamins, hair styling aids, shower gels/creams, toilet bars, antiperspirants, deodorants, depilatories, all colour cosmetics including, but not limited to, lipsticks and foundations, sunless tanners or sunscreen lotions, shampoos, conditioners, hair tonics, mousses, gels, liposomes, or other topically suitable forms.

Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening, emulsifying and/or gelling agents (e.g. emulsion of oil in water, emulsion of water in oil or emulsion of water in silicone). Lotions may be formulated with an aqueous or oily base and generally also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, colouring agents or preservatives (e.g. methyl paraben, propyl paraben, phenoxyethanol). Ointments, creams, lotions and the like may also be formulated to comprise C₁₋₃-alkoxylated oils and waxes (e.g. ethoxylated vegetable oils, ethoxylated jojoba oil/wax, ethoxylated lanolin oil, ethoxylated coconut oil, ethoxylated cocoa butter).

Any cosmetic product that contains water (i.e. creams, gels, lotions, etc) has the potential for bacteria, yeast, and mould to grow, and may in some embodiments require a preservative. Exemplary preservatives for the composition of the present disclosure include ethylhexylglycerine, phenoxyethanol, chlorphensin and sodium metabisulfite. Exemplary emollients include C₁₂₋₁₅ alkyl benzoate, PPG-3 myristyl ether, isopropyl lauroyl sarcosinate, glycerine and bis-PEG-18 methyl ether dimethyl silane. An exemplary humectant is 1,2-hexane diol or betaine. An exemplary rheological modifier includes hydroxyethyl acrylate, sodium acryloyldimethyl taurate copolymer, or mixtures thereof.

In some embodiments, the composition of the present disclosure comprises one or more of the following: water, an alkyl benzoate, PPG-3 myristyl ether, hydroxyacrylate/sodium acryloyldimethyl taurate copolymer, ethylhexylglycerin, phenoxythanol, methylpropanediol, betaine, sclerotium gum, sodium polyacryloyldimethyl taurate, disodium EDTA, sodium hyaluronate, bis-PEG-18 methyl ether dimethyl silane, PEG-12 dimethicone, glycerine, chlorphenesin, sodium metabisulfite, lactic acid, dicaprylyl carbonate, and polyacrylate crosspolymer-6.

The compositions of the present invention may be compositions suitable for topical use. In some embodiments, the composition is for topical application. Advantageously, the ascorbic acid or a derivative and/or a salt thereof and anti-melanogenic agents are capable of penetrating through the stratum corneum to reach the basal layer of the epidermis (where melanocytes are located), to effectively inhibit melanogenesis. Accordingly, topical compositions comprising of 3-*O*-ethyl ascorbic acid and anti-melanogenic agents are expected to improve skin hyperpigmentation clinically, for example conditions such as acne-induced pigmentation and aged-induced pigmentation.

The topical use could for example be by way of incorporating ascorbic acid or a derivative and/or a salt thereof and anti-melanogenic agent in a leave-on or in a rinse off product that is applied to the human body primarily for improving skin appearance and general aesthetic benefit. "Skin" as used herein, is meant to include the external surface of mammals, especially humans and includes skin, scalp and hair. The use of the composition of the present disclosure may for example be by way of incorporation in a leave-on composition.

The composition can be in the form of a liquid, lotion, cream, foam, scrub, gel, soap bar, toner, or applied with an implement or via a face mask, pad or patch in any form of materials. Non-limiting examples of such compositions include leave-on skin lotions and creams in the forms of oil/silicone in water emulsion, water in oil/silicone emulsion and multiple emulsions, serums, shampoos, conditioners, shower gels, toilet bars, antiperspirants, deodorants, depilatories, lipsticks, foundations, mascara, sunscreen lotions, ointments, sprays, pastes, mousses, foams, gels, liposomes, or other topically suitable forms.

In some embodiments, the composition of the present disclosure is in the form of a cream, lotion, paste, wax, liquid, semisolid composition or a sprayable composition.

In the case where the composition is in the form of a spray, the carrier is suitably water, and the composition may further comprise preservative such as benzyl alcohol (for example, in an amount of from 0.1 – 5 wt%), co-solvents such as propylene glycol (for example, in an amount of from 1 – 20 wt%), and surfactants such as PEG-40 hydrogenated castor oil (for example, in an amount of from 1 – 20 wt%).

In the case where the composition is in the form of a lotion, it may further comprise water in the presence of a suitable emulsifying agent. Examples of suitable emulsifying agents are cetareth-20, PEG-100 stearate, glyceryl stearate, sodium cetearyl sulfate. The composition may further comprise emollients in the form of esters and/or hydrocarbons and/or silicones and/or plant oils and fragrances, preservatives (such as benzyl alcohol at levels of, for instance, 0.1 – 10%) and humectant and/or excipients (such as glycerol, for instance at levels of 0.5 – 10% and cyclomethicone at levels of between 0.1 – 20%).

When the composition is in the form of an ointment, it may further comprise a conventional ointment base to which the active ingredient is added. The ointment base may be a paraffin, such as soft paraffin, or a combination of soft and liquid paraffin. Other ointment bases may also be used such as polyalkylene glycol (such as polyethylene or polypropylene glycol) base. Other possible components of an ointment composition include

emulsifying wax (for example, in an amount of between 1 – 40%, preferably 5 – 40%), and optionally one or more preservatives.

When the composition is in the form of a liquid soap, any known liquid soap may be used. A preferred liquid soap with key composition saponified fatty acids derived from palm kernel oil or coconut oil. The pH of the liquid soap base may be adjusted to that which is suitable for topical use.

When the composition is an anhydrous cream, any suitable cream base may be used. A preferred cream base is petrolatum cream and/or natural plant oils and waxes with introduction of consistency factors.

When the composition is a shampoo or conditioner, it may further comprise one or more of a surfactant, a thickening agent, a pH adjuster/buffer, an aesthetic additive, water, a conditioner, a preservative and moisturizers/vitamins in addition to the active ingredients. Shampoos may also include other active components known to those skilled in the art as having some advantage when included in a shampoo formula or in a medicinal combination for treating a hyperpigmentation disorder. A shampoo may comprise a primary surfactant to provide flash foam for cleaning the hair by removing dirt and other impurities. A secondary surfactant may be included to provide stable foam and to reduce the harshness of the primary surfactant. A surfactant may be used that includes a charged, hydrophilic head group and a long, hydrophobic alkyl chain tail. Surfactants are configured to reduce surface tension of an interphase between dirt and hair and allowing the dirt to be transported into an aqueous medium to be rinsed free from the hair and scalp. Examples of surfactants that may be contained in a shampoo in accordance with certain embodiments include sodium laureth sulphate, ammonium laureth sulfate, and sodium cocoyl isethionate. Examples of co-surfactants include cocamide MEA and cocoamidopropyl betaine. Suitable thickening or suspending agents include carbomer and PEG 150 distearate. The thickening agent may be included to stabilize the shampoo during storage and/or to prevent the settling or dumping of pigments and silicone. Suitable pH adjusters or buffers include citric acid, tartaric and sodium hydroxide. The pH adjuster or buffer is configured to cause the shampoo to be gentle to the skin. A lower pH may cause hair to be compact and to shine and to protect the surfactant from hydrolysis, and as such, the pH

Advantageously, the ascorbic acid or derivative and/or salt thereof or the anti-melanogenic agent can be prepared in stabilized topical composition and applied on the skin. Accordingly, the ascorbic acid or derivative and/or salt thereof or the anti-melanogenic agent

may exist in an encapsulated format within the composition. Advantageously, the encapsulation technology may be used to protect and enhance the transdermal delivery of the ascorbic acid or derivative and/or salt thereof or the anti-melanogenic agent. Suitable encapsulation technologies may be selected from the group consisting of liposomes, niosomes, nano-emulsions, and cyclodextrin inclusion technologies. Suitable encapsulation technologies may be produced by any means known in the art and are not limited to the present disclosure.

The ascorbic acid or derivative and/or salt thereof or the anti-melanogenic agent may be encapsulated in an encapsulation material comprising at least one emulsifier and a stabilizer. The encapsulation material may comprise an emulsifier and a co-emulsifier. The emulsifier may be selected from glyceryl esters of different fatty chains, including, but not limited to, glyceryl citrate, glyceryl lactate, glyceryl myristate, glyceryl laurate, glyceryl linoleate, glyceryl oleate or any combination thereof or any mixed glyceryl esters of fatty acids including citrate, lactate, myristate, laurate, linoleate and/or oleate. In one embodiment, the emulsifier is glyceryl citrate/lactate/linoleate/oleate (a mixed ester of citric acid, lactic acid, linoleic acid and oleic acid with glycerol). In some embodiments, the co-emulsifier is a di or polyglyceryl ester selected from, but not limited to, diglyceryl monooleate, polyglyceryl monooleate, polyglyceryl monolinoleate, or any combination thereof. In particular embodiments, the co-emulsifier is diglyceryl monooleate.

Where used, the stabilizer can for example be selected from organic substances of any kind that may solubilize the emulsifiers. Suitable examples include alcohols or glycols, including, but not limited to, ethanol, isopropanol, propylene glycol, pentylene glycol, caprylyl glycol, especially pentylene glycol.

Therapeutic and Non-Therapeutic Methods and Uses

The present disclosure further relates to methods and uses for treating skin pigmentation disorders, and for degrading melanin in dermal fibroblasts, using the compositions and agents described herein. The composition may be used to treat, control, prevent and ameliorate skin hyperpigmentation in safe and non-invasive way. As discussed above, it has now been discovered that fibroblast cells in the dermal layer of skin are involved in uptake of melanosomes, and that melanin can be retained in fibroblast cells and may be degraded at a slower rate, thus contributing to a persistently darker skin tone. Advantageously, the novel findings by the present inventors have emerged as a strategy for

treating hyperpigmentation by promoting the clearance of melanin in fibroblasts which can be used to supplement existing anti-melanogenesis strategies. Accordingly, it has been identified that 3-*O*-ethyl ascorbic acid, an ascorbic acid derivative, is particularly effective in reducing the melanin content in fibroblast cells in a biomimetic skin melanosis model where it provides potent anti-hyperpigmentation efficacy in diminishing dermal melanin. It has further been found that the combination of 3-*O*-ethyl ascorbic acid together with certain anti-melanogenic agents provides superior anti-hyperpigmentation properties, providing superior results in diminishing melanin content in fibroblast cells. For example, 3-*O*-ethyl ascorbic acid and resveratrol have been shown to synergistically inhibit melanogenesis activity in the epidermis and reduced melanin content in melanosome internalized fibroblasts in a human skin model of dermal melanosis.

In addition to diminishing melanin content in fibroblast cells, the composition comprising ascorbic acid or a derivative and/or salt thereof and an anti-melanogenic agent, may also act to promote depigmentation via alternative mechanisms, including, but not limited to, autophagic activity, which accelerates melanin clearance at both epidermal and dermal skin layers, and phagocytic activity by melanophages, which aid in melanosome clearance at both epidermal and dermal skin layers.

In accordance with recent investigation conducted by Ando *et al.*, (2020), melanosomes were shown to be internalized by dermal fibroblasts *via* an actin-dependent endocytosis process. In normal skin physiology, internalized melanosomes in keratinocytes can be differentiated and shed or removed via exfoliating agents like glycolic acid, salicylic acid etc. However, unlike keratinocytes in the epidermis, internalized melanosomes in fibroblasts are retained within the dermis and degrade at a slower rate which may contribute to persistent darker skin spots. The present inventors have thus determined that promoting the clearance of melanin in fibroblasts has emerged as new dimension and strategy for treating hyperpigmentation.

Accordingly, in one aspect, there is provided a method of preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder in a subject, or a method of degrading melanin in dermal fibroblasts of a subject, comprising administering an effective amount of a composition as defined herein to the subject.

Advantageously, application of the composition of the present disclosure in a method as defined herein demonstrates minimal negative effects on tissue viability of reconstituted skin and cell viability of the fibroblasts, which indicates that the inhibitory activity on

melanin reduction mediated by the compositions of the present disclosure are not due to cytotoxic effects.

In another aspect, there is provided a use of a composition as defined herein for the manufacture of a medicament for the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder in a subject, or for degrading melanin in dermal fibroblasts of a subject.

In another aspect, there is provided a composition as defined herein for use in the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder.

In another aspect, there is provided a composition as defined herein for use in degrading melanin in dermal fibroblasts.

The composition of the present disclosure can be used to treat skin pigmentation disorders including, but not limited to, Nevus of Ota, Mongolian spots, Café-au-lait spots, melasma, post-inflammatory hyper- or hyperpigmentation, solar lentigo, nevus of Ota, nevus of Ito, progressive pigmentary purpura, age spots, a canthosis nigricans, and incontinentia pigmenti.

In some embodiments, the skin pigmentation disorder is selected from the group consisting of skin hyperpigmentation, melasma, solar lentigo, nevus of Ota, nevus of Ito, and Mongolian spots.

In some embodiments, the composition is applied topically, for example to the skin of a subject.

As discussed above, the composition of the present disclosure comprising ascorbic acid or a derivative and/or salt thereof, as the sole active agent, potently reduces melanin content in melanosome internalized dermal fibroblasts.

Accordingly, in another aspect there is provided a method of preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder in a subject, or of degrading melanin in dermal fibroblasts of a subject, comprising administering an effective amount of

a) ascorbic acid or a derivative and/or salt thereof,
in an amount effective to degrade melanin in dermal fibroblasts.

In some embodiments, the ascorbic acid or derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

In another aspect, there is provided ascorbic acid or a derivative and/or salt thereof, for use in the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder, wherein the ascorbic acid or derivative and/or salt thereof is administered in an amount effective to degrade melanin in dermal fibroblasts.

5 In some embodiments, the ascorbic acid or derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

In another aspect, there is provided ascorbic acid or a derivative and/or salt thereof, for use in degrading melanin in dermal fibroblasts.

10 In some embodiments, the ascorbic acid or a derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

In another aspect, there is provided an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for use in the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

20 In another aspect, there is provided an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for use in degrading melanin in dermal fibroblasts, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

In another aspect, there is provided a use of ascorbic acid or a derivative and/or salt thereof, for the manufacture of a medicament, for preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder, or for degrading melanin in dermal fibroblasts, wherein the ascorbic acid or derivative and/or salt thereof is administered in an amount effective to degrade melanin in dermal fibroblasts.

In some embodiments, the ascorbic acid or derivative and/or salt thereof is administered in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

30 In another aspect, there is provided a use of an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for the manufacture of a medicament, for preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder, or for degrading melanin in dermal

fibroblasts, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

In some embodiments, the skin pigmentation disorder is selected from the group consisting of skin hyperpigmentation, melasma, solar lentigo, nevus of Ota, nevus of Ito,
5 and Mongolian spots.

In some embodiments, the anti-melanogenic agent is applied topically to the skin of the subject.

The present disclosure also relates to non-therapeutic methods for lightening skin, for example, the composition of the present disclosure may be applied in cosmetic uses to
10 lighten skin colour.

Accordingly, in one aspect, there is provided a non-therapeutic method of skin lightening of a subject, comprising administering an effective amount of a composition as defined herein.

In some embodiments, the composition is applied topically to the skin of the subject.

15 In another aspect, there is provided a non-therapeutic method of skin lightening of a subject, comprising administering an amount of ascorbic acid or a derivative and/or salt thereof to the subject effective to degrade melanin in dermal fibroblasts.

In some embodiments, the ascorbic acid or derivative and/or salt thereof is administered topically to the skin of the subject.

20 In some embodiments, the ascorbic acid or derivative and/or salt thereof is administered in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

In some embodiments, the anti-melanogenic agent is administered topically to the skin of the subject.

25 The composition of the present disclosure may for example be a topical composition that is to be applied on an external surface of a subject. In particular embodiments, the external surface may be one or more of the scalp, face, arms, legs or torso of a subject. Advantageously, the composition of the present disclosure demonstrates effective skin bioavailability which allows skin penetration to lighten skin tone, which has been
30 demonstrated in human skin penetration and efficacy studies and the biomimetic skin model of dermal melanosis.

In some embodiments, the composition of the present disclosure is administered once daily, or twice daily, or three times daily, or four times daily, for example for a period

of time sufficient to bring about a desired level of improvement in hyperpigmentation. Alternatively, the compositions may be used less frequently if that will provide the required effects. For example, it may be applied once every other day or once, twice, three or four times a week, or on an ad hoc or as needed basis.

5 For example, a user may topically administer a composition of the present invention directly to the skin where decreased pigmentation is desired by gently massaging the composition of the present invention into the desired area. In some embodiments, the composition of the present invention is left on the skin or other area where decreased pigmentation is desired between applications occurring on the same day.

10 Topical application of the compositions of the present disclosure may continue for any suitable period of time. For example, within a few days to a few weeks, or within a few days to 10 weeks of the initial application, a user may notice a reduction in pigmentation. Advantageously, topical treatment with the composition of the present disclosure demonstrates anti-hyperpigmentation effects by significantly reducing the skin tone of
15 identified acne-related post-inflammatory hyperpigmentation in, for example, as short as 2-weeks of product application, and by significantly reducing the skin tone of other identified pigmented spots after 8-weeks of product application.

 It should be appreciated that the frequency with which the compositions of the present invention should be applied will vary depending on the desired effect. In some
20 embodiments, the degree of therapeutic and/or cosmetic enhancement will vary directly with the total amount of composition used. Topical application of the compositions may be administered in any amount sufficient to bring about the desired level of improvement in the hyperpigmentation.

 For example, the compositions may be applied in amounts ranging from about 0.5g
25 to about 50g, or from about 0.5 g to about 40g, or from about 0.5g to about 30g, or from about 0.5g to about 20g, or from about 0.5g to about 15g, or from about 0.5g to about 10g, or from about 0.5g to about 5g, or from about 0.5g to about 2.5g, or from about 0.5g to about 1g.

 When topically applied, the compositions of the present invention may be applied to
30 the area to be treated, for example, the face, by spraying, dabbing, swabbing, rubbing or massaging. For example, in one embodiment, a composition of the present invention may be provided in a liquid, lotion, cream, foam, scrub, gel, soap bar, toner, or applied with an

implement or via a face mask, pad or patch in any form of materials or other leave-on product formulation, which preferably may be applied on at least a daily basis.

In some embodiments, the "subject" is a mammal. In some embodiments, the subject is human, for example an adult human.

5

Preparation of compositions

The compositions of the present disclosure may be prepared by any suitable method known to a person skilled in the art.

10 In some embodiments, the compositions are prepared as compositions suitable for topical use.

In some embodiments, the compositions are prepared as emulsions. For example, the compositions of the present disclosure may be prepared by combining an aqueous phase and a lipid phase, followed by homogenisation.

15 The composition may comprise suitable solvents or agents which act to enhance the absorption of other ingredients into the skin, humectants and anti-irritants, gums for producing a gel base that may have skin smoothing and soothing properties, emulsion stabilising and viscosity controlling agents, agents which act to prevent deterioration of the formulation, moisture attracting agents, emollients, hair/skin-conditioning agents, agents which help improve the overall texture and finish of skin care formulae, gelling agents, 20 preservatives, agents which reduce the appearance of large pores, age spots and hyperpigmentation, including lactic acids, together with ascorbic acid or a derivative and/or salt thereof, and anti-melanogenic agent, and subjecting this mixture to homogenisation. In some embodiments, the ascorbic acid or derivative and/or salt thereof, and anti-melanogenic agent may be in an encapsulated format.

25 In some embodiments, the compositions are prepared as compositions suitable for topical use, by combining suitable solvents which may enhance the absorption of other ingredients into skin, such as methylpropanediol, humectants and anti-irritants, such as betaine, natural gums such as sclerotium gum, emulsion stabilizing and viscosity agents such as sodium polyacryloyldimethyl taurate, anti-deterioration, agents such as disodium EDTA, 30 moisture attractants, such as sodium hyaluronate, water-dispersible silicones, such as bis-PEG-18 methyl ether dimethyl silane, which may act as emollients and with added foam-boosting properties, hair/skin conditioning agents, such as PEG-12 dimethicone, other suitable emollients selected from C₁₂₋₁₅ alkyl benzoate, PPG-3 myristyl ether and glycerine,

gelling agents selected from hydroxyethyl acrylate/sodium acryloyldimethyl taurate copolymer, agents which reduce the appearance of large pores, age spots and hyperpigmentation, including lactic acids, preservatives selected from ethylhexylglycerine and phenoxyethanol, antioxidants including sodium metabisulfite, biocides including chlorophensin with deionized water, and ascorbic acid or a derivative and/or salt thereof, and anti-melanogenic agent, and subjecting this mixture to homogenisation. In some embodiments, the ascorbic acid or derivative and/or salt thereof, and anti-melanogenic agent are in an encapsulated format.

Those skilled in the art will appreciate that the disclosure herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications.

Examples

The present disclosure is further illustrated by the following non-limiting examples.

Example 1 – Development of a dermal melanosis model.

1.1 Objective

To understand the ability of fibroblast cells to internalize melanosome, and to build a biomimetic skin model of dermal melanosis

1.2 Methodology

1.2.1 Establishment of the fibroblast cells with internalized melanosome

The mouse melanoma cells B16-F10 (ATCC, USA) at concentration of 5×10^5 cells were seeded in a T75 flask and maintained in complete Dulbecco's Modified Eagle's Media (DMEM from Nacalai, Japan) supplemented with 10% FBS, sodium pyruvate (1 mM), L-glutamine (2mM), streptomycin (50 μ g/mL) and penicillin (50 U/mL) at 37 °C and 5% CO₂ for 5 days. Then, the culture media was centrifuged at 300 xg to remove cell debris and the supernatant comprising melanosomes were collected (melanosome conditioned media). Then, 7×10^5 of human skin fibroblast HS-68 cells (ATCC, USA) were seeded in a T25 flask and maintained in DMEM media supplemented with 10% FBS sodium pyruvate (1 mM), L-glutamine (2mM), streptomycin (50 μ g/mL) and penicillin (50 U/mL) at 37 °C and 5% CO₂ for 24 hours. Then, the culture media was replaced with melanosome conditioned

media and cultured for another 24 hours. Lastly, the cell viability of treated fibroblast cells was determined by using MTT assay. The fibroblast cells treated with melanosome conditioned media were then trypsinized and harvested by centrifugation at 10,000 xg for 10min. The collected cell pellets were lysed by re-suspending with 1M of NaOH (Merck, Germany) and incubated at 80 °C for 1 hour. Lastly, the lysed samples were centrifuged at 3000 xg for 5 min, and the supernatant was collected for melanin and protein quantification.

1.2.2 Co-culture model of reconstituted skin and fibroblast cells with internalized melanosomes

Human skin fibroblast HS-68 cells were seeded in a 12-well hanging top plate at a cell concentration of 1×10^5 cells/well and cultured for 24h. Then, the culture media was replaced with melanosome conditioned media and cultured for another 24h. On the same day, reconstituted skin with melanocytes (Melanoderm from Mattek, USA) was maintained with the long-life maintenance media (LLMM from Mattek, USA) and cultured at 37 °C and 5% CO₂ overnight. The next day, the melanosome conditioned media was discarded and rinsed with PBS, followed by addition of the co-culture of Melanoderm skin to the 12-well hanging top plate containing melanosome conditioned media-treated fibroblast cells. The co-culture system was maintained in LLMM media for a total duration of 7 days and media was refreshed every 2 days. Lastly, fibroblast cells were trypsinized and harvested for melanin quantification. The cell viability of treated skin and fibroblast cells was determined by using MTT assay.

1.2.3 Melanin and protein quantification

Briefly, 150 µL of collected supernatant containing melanin was treated with 45 µL of hydrogen peroxide (Merck, Germany) and incubated at room temperature in the absence of light. Then, the mixture was centrifuged at 3000 xg for 5 min and the fluorescence intensity of the collected supernatant was measured at an excitation wavelength of 470nm and emission wavelength of 550nm by using Cytation 5 multi-mode reader (Biotek Instrument, USA). The amount of melanin in the samples was calculated from a standard curve (0-100 µg/mL) of a freshly prepared melanin standard (Sigma, USA). The amount of the protein was quantified using the Bradford assay. Lastly, the melanin content was

normalized with the protein content and expressed in total melanin (μg) per one mg of protein ($\mu\text{g}/\text{mg}$ protein).

1.3 Results and Discussion

5 To understand the melanosome uptake capability of fibroblast cells, fibroblast cells were treated with conditioned media containing melanosome. As demonstrated in **Figure 1A**, fibroblast cells treated with conditioned media containing melanosome showed a significant increase in melanin content when compared with the control group that was pre-treated with media only. In addition, fibroblast cells showed no significant cytotoxicity effects after treatment with conditioned media containing melanosome (**Figure 1B**). This
10 observation demonstrated the ability of fibroblast cells to internalize melanosomes and aligns with recent findings of Ando *et al.*, (2020), that demonstrated uptake of melanosomes by dermal fibroblasts via actin-dependent endocytosis.

A biomimetic skin dermal melanosis model was further established by co-culturing
15 the melanosome internalized fibroblasts and reconstituted skin model for a duration of 7 days. Similarly, a significant increase in melanin content was found in conditioned media treated fibroblasts with no significant cytotoxicity effects on the fibroblasts and skin tissue in the established co-culture system (**Figure 2**). This skin model enables evaluation of the product efficacy of a topical formulation on the impact of the melanin content in both the
20 epidermis and dermis.

1.4 Conclusion

Fibroblast cells demonstrate the ability to internalize melanosomes and increase their intracellular melanin content. A biomimetic skin model of dermal melanosis was developed
25 to study the efficacy of topical formulations on the melanosome internalized fibroblast cells.

Example 2 – 3-O-Ethyl ascorbic acid (3-EAA) as potent molecule in diminishing melanin content in melanosome internalized fibroblast cells.

30 2.1 Objective

To investigate the effects of 3-O-ethyl ascorbic acid (3-EAA) in reducing melanin content in melanosome internalized fibroblasts.

2.2 Methodology

Establishment of the fibroblast cells with internalized melanosomes was carried out as described at 1.2.1 above with slight modification. After incubation of the fibroblast cells with melanosome conditioned media for 24 hours, the conditioned media was removed and replaced with complete culture media containing 3-EAA at a final concentration of 500 μ M and 1000 μ M. For the control group, complete culture media without 3-EAA was used for comparison.

2.3 Results and Discussion

As shown in **Figure 4**, treatment of 3-EAA at both tested concentrations (500 μ M and 1000 μ M) significantly reduced the melanin content in melanosome internalized fibroblast cells when compared with the control group.

2.4 Conclusion

3-*O*-Ethyl ascorbic acid potently reduces melanin content in melanosome internalized fibroblast cells.

Example 3 – Synergism of 3-*O*-ethyl-ascorbic acid and resveratrol in reducing melanogenesis activity in epidermis and melanin content of melanosome internalized fibroblast cells in a skin model of dermal melanosis.

3.1 Objective

To investigate the synergistic effects of 3-EAA and resveratrol in reducing melanogenesis activity in the epidermis and melanin content in melanosome internalized fibroblasts in a skin model of dermal melanosis

3.2 Methodology

3.2.1 Composition preparation

Premix B was added into water phase (A) and homogenized at 2500 rpm for 5 minutes. Premix C and D were prepared separately until fully dissolved. Then, premix C was added into the bulk (A+B) and stirred at 100-200 rpm for 5 minutes. Lastly, premix D was added into the bulk (A+B+C) and stirred at 100-200 rpm for 30 minutes.

Table 1 – Formulae 1-4

Phase	Ingredient	Formula 1 (%)	Formula 2 (%)	Formula 3 (%)	Formula 4 (%)
A	Deionized water	qs 100	qs 100	qs 100	qs 100
B	C ₁₂₋₁₅ Alkyl Benzoate	5	5	5	5
	PPG-3 Myristyl Ether	5	5	5	5
	Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer	3	3	3	3
C	Deionized water	20	18	18	18
	3-O-Ethyl Ascorbic Acid	-	2	-	1
	Encapsulated Resveratrol (*5%)	-	-	2 (*0.1%)	1 (*0.05%)
D	Ethylhexylglycerin	0.3	0.3	0.3	0.3
	Phenoxyethanol	0.4	0.4	0.4	0.4

*Active level of compound

5 3.2.2 Topical treatment on co-culture system

Establishment of the fibroblast cells with internalized melanosomes was carried out as described at 1.2.2 with a slight modification on the topical formulation. The co-culture system of melanosome internalized fibroblasts and reconstituted skin were maintained in LLMM for a duration of 7 days. During the maintenance of the co-culture system, the LLMM media was refreshed, followed by topical application of the formulation on skin for two hours and washing off. Both media refreshment and topical treatment were conducted every 2 days. Upon completion of 7 days' culture, fibroblast cells were trypsinized and harvested for melanin quantification. In addition, the treated skins were fixed in 4% paraformaldehyde for melanin index and skin tone measurement using Mexameter (Courage-Khazaka electronic GmbH, Germany). Lastly, the cell/tissue viability of treated skin and fibroblast cells was determined by using MTT assay.

3.3 Results and Discussion

As demonstrated in Table 2, formula 2 comprising 2% 3-EAA demonstrated a significant reduction of melanin content in fibroblast cells but not melanin index in reconstituted skin. In contrast, formula 3 comprising 0.1% resveratrol demonstrated a significant reduction of melanin index in reconstituted skin, but not melanin content in fibroblast cells. Interestingly, Formula 4 comprising a relatively lower dose of active level (1% 3-EAA and 0.05% resveratrol) is shown to significantly diminish melanin index in reconstituted skin and melanin content in dermal fibroblast cells. In comparison with formulae 2 and 3, the inhibitory activity of formula 4 is superior despite a lower level of actives. Furthermore, the tissue viability of reconstituted skin and fibroblasts was more than 80%, indicating that the inhibitory activity on melanin reduction mediated by the formulation was not due cytotoxic effects (data not shown). Taken together, these data indicate that both EAA and resveratrol work synergistically by inhibiting melanogenesis activity in the epidermis and reduced form melanin in dermal fibroblast cell.

Table 2 – Effects of selected formulae on skin luminosity and melanin index of reconstituted skin as well as melanin content in melanosome internalized fibroblast cells of biomimetic skin models of dermal melanosis.

No.	Product	Active Level (%)			Product Efficacy (Δ percentage versus formula 1)	
		3-EAA	Resveratrol	Total	Reduction of melanin index in reconstituted skin	Reduction of melanin content in fibroblast cells
1	Formula 1	-	-	-	0	0
2	Formula 2	2	-	2	-7.70 ± 0.97	-41.4 ± 0.29*
3	Formula 3	-	0.1	0.1	-13.26 ± 0.22**	-34.19 ± 6.33
4	Formula 4	1	0.05	1.05	-21.32 ± 4.71***	--49.7 ± 9.76*

All obtained data were normalized with placebo-treated group (formula 1) and product efficacy of the formulation was interpreted as the percentage change versus the placebo treated group (formula 1). All data are expressed as mean ± S.E.M of two independent experiments (n=2). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ when compared to placebo-treated group (formula 1) by one-way ANOVA.

3.4 Conclusion

3-*O*-Ethyl ascorbic acid and resveratrol work synergistically to inhibit melanogenesis activity in the epidermis and act to diminish melanin content in melanosome internalized fibroblast cells in skin model of dermal melanosis.

- 5 **Example 4 – 4-*n*-Butylresorcinol as a functional booster to the activity of 3-*O*-ethyl-ascorbic acid in diminishing melanin content of melanosome internalized fibroblast cells in a skin model of dermal melanosis.**

4.1 Objective

- 10 To investigate the boosting effects of 4-*n*-butylresorcinol on activity of 3-EAA in diminishing melanin content in melanosome internalized fibroblast cells in skin model of dermal melanosis.

4.2 Methodology 4.2.1 Composition preparation

- 15 Premix B was added into water phase (A) and homogenized at 2500 rpm for 5 minutes. Premixes C and D were prepared separately until fully dissolved. Then, premix C was added into the bulk (AB) and stirred at 100-200 rpm for 5 minutes. Lastly, premix D was added into the bulk (AB) and stirred at 100-200 rpm for 30 minutes.

20 **Table 3 – Formulae 1 and 5-7**

Phase	Ingredient	Formula 1 (%)	Formula 5 (%)	Formula 6 (%)	Formula 7 (%)
A	Deionized water	qs 100	qs 100	qs 100	qs 100
B	C ₁₂₋₁₅ Alkyl Benzoate	5	5	5	5
	PPG-3 Myristyl Ether	5	5	5	5
	Hydroxyethyl Acrylate/Sodium Acryloyldimethyl Taurate Copolymer	3	3	3	3
C	Deionized water	20	19	12.5	11.5
	3- <i>O</i> -Ethyl Ascorbic Acid	-	1	-	1
	Encapsulated 4- <i>n</i> -butylresorcinol (*4%)	-	-	7.5 (0.3*)	7.5 (0.3*)

D	Ethylhexylglycerin	0.3	0.3	0.3	0.3
	Phenoxyethanol	0.4	0.4	0.4	0.4

*Active level of compound

4.2.2 Topical treatment on co-culture system

This was carried out in accordance with the procedure outlined in 3.2.2.

5

4.3 Results and Discussion

As demonstrated in Table 4, formula 5 comprising 1% 3-EAA demonstrated a reduction trend of melanin content in fibroblast cells, but not melanin index in reconstituted skin. On the contrary, formula 6 comprising 0.3% 4-n-butylresorcinol demonstrated a significant reduction of melanin index in reconstituted skin, but not melanin content in fibroblast cells. Interestingly, formula 7 comprising a combination of both actives (1% 3-EAA and 0.3% 4-n-butylresorcinol) was shown to significantly diminish both melanin index in reconstituted skin and melanin content in fibroblast cells. In comparison with formulae 5 and 6, the inhibitory activity of formula 7 was more superior in reducing melanin content in fibroblast cells. The tissue viability of reconstituted skin and cell viability of the fibroblasts were more than 80%, which indicates that the inhibitory activity on melanin reduction mediated by the formulation was not due to cytotoxic effects (data not shown). Taken together, these data indicate that 4-n-butylresorcinol is a potent booster to 3-EAA activity in reducing form melanin in dermal fibroblast cells.

20

Table 4 – Effects of selected formulae on skin luminosity and melanin index of reconstituted skin as well as melanin content in melanosome internalized fibroblast cells of a biomimetic skin model of dermal melanosis.

No.	Product	Active Level (%)			Product Efficacy (Δ percentage versus formula 1)	
		EAA	4-n-BR	Total	Reduction of melanin index in reconstituted skin	Reduction of melanin content in fibroblast cells
1	Formula 1	-	-	-	0	0
2	Formula 5	1	-	1	0	-18.83 $\pm$ 16.04
3	Formula 6	-	0.3	0.3	-13.14 $\pm$ 2.10*	-26.52 $\pm$ 15.06
4	Formula 7	1	0.3	1.3	-15.18 $\pm$ 1.70**	-41.53 $\pm$ 8.57*

All obtained data were normalized with placebo-treated group (formula 1) and product efficacy of formulation was interpreted as the percentage change versus the placebo treated group (formula 1). All data are expressed in mean $\pm$ S.E.M of two independent experiments (n=2). * p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001 when compared to placebo-treated group (formula 1) by one-way

5 ANOVA.

4.4 Conclusion

4-*n*-Butylresorcinol boosts the activity of 3-*O*-ethyl ascorbic acid in diminishing melanin content of melanosome internalized fibroblast cells in a skin model of dermal
10 melanosis.

Example 5 – Clinical anti-post-inflammatory hyperpigmentation efficacy of a topical composition comprising of 3-EAA and resveratrol.

15 5.1 Objective

To clinically investigate the anti-post-inflammatory hyperpigmentation activity of a topical formulation comprising 3-EAA and resveratrol.

5.2 Methodology

20 5.2.1 Composition preparation (Formula 8)

Premix A and premix B were prepared separately until fully dissolved. Then, Premix B was added into premix A and stirred at 2500 rpm for 10 minutes. Premix C was prepared by firstly warming up to 40 °C followed by addition into the bulk (A+B) and the mixture stirred at 2500 rpm until fully dispersed. Lastly, ingredients in phase D were added
25 sequentially into the bulk (A+B+C) and stirred at 100-200 rpm for 10 minutes.

Table 5 – Formula 8

Phase	Ingredient	Dosage (%)
A	Water	qs 100
	Methylpropanediol	3
	Betaine	2
	Sclerotium Gum	0.3
	Sodium polyacryloyldimethyl taurate	0.3
	Disodium EDTA	0.05
	Sodium hyaluronate	0.08
B	Water	10
	Ethyl ascorbic acid	2
	Encapsulated Resveratrol (*1%)	1 (*0.1%)
C	Water	10
	Bis-PEG-18 Methyl Ether Dimethyl Silane	0.15
D	PEG-12 dimethicone	0.48
	Glycerin	0.07
	Phenoxyethanol	0.5
	Chlorphenesin	0.14
	Sodium metabisulfite	0.05
	Lactic acid	0.45

5.2.2 Clinical trial

- 5 The product efficacy on improvement of acne markings (post-inflammatory hyperpigmentation) was conducted on a group of 12 women with ages ranging from 22 to 23 years old with obvious acne markings. In this study, the recruited panellists were

instructed to apply formula 8 topically twice daily for two months duration. Then, photographs of the panellists were captured by using VISIA Complexion Analysis system (Canfield, USA) at baseline (T_0), post 2-weeks (T_{2w}), 4-weeks (T_{4w}) and 8-weeks (T_{8w}) of product application. Lastly, the skin tone of the individual acne markings was marked as a region of interest and further analyzed by determining its individual topology angle (ITA°).

5.3 Results and Discussion

As illustrated in Table 6, the results demonstrate that topical treatment with Formula 8 reduced the skin tone of identified acne-related post-inflammatory hyperpigmentation. This reduction is significantly demonstrated in as little as 2-weeks post product application. The progressive anti-hyperpigmentation effect of the product is also shown throughout 8 weeks of product application.

Table 6 – Effects of Formula 8 on improvement of skin tone of acne mark (post-inflammatory hyperpigmentation) which determined by using Individual Topology Angle (ITA°).

ITA° of	Formula 8 (2% 3-EAA and 0.1% resveratrol)			
Acne Mark	Baseline (T_0)	2-weeks (T_{2w})	4-weeks (T_{4w})	8-weeks (T_{8w})
Mean ITA°	25.88 ± 2.93	27.05 ± 3.03	28.75 ± 2.75	28.52 ± 2.44
Δ ITA° (vs T_0)	-	1.37 ± 0.38	2.87 ± 0.55	2.64 ± 0.82
Statistical analysis (vs T_0)	-	** P < 0.01	*** P < 0.001	* P < 0.05

All values are expressed in mean ± SEM of group of test panelist (n =12). * p <0.05 ** p <0.01 *** p <0.001, indicates significantly different compared to baseline by utilizing Wilcoxon Matched Pairs Signed Rank Test.

5.4 Conclusion

A topical formulation comprising 3-EAA and resveratrol demonstrate efficacy to clinically reduce acne marks (post-inflammatory hyperpigmentation).

Example 6 – Clinical anti-hyperpigmentation efficacy of composition comprising 3-EAA and 4-n-butylresorcinol.

6.2 Objective

To clinically validate the anti-hyperpigmentation activity of a topical formulation comprising 3-EAA and 4-n-butylresorcinol.

6.2 Methodology

5 6.2.1 Composition preparation (Formula 9)

Premix A (aqueous phase) and premix B (lipid phase) were prepared separately until fully dissolved. Then, Premix B was added into premix A and homogenized at 2500 rpm for 10 minutes. Premix C and D were also prepared separately. Then, premix C and D were added sequentially into the bulk (A+B) and homogenized at 2500 rpm for 5 minutes,
10 followed by stirring at 100-200 rpm for 10 minutes.

Table 7 – Formula 9

Phase	Ingredient	Dosage (%)
A	Water	qs 100
	Disodium EDTA	0.05
	Sodium metabisulfite	0.05
	Betaine	4
	Glycerin	1
	Ethylhexylglycerin, Propanediol, Caprylyl Glycol	1
	Sclerontium Gum	0.8
B	Dicaprylyl Carbonate	1
	Polyacrylate Crosspolymer-6	0.3
C	Water	5
	Ethyl Ascorbic Acid	2
D	Water	10
	Encapsulated 4-n-butylresorcinol (*4%)	7.5 (*0.3%)

*Active level of compound

6.2.2 Clinical trial

The product efficacy on improvement of hyperpigmentation was conducted on a group of 11 women with ages ranging from 22 to 35 years old and obvious pigmented skin spots. In this study, recruited panellists were instructed to apply formula 9 topically twice daily for two months' duration. Photographs of the panellists were captured by using VISIA Complexion Analysis system (Canfield, USA) on baseline (T_0), 4-weeks (T_{4w}) and 8-weeks (T_{8w}) of product application. Lastly, the skin tone of the individual pigmented spot was marked as region of interest and further analysed by determining its individual topology angle (ITA).

6.3 Results and Discussion

Results illustrated in Table 8 show that topical treatment of Formula 9 reduces the skin tone of identified pigmented spots and its reduction is significantly demonstrated after 8 weeks of product application.

Table 8 – Effects of formula 9 on improvement of skin tone of pigmented spot as determined using Individual Topology Angle (ITA°).

ITA° of Pigmented spot	Formula 9 (2% EAA and 0.3% 4-n-butylresorcinol)		
	Baseline (T_0)	4-weeks (T_{4w})	8-weeks (T_{8w})
Mean ITA°	-1.13 ± 5.94	-0.40 ± 4.71	1.05 ± 5.12
Δ ITA° (vs T_0)	-	0.73 ± 2.32	2.19 ± 1.58
Statistical analysis (vs T_0)	-	$P > 0.05$	$**P < 0.01$

All values are expressed in mean $\pm$ SEM of group of test panelist (n=11). $**p < 0.01$, indicates significantly different compared to baseline by utilizing Wilcoxon Matched Pairs Signed Rank Test.

6.4 Conclusion

Topical formulation 9 comprising 3-EAA and 4-n-butylresorcinol demonstrates clinically efficacy in reducing pigmented spot (hyperpigmentation).

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the above-described embodiments, without departing from the broad general scope of the present disclosure. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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Claims

1. A composition for preventing and/or treating a skin pigmentation disorder, the composition including:
 - a) ascorbic acid or a derivative and/or salt thereof; and
 - 5 b) an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof; in an amount effective to degrade melanin in dermal fibroblasts.
2. A composition as claimed in claim 1, wherein component a) is selected from the
10 group consisting of 3-O-ethyl ascorbic acid, L-ascorbic acid, 2-O-ethyl ascorbic acid, ascorbyl glucoside, ascorbyl tetraisopalmitate, magnesium ascorbyl phosphate, sodium ascorbic phosphate, caprylyl 2-glyceryl ascorbate, 3-glyceryl ascorbate, bis-glyceryl ascorbate, hexyl 3-glyceryl ascorbate, myristyl 3-glyceryl ascorbate, and lauryl glyceryl ascorbate.
- 15 3. A composition as claimed in claim 2, wherein component a) is selected from the group consisting of 3-O-ethyl ascorbic acid, L-ascorbic acid, 2-O-ethyl ascorbic acid, ascorbyl tetraisopalmitate, sodium ascorbic phosphate, caprylyl 2-glyceryl ascorbate, 3-glyceryl ascorbate, bis-glyceryl ascorbate, hexyl 3-glyceryl ascorbate, myristyl 3-glyceryl
20 ascorbate, and lauryl glyceryl ascorbate.
4. A composition as claimed in claim 3, wherein component a) is 3-O-ethyl ascorbic acid.
- 25 5. A composition as claimed in any of claims 1 to 4, wherein component b) is resveratrol.
6. A composition as claimed in any of claims 1 to 4, wherein component b) is pterostilbene.
- 30 7. A composition as claimed in any of claims 1 to 4, wherein component b) is sclareolide.

8. A composition as claimed in any of claims 1 to 4, wherein component b) is 4-*n*-butylresorcinol.

9. A composition as claimed in any of claims 1 to 8, wherein the amount of component
5 a) is in the range of from 0.01% to 50% by weight of the composition, optionally from 0.5% to 5% by weight of the composition, optionally from 0.5% to 1.5% by weight of the composition.

10. A composition as claimed in any of claims 1 to 9, wherein the amount of component
10 b) is in the range of from 0.01% to 5% by weight of the composition, optionally from 0.1% to 0.5% by weight of the composition, or optionally from 0.2% to 0.35% by weight of the composition.

11. A composition as claimed in any of claims 1 to 10, wherein the weight ratio of
15 component a) to component b) is in the range of from 2:1 to 200:1, optionally from 2:1 to 50:1, or optionally from 3:1 to 5:1.

12. A composition as claimed in any of claims 1 to 11, wherein the composition does
not contain any anti-melanogenic agent other than a) and b).

20 13. A composition as claimed in any of claims 1 to 12, wherein the composition comprises 0.5 to 1.5% by weight of 3-O-ethyl ascorbic acid as component a) and comprises 0.2 to 0.35% by weight of resveratrol as component b), and wherein the weight ratio of component a) to component b) is in the range of from 3:1 to 5:1.

25 14. A composition as claimed in any of claims 1 to 13, wherein the composition is for topical application.

15. A composition as claimed in any of claims 1 to 14, wherein the composition
30 comprises one or more of the following: an anti-inflammatory agent, a sunscreen agent, an antioxidant, an anti-aging peptide, a desquamation agent, and an exfoliating agent.

16. A composition as claimed in any of claims 1 to 15, wherein the composition comprises one or more of the following: water, an alkyl benzoate, PPG-3 myristyl ether, hydroxyacrylate/sodium acryloyldimethyl taurate copolymer, ethylhexylglycerin, phenoxythanol, methylpropanediol, betaine, sclerotium gum, sodium polyacryloyldimethyl taurate, disodium EDTA, sodium hyaluronate, bis-PEG-18 methyl ether dimethyl silane, PEG-12 dimethicone, glycerine, chlorphenesin, sodium metabisulfite, lactic acid, dicaprylyl carbonate, and polyacrylate crosspolymer-6.
17. A composition as claimed in any of claims 1 to 16, wherein the composition is in the form of a cream, lotion, paste, wax, liquid, semisolid composition or a sprayable composition.
18. A method of preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder in a subject, or a method of degrading melanin in dermal fibroblasts of a subject, comprising administering an effective amount of a composition as defined in any of claims 1 to 17 to the subject.
19. Use of a composition as defined in any of claims 1 to 17 for the manufacture of a medicament for the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder in a subject, or for degrading melanin in dermal fibroblasts of a subject.
20. A composition as defined in any of claims 1 to 17 for use in the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder.
21. A composition as defined in any of claims 1 to 17 for use in degrading melanin in dermal fibroblasts.
22. A method, use, or composition for use, as claimed in any of claims 18-20, wherein the skin pigmentation disorder is selected from the group consisting of skin hyperpigmentation, melasma, solar lentigo, nevus of Ota, nevus of Ito, and Mongolian spots.

23. A method, use, or composition for use, as claimed in any of claims 18 to 22, wherein the composition is applied topically to the skin of the subject.
24. A method of preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder in a subject, or of degrading melanin in dermal fibroblasts of a subject, comprising administering an effective amount of
- 5 b) ascorbic acid or a derivative and/or salt thereof, in an amount effective to degrade melanin in dermal fibroblasts.
- 10 25. A method as claimed in claim 24, wherein the ascorbic acid or a derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.
26. Ascorbic acid or a derivative and/or salt thereof, for use in the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder, wherein the ascorbic acid or derivative and/or salt thereof is administered in an amount effective to degrade melanin in dermal fibroblasts.
- 15 27. Ascorbic acid or a derivative and/or salt thereof, for use as claimed in claim 26, wherein the ascorbic acid or derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.
- 20 28. Ascorbic acid or a derivative and/or salt thereof, for use in degrading melanin in dermal fibroblasts.
- 25 29. Ascorbic acid or a derivative and/or salt thereof, for use as claimed in claim 28, wherein the ascorbic acid or derivative and/or salt thereof is used in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.
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30. An anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for use in the prevention, treatment, and/or reduction of a symptom associated with, a skin pigmentation disorder, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

31. An anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for use in degrading melanin in dermal fibroblasts, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

32. Use of ascorbic acid or a derivative and/or salt thereof, for the manufacture of a medicament, for preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder, or for degrading melanin in dermal fibroblasts, wherein the ascorbic acid or derivative and/or salt thereof is administered in an amount effective to degrade melanin in dermal fibroblasts.

33. Use as claimed in claim 32, wherein the ascorbic acid or derivative and/or salt thereof is administered in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof.

34. Use of an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and sclareolide, or a salt thereof, for the manufacture of a medicament, for preventing, treating, and/or reducing a symptom associated with, a skin pigmentation disorder, or for degrading melanin in dermal fibroblasts, wherein the anti-melanogenic agent is administered in combination with ascorbic acid or a derivative and/or salt thereof.

35. A method, use, or composition for use, as claimed in any of claims 24-27, 30 or 32-34, wherein the skin pigmentation disorder is selected from the group consisting of skin hyperpigmentation, melasma, solar lentigo, nevus of Ota, nevus of Ito, and Mongolian spots.

36. A method, use, or composition for use, as claimed in any of claims 24 to 35, wherein the composition is applied topically to the skin of the subject.

37. A non-therapeutic method of skin lightening of a subject, comprising administering
5 an effective amount of a composition as defined in any of claims 1 to 17 to the subject.

38. A non-therapeutic method as claimed in claim 35, wherein the composition is applied topically to the skin of the subject.

10 39. A non-therapeutic method of skin lightening of a subject, comprising administering an amount of ascorbic acid or a derivative and/or salt thereof to the subject effective to degrade melanin in dermal fibroblasts.

40. A non-therapeutic method as claimed in claim 39, wherein the ascorbic acid or
15 derivative and/or salt thereof is administered topically to the skin of the subject.

41. A non-therapeutic method as claimed in claim 39 or 40, wherein the ascorbic acid or derivative and/or salt thereof is administered in combination with an anti-melanogenic agent selected from the group consisting of 4-*n*-butylresorcinol, resveratrol, pterostilbene and
20 sclareolide, or a salt thereof.

42. A non-therapeutic method as claimed in claim 41, wherein the anti-melanogenic agent is administered topically to the skin of the subject.