

## METHOD AND COMPOSITIONS FOR SKIN MICROBIOME NORMALIZATION IN SENSITIVE SKIN

### Field of the Invention

- 5 The present invention relates to the use of a topical composition for improving the subclinical symptoms of sensitive skin and normalization of the skin microbiome profile. More particularly, the composition comprises a stabilized and encapsulated form of boswellic acid and/or derivatives thereof which are obtained from or contained within an extract of *Boswellia serrata*. The present invention also provides method of improving skin condition in a population with sensitive skin.

### 10 Background Description

- Sensitive skin is a condition of cutaneous hyper-reactivity to environmental factors and exaggerated skin reactions (e.g. stinging, itching, burning, erythema and dryness) which can be triggered through contact with allergens such as cosmetic ingredients or by extreme climate conditions. The pathophysiology of sensitive skin is well accepted and is characterized by impairment of skin barrier function that is correlated with a thin permeable stratum corneum and hypersensitive reactions that are correlated to the nerve fibres innervating the epidermis. Both impairment of the skin barrier and heightened neurosensitivity enable more susceptibility to the chemical irritation and allergy response in sensitive skin when compared to healthy skin (Berardesca *et al.*, 2013.)

- Several studies have shown the dysbiosis of cutaneous microbiota to be associated with skin disorders including psoriasis and atopic dermatitis (Alekseyenko *et al.*, 2013, Byrd *et al.*, 2017 and William & Gallo, 2015). Givaudan demonstrated significant increase of pathogenic strains in the Caucasian population with sensitive skin, including *Corynebacterium*, *Chryseobacterium*, *Smadgrassella*, *Kocuria*, *Micrococcus* and *Lawsonella* genera (Jarri *et al.*, 2020). In addition, the composition of the skin microbiome among the Japanese population was demonstrated to have potential effects on skin hypersensitivity development (Shinohara & Masaki, 2019). Therefore, a maintenance or restoration of the skin microbiome balance is critical for overall skin health to avoid development of sensitive skin.

- Gum-resin extracts of *Boswellia serrata* have been used in traditional folk medicine for centuries for the treatment of various chronic inflammatory diseases. *Boswellia serrata* consists of four major pentacyclic triterpenic acids, namely boswellic acid derivatives, including, but not limited to,  $\beta$ -boswellic acid (BBA), acetyl- $\beta$ -boswellic acid (ABA), 11-keto- $\beta$ -boswellic acid (KBBA) and acetyl-11-keto- $\beta$ -boswellic acid (AKBA). These active compounds are responsible for inhibition

- of lipooxygenase-5 (LOX-5) which is a pro-inflammatory enzyme (M. Z. Siddiqui, 2010) and have been used as oral supplements in the treatment of chronic inflammatory disease (US20190175678A1) and photo-aged skin (Calzavara-Pinton *et al.*, 2010). In addition, *Boswellia serrata* extract has been used in a topical formulation for treating eczematous symptoms of psoriatic patients (Togni *et al.*, 2014). Furthermore, specific active compounds such as AKBA have been proven to exhibit potential anti-bacterial and anti-biofilm activity by interrupting the membrane integrity of *Staphylococcus aureus* and *Staphylococcus epidermidis*, which reveal its potential application for use in the treatment of *Staphylococcus aureus* infections (Raja *et al.*, 2011).
- 10 There is a need for compositions that are able to treat the subclinical symptoms of sensitive skin and thereby restore sensitive skin to healthy skin. However, boswellic acid and derivatives thereof are chemically unstable and are thus prone to discolouration and degradation upon prolonged storage which limits their industrial applicability in the Fast-Moving Consumer Good (FMCG) sector that requires established product shelf life. There is a need for stabilized compositions
- 15 comprising boswellic acid and/or derivatives thereof.

The present invention is predicated at least in part on the discovery of a composition comprising a stabilized form of boswellic acid and/or its derivatives and that this composition can improve skin barrier function and calm neurosensitivity and restore the skin microbiome of sensitive skin towards that found in healthy skin.

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#### Summary of Invention

- In one aspect, the present invention provides a topical composition comprising boswellic acid, one or more derivatives of boswellic acid, or a mixture thereof, wherein the boswellic acid, one or more derivatives of boswellic acid, or mixture thereof are stabilised *via* encapsulation in a
- 25 nanoemulsion having a liquid lipid core and a negative surface charge.

In another aspect of the present invention there is provided a composition according to the invention, for use in alleviating a subclinical symptom of sensitive skin in a subject.

In another aspect of the present invention there is provided a composition of the invention, for use in normalizing the skin microbiome profile of a subject with sensitive skin.

- 30 In yet another aspect of the present invention there is provided a composition of the invention, for use on a subject with healthy skin without causing disruption of skin microbiome profile.

In yet another aspect of the present invention there is provided a method of treating a subclinical symptom of sensitive skin in a subject, comprising applying to the skin of said subject the composition of the present invention.

5 In a further aspect of the present invention there is provided a use of a composition of the invention in the manufacture of a medicament for treating a subclinical symptom of sensitive skin.

In yet a further aspect, there is provided a composition according to the invention for use in treating or preventing acne skin or acne prone skin, oily skin, oily scalp, dry skin, dry scalp, irritated skin, irritated scalp, hair loss, hair greying, aged skin, aged scalp, hyper-pigmented skin, dandruff, atopic dermatitis or seborrheic dermatitis.

10 In another aspect of the present invention there is provided a method of treating or preventing acne skin or acne prone skin, oily skin, oily scalp, dry skin, dry scalp, irritated skin, irritated scalp, aged skin, aged scalp, hair loss, hair greying, hyper-pigmented skin, dandruff, atopic dermatitis or seborrheic dermatitis, comprising applying to the skin of a subject the composition of the invention.

15 In yet another aspect of the present invention there is provided a use of a composition of the invention in the manufacture of a medicament for treating or preventing acne skin or acne prone skin, oily skin, oily scalp, dry skin, dry scalp, irritated skin, irritated scalp, aged skin, aged scalp, hair loss, hair greying, hyper-pigmented skin, dandruff, atopic dermatitis or seborrheic dermatitis.

#### **Brief Description of the Figures**

20 **Figure 1** provides a graphical representation of the differential skin microbiome profile between healthy skin and sensitive skin at Phylum level (A) and Species level (B). \* $p < 0.05$  is statistically difference between groups based on Wilcoxon Match Pair Signed Rank Test.

**Figure 2** provides a carton illustration of a nano-encapsulated boswellic acid derivative, in which the size of the nanoemulsion is within the range of 60-180 nm and the nanoemulsion has a surface charge of -65 to -75 mV.

**Figure 3** provides a graphical representation of the effects of topical application of Formula 1 on skin neuro-sensitivity (A) based on a Virtual Analogue Score (VAS) of the lactic acid stinging test (LAST) and improvement of skin barrier function (B) based on trans-epidermal water lost (TEWL) measurement. All values are expressed in mean  $\pm$  S.E.M of the group of panellists (n=12).

30 \* $p < 0.05$ , is statistically different between group based on Wilcoxon Match Pair Signed Rank Test.

**Figure 4** provides a graphical representation of the effects of topical application of Formula 1 on

the skin microbiome profile, including abundance of bacteria belonging to Firmicutes phylum and *Staphylococcus epidermidis*. All values are expressed in median of group of panellists (n=12).

\*p<0.05, is statistically different between group based on Wilcoxon Match Pair Signed Rank Test.

#### **Detailed Description of the Invention/Embodiments**

##### **5 Definitions**

It is to be understood that this invention is not limited to any particular methodology or protocols described herein. Further, unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which this invention belongs. It is also to be understood that the terminology used herein is for describing  
10 particular embodiments only and is not intended to limit the scope of the present invention, which will be limited only by the claims.

In the specification, except where the context requires otherwise due to express language or necessary implication, the word "comprise" or variations such as "comprises" or "comprising" is used in an inclusive sense, i.e. to specify the presence of the stated features but not to preclude the  
15 presence or addition of further features in various embodiments of the invention. In other words, the listed steps or options need not be exhaustive.

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, in Malaysia or any other country.

20 Unless otherwise indicated, the present invention employs conventional chemistry, molecular biology and microbiology techniques within the capacity of those skilled in the art. Such techniques are well known to the skilled worker and are explained fully in the literature.

As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a  
25 carrier" may include a plurality of carriers, and a reference to "a subject" may be a reference to one or more subjects, and so forth.

The term "(s)" following a noun contemplates the singular or plural form, or both. The term "and/or" can mean "and" or "or".

Unless the context requires otherwise, all percentages referred to herein are percentages by weight  
30 of the composition.

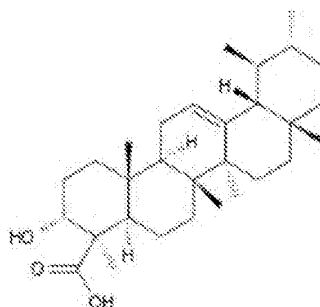
Various features of the invention are described and/or claimed with reference to a certain value, or range of values. These values are intended to relate to the results of the various appropriate measurement techniques, and therefore should be interpreted as including a margin of error inherent in any particular measurement technique. Some of the values referred to herein are denoted by the term "about" to at least in part account for this variability. The term "about", when used to describe a value, preferably means an amount within  $\pm 25\%$ ,  $\pm 10\%$ ,  $\pm 5\%$ ,  $\pm 1\%$  or  $\pm 0.1\%$  of that value.

Where a range of values are expressed, it will be clearly understood that this range encompasses the upper and lower limits of the range, and all values in between these limits.

### 10 *Compositions*

- In a first aspect of the present invention there is provided a topical composition comprising boswellic acid, one or more derivatives of boswellic acid or a mixture thereof, wherein the boswellic acid, one or more derivative of boswellic acid and/or mixture thereof is encapsulated in a nanoemulsion having a liquid lipid core and a negative surface charge.
- 15 In some embodiments, the boswellic acid or one or more derivative(s) thereof is substantially pure. In other embodiments, the boswellic acid or one or more derivative(s) thereof is present in a natural extract, including, but not limited to, *Boswellia serrata* extract, *Boswellia sacra* extract, *Boswellia papyrifera* extract, *Boswellia frereana* extract and *Boswellia carterii* extract.

Boswellic acid has the structure as given below:



- 20 Boswellic acid, or derivatives thereof, are present in several plant products, for example, boswellic acid and/or derivative(s) thereof, are available as extracts from *Boswellia serrata* or *Boswellia carterii*. Commercial sources are available, for example, from Active International LLC, USA (Trade name: Viapure Boswellia), Sophartops, Germany (Trade name: Sophartops Boswellin),
- 25 Sabinsa, India (Trade name: Boswellin CG), Ambe Phytoextracts, India (Trade name: AKBA ultra). Alternatively, extracts comprising boswellic acid and/or derivative(s) thereof, that may be

used in the composition of the present invention may be obtained using any known extraction methods, for example, by those skilled in the art of phytochemistry. As used herein, an "extract" is a substance made by extracting a part of a raw material, such as a plant. Those of skill in the art will appreciate that there are many other extraction processes, both known in the art and described in various patents and publications that can be used to obtain the extracts to be used in practicing the present invention. For example, the extraction procedures described in the following references, which are incorporated herein by reference, could be used in practicing the present invention; S.S. Niphadkar *et al.*, Extraction of 3-*O*-acetyl-11-keto- $\beta$ -boswellic acid (AKBA) from *Boswellia serrata* plant oleo gum resin using novel three phase partitioning (TPP) technique, 2017, *J. Appl. Res. Med. Aromat. Plants*, (7):41-47; P. Shah *et al.*, An improved process for preparation and purification of boswellic acids, WO2014195976A2, 2014; D Kumar *et al.*, Boswellic acid – Potential tumour suppressant terpenoid – Photochemistry, Extraction and Isolation Methods -A comprehensive review study, *J. Pharmacogn. Phytochem.* 2016; 5(3): 231-239.

The derivatives of boswellic acid encompassed by the present invention include, but are not limited to, triterpenic acids such as  $\alpha$ -boswellic acid, acetyl- $\alpha$ -boswellic acid,  $\beta$ -boswellic acid (BBA), acetyl- $\beta$ -boswellic acid (ABA), 9,11-dehydro- $\alpha$ -boswellic acid, acetyl-9,11-dehydro- $\alpha$ -boswellic acid, 9,11-dehydro- $\beta$ -boswellic acid, acetyl-9,11-dehydro- $\beta$ -boswellic acid, lupeolic acid, acetyl-lupeolic acid, 11-keto- $\beta$ -boswellic acid (KBBA), 3-*O*-acetyl-11-keto- $\beta$ -boswellic acid (AKBA). The derivatives of boswellic acid encompassed by the present invention may also further include, but are not limited to, derivatives of boswellic acid wherein one or more hydrogen atoms are replaced with one or more substituents selected from alkyl, alkenyl, alkynyl, aryl, halo, haloalkyl, haloalkenyl, haloalkynyl, haloaryl, hydroxy, alkoxy, alkenyloxy, aryloxy, benzyloxy, haloalkoxy, haloalkenyloxy, haloaryloxy, nitro, nitroalkyl, nitroalkenyl, nitroalkynyl, nitroaryl, nitroheterocyclyl, amino, alkylamino, dialkylamino, alkenylamino, alkynylamino, arylamino, diarylamino, benzylamino, dibenzylamino, acyl, alkenylacyl, alkynylacyl, arylacyl, acylamino, diacylamino, acyloxy, alkylsulphonyloxy, arylsulphenyloxy, heterocyclyl, heterocycloxy, heterocycylamino, haloheterocyclyl, alkylsulphenyl, arylsulphenyl, carboalkoxy, carboaryloxy, mercapto, alkylthio, benzylthio, acylthio, and phosphorus-containing groups, as well as heterocyclic derivatives of boswellic acids.

In a particular embodiment, the composition comprises AKBA.

In some embodiments, the boswellic acid and/or derivative(s) thereof may be used in "substantially pure form". This means that the boswellic acid or derivative(s) thereof, is used in a form that is substantially free of the other principal components of the plant from which it is obtained, such as *Boswellia serrata*. In this context, substantial means that the boswellic acid

- and/or derivative(s) thereof used in the composition may contain one or more of boswellic acid and/or derivative(s) thereof but contains no more than 20% w/w of the non-boswellic acid and/or derivative components of said plant, or no more than 15% w/w, no more than 10 % w/w, no more than 5% w/w of the other principal components of said plant. In some embodiments, the
- 5 substantially pure form means that the boswellic acid and/or derivative(s) thereof composition contains at least 80% w/w the boswellic acid and/or derivative(s) thereof, at least 85% w/w of the boswellic acid and/or derivative(s) thereof, at least 90% w/w of the boswellic acid and/or derivative(s) thereof, at least 95% w/w of the boswellic acid and/or derivative(s) thereof, at least 96% w/w of the boswellic acid and/or derivative(s) thereof, at least 97% w/w of the boswellic acid
- 10 and/or derivative(s) thereof, at least 98% w/w of the boswellic acid and/or derivative(s) thereof, at least 99% w/w of the boswellic acid and/or derivative(s) thereof, at least 100% w/w of the boswellic acid and/or derivative(s) thereof. Thus, the compositions may comprise pure boswellic acid and/or a derivative(s) thereof, and extracts retaining small levels of impurities from the plant from which it is obtained, such as *Boswellia serrata*.
- 15 In other embodiments, the boswellic acid and/or derivative(s) thereof are in the form of a plant extract. In these embodiments, the extract comprises at least 30%, especially at least 40%, more especially 50% boswellic acid and/or derivative(s) thereof. For example, the extract may comprise 30 to 85%, 35 to 80%, 40 to 75%, 45 to 70% or 50 to 65% boswellic acid and/or derivative(s) thereof.
- 20 In other embodiments, the boswellic acid or derivative thereof may be produced synthetically (Shamraiz *et al.*, 2018), in which case it would not be expected that any of the non-boswellic acid components of a plant extract would be present.
- In some embodiments the boswellic acid derivative is AKBA in substantially pure form. In other embodiments, the boswellic acid and/or derivative(s) thereof comprises AKBA. In yet other
- 25 embodiments, the boswellic acid and/or derivative(s) thereof is in the form of an extract, especially a *Boswellia serrata* extract.
- In some embodiments, the composition comprises boswellic acid and/or a derivative(s) thereof, in an amount in the range of from 0.0001-2.0 %, especially 0.0005-1.0 %, more especially 0.001-0.50 %, and most especially 0.010 - 0.10% by weight of the composition.
- 30 In some embodiments, the composition comprises AKBA in an amount of 0.0001 to 0.5 %, especially 0.001 to 0.1% and more especially 0.01 to 0.05 % by weight of the composition.
- The boswellic acid, derivative(s) of boswellic acid or mixture thereof is encapsulated in a nanoemulsion having a liquid lipid core and a negative surface charge. The boswellic acid and/or

derivative(s) thereof are hydrophobic and are in the form of an oil. In some embodiments, the oil is a neat extract comprising the boswellic acid and/or derivative(s) thereof. In other embodiments, the boswellic acid and/or derivative(s) thereof is dissolved in an oil/emollient without recrystallisation, preferably an ester oil. Any ester oil/emollient suitable for use in a pharmaceutical composition for topical application to the skin may be used, including, but not limited to, caprylic/capric triglyceride, coco-caprylate, coco-caprylate caprate, dicaprylyl carbonate or any combination thereof.

In some embodiments, other encapsulation technologies may be used to protect and enhance the transdermal delivery of boswellic acid and/or derivative(s) thereof including, but not limited to, liposomes, neosomes or phytosomes.

The boswellic acid and/or derivative(s) thereof are encapsulated in an encapsulation material comprising at least one emulsifier and a stabilizer. In some embodiments, the encapsulation material comprises an emulsifier and a co-emulsifier. In some embodiments, the emulsifier is 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.

In some embodiments, the stabilizer can be selected from organic substances of any kind that may solubilize the emulsifiers. Suitable examples are selected from alcohols or glycols, including, but not limited to, ethanol, isopropanol, propylene glycol, pentyleneglycol, caprylyl glycol, especially pentyleneglycol.

In some embodiments, the encapsulated boswellic acid and/or derivative(s) thereof comprises one or more emollients. In some embodiments, the emollient is a  $C_{12-15}$  alkylbenzoate. In some embodiments, the emollient is present in the lipid core. In other embodiments, the emollient is present in the encapsulation material. In yet other embodiments, the emollient is present in both the lipid core and the encapsulation material.

The pharmaceutical or cosmetic composition is formulated as a topical composition, suitable to be applied topically to the skin.

In some embodiments, the composition comprises a pharmaceutically or cosmetically acceptable vehicle. The pharmaceutically or cosmetically acceptable vehicle may be any pharmaceutically or cosmetically acceptable vehicle, including pharmaceutically and/or dermatologically acceptable carriers, including surfactants.

- 5 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. 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.
- 10 Emollients suitable for use in the vehicle or carrier for the compositions of the invention include, for example,  $C_{12-15}$  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, Ceteareth-20 (cetostearyl alcohol, which is polyethylene glycol ether of cetearyl alcohol). Rheological modifiers
- 15 suitable for use in the vehicle or carrier for the compositions of the invention include, for example, carbomer, glyceryl polyacrylate, and acrylates/ $C_{16-20}$  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,
- 20 cetearyl alcohol, 2-octyldodecanol, benzyl alcohol, sodium hyaluronate, hyaluronic acid, and water.

The composition of the present invention may also include other suitable ingredients including thickeners, fragrances, pearlescent agents, preservatives (e.g. ethylhexylglycerin, phenoxyethanol, or hydroxyacetophenone), sunscreens, vitamins, silicones, gelling agents (e.g.

25 hydrophilic or lipophilic gelling agents), antioxidants, solvents, fillers, odorabsorbers, coloring agents, pH adjusters (e.g. sodium hydroxide), solubilisers (e.g. methylpropanediol), sunscreens, skin-lightening agents, skin tanning agents, perfumes, opacifiers and preservatives. These components may make up between 0 to 50% w/w of the formulation.

For topical administration, the compositions of the present invention may be formulated as

30 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.

- 5 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 C<sub>12-18</sub>-alkoxylated oils and waxes (e.g.
- 10 ethoxylated vegetable oils, ethoxylated jojoba oil/wax, ethoxylated lanolin oil, ethoxylated coconut oil, ethoxylated cocoa butter).

In some embodiments, 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.

- 15 The compositions may additionally comprise carriers and/or vehicles to formulate the encapsulated boswellic acid and/or derivative(s) thereof into suitable pharmaceutical or cosmetic formulations. The compositions may also contain various known and conventional additives so long as they do not detrimentally affect the encapsulated boswellic acid and/or derivative(s) thereof or the microbiome-balancing capabilities provided by the compositions of the present invention. The
- 20 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 are suitable for use in the compositions of the present invention. Examples of suitable formulation ingredients include: antioxidants, soothing or calming agents, moisturizing actives, binders, biological additives, buffering agents, colorants or colour pigments, thickeners, polymers,
- 25 astringents, fragrance, humectants, opacifying agents, conditioners, exfoliating agents, pH adjusters, preservatives, rheological modifiers, foaming agents, surfactants, emollients, natural extracts, essential oils, skin sensates, skin soothing agents, and skin healing agents. The skin microbiome balancing activity of the composition comprising formulation base may also comprise other skin conditioning agents, including, but not limited to, vitamins or vitamin derivatives and
- 30 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. Exemplary preservatives include ethylhexylglycerine and phenoxyethanol. An exemplary humectant is 1,2-hexane diol.

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%), cosolvents 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  
5 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  
10 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  
15 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 preservative.

When the composition is in the form of a liquid soap, any known liquid soap may be used. A  
20 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  
25 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  
30 included in a shampoo formula or in a medicinal combination for treating a skin condition such as psoriasis, eczema, dermatitis or hair loss or another head or scalp disorder or ailment. 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

#### Methods

In one aspect, the present invention provides a composition as described above, for use in treating a subclinical symptom of sensitive skin in a subject.

In another aspect there is provided a method of treating a subclinical symptom of sensitive skin in a subject comprising applying to the skin of the subject a composition described above.

In yet a further aspect, the present invention provides a use of a composition described above in the manufacture of a medicament for treating a subclinical symptom of sensitive skin.

The subclinical symptoms of sensitive skin include impaired skin barrier function, which affects the outer surface of the skin including the stratum corneum, the outermost layer of the epidermis, together with heightened skin neurosensitivity. The prevalence of *Staphylococcus epidermidis* on the skin has been linearly correlated to Trans Epidermal Water Loss (TEWL), an indicator of skin barrier function impairment and higher Lactic Acid Stinging (LAST) virtual analogue scores (VAS) which is an indicator of skin neurosensitivity. By normalising the number of the *S. epidermidis* on the skin, the composition of the present invention can significantly reduce TEWL and VAS scores in the population with sensitive skin. The microbiome normalizing ability of invention also can be applied to other skin and scalp problems with a disrupted microbiome profile, including but not limited to, acne skin or acne prone skin, oily skin, dry skin, hair loss, hair greying, hair thinning, dandruff, sensitive scalp, irritated scalp and aged scalp, by preventing/inhibiting, the growth of microorganisms, specifically, sensitive-skin-causing microorganisms associated with TEWL and LAST.

In some embodiments, the composition of the invention adjusts the skin microbiome of a person with sensitive skin towards the microbiome of a person with healthy skin. In some embodiments, the composition of the invention normalizes the skin microbiome of a person with sensitive skin to provide a skin microbiome of healthy skin.

- 5 The phrase "microbiome balancing" or "microbiome normalisation" as used herein means selectively reducing harm causing microbes existing on the skin while maintaining or increasing the beneficial microbes.

Examples of bacteria whose growth may be reduced include, but are not limited to, bacterium in the Firmicutes Phylum. The Firmicutes phylum is composed of more than 200 different genera  
10 such as *Streptococcus*, *Lactobacillus*, *Bacillus*, *Clostridium*, *Enterococcus*, and *Ruminococcus*. Examples of bacteria whose growth may be prevented or inhibited according to the present invention include, but are not limited to, *Staphylococcus epidermidis*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus aureus*, *Staphylococcus hominis*, *Staphylococcus lentus*, *Staphylococcus lugdunensis*, *Staphylococcus massiliensis*, and *Staphylococcus simulans*,  
15 *Streptococcus* spp, *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus infantis*, and *Streptococcus periodonticum*.

In some embodiments, the methods and uses of the invention balance the skin microbiome for the treatment of sensitive skin. In other embodiments, the microbiome normalizing ability of  
20 invention also can be applied to other skin or scalp problems with a disrupted microbiome profile, including but not limited to, acne skin or acne prone skin, oily skin, dry skin, hair loss, hair thinning, dandruff, sensitive scalp, irritated scalp, hair loss, hair greying, hyper-pigmented skin, dandruff, atopic dermatitis or seborrheic dermatitis.

The composition is a topical composition and is applied on an external surface of the subject. In particular embodiments, the external surface is the skin of a subject.

- 25 Generally, the compositions of the invention are topically administered at least on a daily, and preferably a twice daily, basis for a period of time sufficient to bring about the desired level of improvement in the skin, such as a reduction in skin sensitivity. Alternatively, the compositions may be applied, once, twice, three or four times a week. For example, a user may topically administer a composition of the present invention directly to the skin where decreased sensitivity  
30 is desired by gently massaging the composition of the present invention into the desired area. This process may be repeated later the same day. In some embodiments, the composition of the present invention is left on the skin where decreased sensitivity is desired between applications occurring on the same day.

Topical application of the compositions of the invention may continue for any suitable period of time. For example, within a few days to a few weeks of the initial application, a user may notice a reduction in skin sensitivity. 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.

5 In some embodiments, the degree of therapeutic enhancement will vary with the total amount of composition used.

When topically applied, the compositions of the present invention may be applied to the area to be treated, for example, the skin in humans, by spraying, dabbing, swabbing, rubbing or massaging.

In some embodiments, the composition is intended to be applied to a localized area or surface of a body part with the intention of having a pharmacodynamic effect on that localized area. The methods and uses of the invention may improve the skin barrier function in a subject. Without wishing to be bound by theory, improvement of skin-barrier function may be mediated at the molecular level or through involvement of protein biomarkers that are proven in the art to be associated with skin barrier function, including but not limited to: increased filaggrin, involucrin, loricrin and transglutaminase-1 expression in keratinocytes cells that are located in the stratum corneum layer of the epidermis, as well as increased claudin-1 and occludin which act on tight junctions to control the permeability of molecules in the inner epidermis. The present invention may also improve skin neuro-sensitivity in a subject. Without wishing to be bound by theory, improvement of skin neuro-sensitivity may be mediated at the molecular level through involvement of protein biomarkers that are proven in the art to be associated with neuro-sensitivity, including but not limited to: reduction of the secretion of nerve growth factor (NGF) that promote the nerve innervation into epidermis, and reduction of the release of neuropeptide, including substance P and calcitonin gene-related peptide (CGRP) by skin neurons, inhibition of receptor activation of keratinocyte cells, including protease-activated receptor 2 (PAR-2) and transient receptor potential vanilloid 1 (TRPV1), and reduction of release of inflammatory mediators, including interleukin-8, interleukin-6, interleukin-1 $\beta$ , tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), thymic stromal lymphopoietin (TSLP), prostaglandin E $_2$  that trigger skin response (stinging, itching or burning) and/or skin neurogenic inflammation. The composition of the present invention may also normalize the growth of other bacterial species implicated in various skin conditions, including, but not limited to,

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*Corynebacterium spp.*, *Chryseobacterium spp.*, *Snodgrassella spp.*, *Kocuria spp.*, *Micrococcus spp.* and *Lawsonella spp.*, which are known to be associated with sensitive skin amongst the Caucasian population; *Corynebacterium spp.*, *Enhydrobacter spp.* and *prevotella spp.* that are associated with skin aging; *Staphylococcus aureus* and *Streptococcus epidermidis* which are known to be associated with atopic dermatitis; *Malassezia restricta* and *Malassezia globosa*,

which known to be associated with dandruff formation; *Cutibacterium acne* which known to be associated with acne skin; *Corynebacterium kroppenstedtii* which known to be associated with skin redness; and *Corynebacterium spp* that are associated with hair loss and grey hair.

The "subject" may be a mammal. In particular embodiments, the subject is human.

- 5 In particular embodiments, the methods and uses of the present invention are for treating sensitive skin, particularly in a subject that has come into contact with external factors including allergens, cosmetic ingredients or extreme climate conditions.

As used herein, "sensitive skin" refers to skin that is more prone to inflammation or adverse reactions. People with sensitive skin may have strong reactions to chemicals, dyes, and fragrances present in products that come into contact with the skin. Sensitive skin on a human subject can be  
10 caused by a higher abundance of harmful microorganisms from the phylum firmicutes, like *S. epidermidis*. Without wishing to be bound by theory, *S. epidermidis*, has been characterised by next generation sequencing analyses to be more abundant on sensitive skin. The abundance of this microbe is also correlated with clinical parameters used to differentiate between healthy skin and  
15 sensitive skin, wherein the abundance of *S. epidermidis* is linearly correlated to the TEWL and LAST score. Thus, *S. epidermidis* is considered to be a "sensitive skin-causing" microbe, which is in contrast to a number of studies that consider *S. epidermidis* to be a skin friendly microbe.

In some embodiments, the composition of the present invention balances the skin microbiome and/or reduces sensitive skin by application of boswellic acid or its derivative(s), optionally in the  
20 form of a *Boswellia serrata* extract or in substantially pure form, by selectively reducing prominent sensitive skin-causing bacteria, thereby maintaining a high level of beneficial microorganisms on the surface such as those belonging to the Proteobacteria Phylum, consequently improving overall skin health and reducing skin sensitivity.

In some embodiments, the microbiome balancing is obtained by selectively reducing at least one  
25 microorganism selected from the Genus *Staphylococcus*. In some embodiments, the microbiome balancing is obtained by selectively reducing the abundance of *S. epidermidis*.

### Examples

The present invention is further illustrated by the following experimental investigations and examples, which should not be construed as limiting. The contents of all references, patents and  
30 published applications cited throughout this patent are hereby incorporated by reference herein.

### Example 1 – Evaluation of the differential skin microbiome profile between Sensitive Skin and Healthy Skin

## Methodology

### *Skin Assessment and Microbial Sampling*

In this study, 24 females were sub-divided into 2 groups, in which the sensitive skin group is classified with impaired skin barrier function of Trans-Epidermal Water Loss (TEWL) more than 15 g m<sup>-2</sup> h<sup>-1</sup> and heightened neuro-sensitivity of Lactic Acid Stinging Test (LAST) score more than 2, whereby the healthy skin group is classified with normal skin barrier function with TEWL less than 15 g m<sup>-2</sup> h<sup>-1</sup> and normal neuro-sensitivity with a LAST score less than 2. All panellists were instructed to use a standard facial cleanser for 3 continuous days prior skin evaluation and microbial sampling. The skin barrier function of each subject was assessed by a TEWA meter TM300 (Courage+Khazaka electronic GmbH, Germany). On the contrary, skin neuro-sensitivity (stinging potential) was assessed by using a Lactic Acid Stinging Test with the qualitative scoring from 0 to 4 in relation to the degree of stinging sensation that was perceived by the panellist and the results were interpreted as a Visual Analogue Scale (VAS) of the stinging response at initial, 2.5 minutes and 5 minutes after 10% w/w lactic acid application on the nasolabial area. In addition, the skin microbial sampling on each panellist was conducted simultaneously and total genomic DNA was extracted from the swabs by using DNeasy PowerSoil DNA Isolation Kit (Qiagen, USA) according to the manufacturer's instructions.

### *Next Generation Sequencing*

Primers 341F (5'- CCTAYGGGRBGCASCAG-3') and 806R (5'- GGACTACNNGGTATCTAAT-3') were used to amplify the V3-V4 hypervariable region of the 16S rRNA gene from bacteria using PCR. Sequencing libraries were generated using NEB Next Ultra DNA Library Pre ® Kit for Illumina, following manufacturer's recommendations and index codes were added. The library quality was assessed on the Qubit® 2.0 Fluorometer (Thermo Scientific, USA) and Agilent Bioanalyzer 2100 system. Then, the library was sequenced on an Illumina platform and 250 bp paired-end reads were generated. Paired-end reads are first removed of sequence adaptors and low-quality reads using BBDuk of the BBTools package (Bushnell, 2016). After this, the forward and reverse reads are merged using USEARCH v11.0.667 (Edgar & Flyvbjerg, 2015). All sequences that are shorter than 150 bp or longer than 600 bp (sequenced on the MiSeq platform) were removed from downstream processing. Reads were then aligned with 16S rRNA (SILVA Release 132) and inspected for chimeric errors using VSEARCH v2.6.2 (Rognes, Flouri, Nichols, Quince, & Mahé, 2016). After these quality assessment steps, reads were clustered *de novo* into Operational Taxonomical Units (OTUs) at 97% similarity using UPARSE v11.0.667 (Edgar, 2013); rare OTUs with less than 2 reads (doubleton) which are often spurious,

were deleted from downstream processing. A single representative sequence from each OTU was randomly chosen, and Pynast (Caporaso, Bittinger, et al., 2010) was used to align and construct a phylogenetic tree against the SILVA 132 16S rRNA database. Taxonomic assignment of OTU was achieved using QIIME V1.9.1 against the Silva database 16S rRNA database (release 132)

- 5 (Caporaso, Kuczynski, et al., 2010). OTUs abundance information were normalized using a standard of sequence number corresponding to the sample with the least sequences. Subsequent analysis of alpha diversity and beta diversity were all performed basing on this output normalized data.

#### *Next Generation Sequencing Analysis*

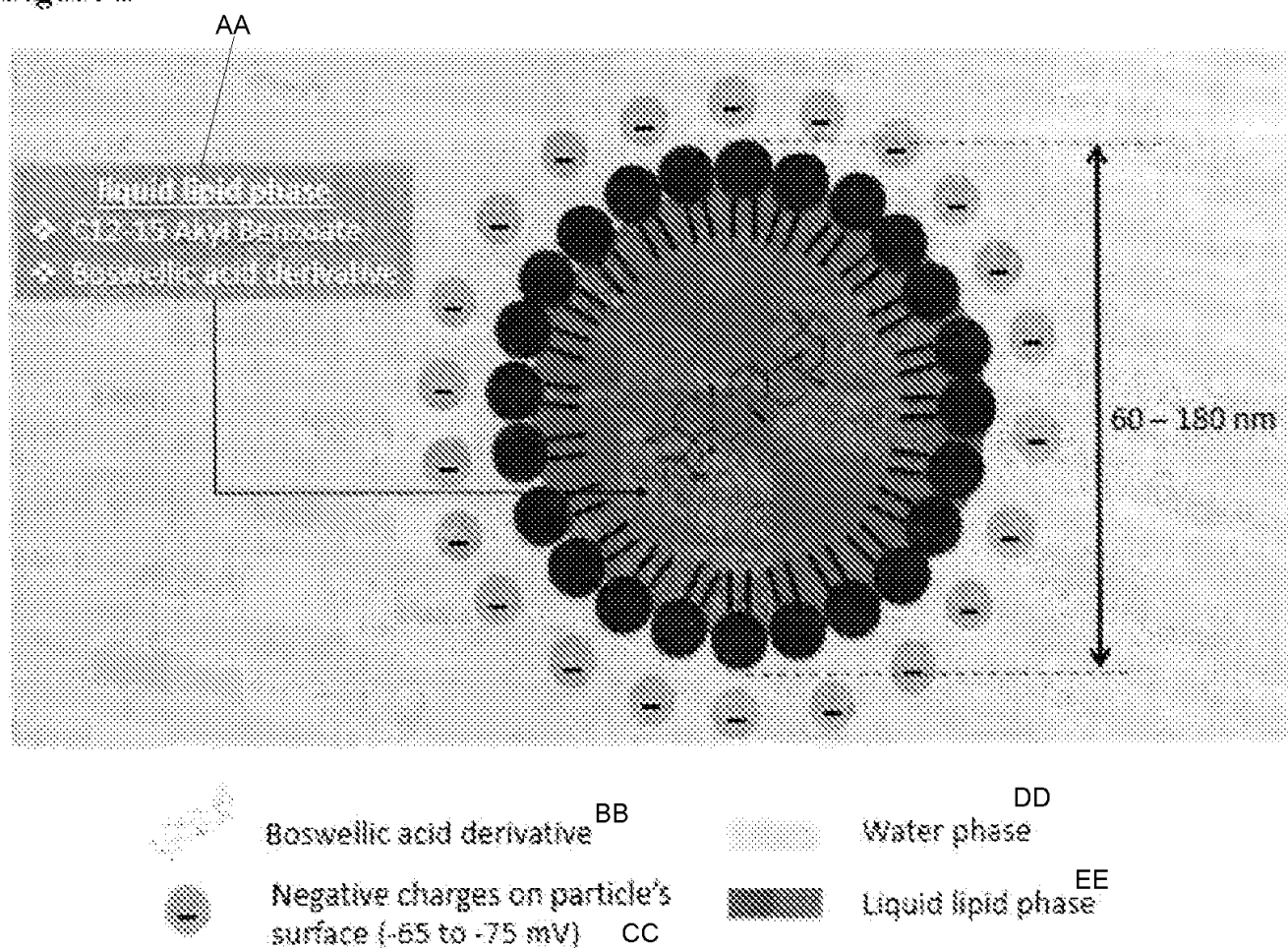
- 10 Alpha diversity was applied in analysing the complexity of species diversity for a sample through 6 indices, including Observed-species, Chao1, Shannon, Simpson, ACE, and Good-coverage. All indices in the samples were calculated with QIIME (Version 1.9.1) and displayed with R software (Version 3.6.1). Beta diversity analysis was used to evaluate differences of samples in species complexity, Beta diversity on both weighted and unweighted unifrac were calculated by QIIME
- 15 software (Version 1.9.1). Cluster analysis was preceded by principal component analysis (PCA), which was applied to reduce the dimension of the original variables using the FactoMineR package and ggplot2 package in R software (Version 3.6.1). Principal Coordinate Analysis (PCoA) was performed to get principal coordinates and visualize from complex, multidimensional data. A distance matrix of weighted or unweighted unifrac among samples obtained before was
- 20 transformed to a new set of orthogonal axes, by which the maximum variation factor is demonstrated by first principal coordinate, and the second maximum one by the second principal coordinate, and so on. PCoA analysis was displayed by WGCNA package, stat packages and ggplot2 package in R software (Version 3.6.1). Unweighted Pair-group Method with Arithmetic Means (UPGMA) Clustering was performed as a type of hierarchical clustering method to
- 25 interpret the distance matrix using average linkage and was conducted by QIIME software (Version 1.9.1). Linear discriminant analysis (LDA) effect size tool-LEfSe was used to identify OTUs with differential relative abundance comparing between normal skin, sensitive skin, and after treatment with SOS 1.1 (Segata et al., 2011). The LDA score was calculated for OTUs with  $p \leq 0.05$  and LDA score threshold  $\geq 2.00$ .

### 30 **Results and Discussion**

#### *Differential Microbiome Profile of Sensitive Skin and Healthy Skin*

As demonstrated in Figure 1, more than 98% of the detected OTU counts fall into the 4 main phyla of skin microbiome, including Bacteroidetes, Proteobacteria, Firmicutes and Actinobacteria.

**Figure 2**



AA ... phase lipidique liquide / benzoate d'alkyle en C12-C15 / dérivé de l'acide boswellique

BB ... Dérivé de l'acide boswellique

DD ... Phase aqueuse

CC ... Charges négatives à la surface de la particule (de -65 à -75 mV)

EE ... Phase lipidique liquide

*Staphylococcus epidermis* was identified as a potential pathogenic bacterium in the development of sensitive skin and its presence shows a positive correlation with TEWL and LAST, which are associated with skin barrier function impairment and heighten neuro-sensitivity.

#### **Example 2 – Composition preparation of an encapsulated boswellic acid derivative**

##### **5 Methodology**

###### *Method of Preparation*

As shown in Table 2, *Boswellia serrata* extract was dissolved in emollients such as C<sub>12-15</sub> alkyl Benzoate, while glyceryl citrate/lactate/linoleate/oleate and diglyceryl monooleate were dissolved in pentylene glycol. Then, the mixture was mixed until it is clear and deionized water is added to the bulk. Lastly, the premix was then processed using high pressure homogenizer (Microfluidizer) at 700 bar for 2 cycles.

**Table 2 – Composition of the encapsulated boswellic acid derivative**

| No. | Category          | Ingredient                                                                                     | Concentration (%) | Concentration range (%) |
|-----|-------------------|------------------------------------------------------------------------------------------------|-------------------|-------------------------|
| 1   | Emulsifier        | Glyceryl citrate/lactate/linoleate/oleate                                                      | 3.5               | 2-7                     |
| 2   | Co-emulsifier     | Diglyceryl monooleate                                                                          | 1.5               | 1-3                     |
| 3   | Stabilizer        | Pentylene glycol                                                                               | 5                 | 1-10                    |
| 4   | Emollients        | C12-15 alkyl benzoate                                                                          | 18                | 15-25                   |
| 5   | Active ingredient | <i>Boswellia serrata</i> extract with purity of total Boswellic acid derivatives more than 40% | 2                 | 0.1 -5                  |
| 6   | Diluent           | Deionized Water                                                                                | Qs to 100%        | Qs to 100%              |

##### **15 Particle size Characterization**

The mean surface charge and size distribution were determined by dynamic light scattering with Zetasizer ZS Nano (Malvern Instruments, Worcestershire, UK). The composition contains nanoemulsion wherein the size of each nanoemulsion is within the range of 60-180 nm and surface

charge of -65 to -75 mV. A cartoon illustration of the nanoemulsion that encapsulates the boswellic acid derivative is shown in Figure 2.

**Example 3 – Composition preparation comprises an encapsulated Boswellic acid derivative**

**Methodology**

5 *Composition Preparation*

Premix A (aqueous phase) and Premix B (lipid phase) were prepared separately by mixing each phase until fully dissolved. Premix B was then added into Premix A and homogenized at 2500 rpm for 15 minutes. Premix C was prepared as set out in Example 2 and was added into the mixture of Premix A and Premix B and the composition homogenized at 2500 rpm for 5 minutes and stirred at 100-200 rpm for 10 minutes. The compositions of Premixes A, B and C are shown in Table 3.

**Table 3**

| Phase | Ingredient                              | Formula 1 (V090)<br>(%) | Formula 2 (V089)<br>(%) |
|-------|-----------------------------------------|-------------------------|-------------------------|
| A     | Deionized water                         | qs 100                  | qs 100                  |
|       | Disodium EDTA                           | 0.05                    | 0.05                    |
|       | Sodium Metabisulfite                    | 0.10                    | 0.10                    |
|       | Betaine                                 | 4.00                    | 4.00                    |
|       | Glycerin                                | 1.00                    | 1.00                    |
|       | Dipropylene glycol                      | 0.45                    | 0.45                    |
|       | Ethyhexylglycerin                       | 0.10                    | 0.10                    |
|       | 1,2-hexanediol                          | 0.50                    | 0.50                    |
|       | Bis-PEG-18 Methyl ether Dimethyl silane | 0.20                    | 0.20                    |
|       | Dimethicone                             | 1.50                    | 1.50                    |
|       | Sclerotium gum                          | 1.00                    | 1.00                    |
| B     | Triethylhexanoin                        | 0.50                    | 0.50                    |
|       | Isononyl Isononanoate                   | 0.50                    | 0.50                    |

|   |                                        |      |       |
|---|----------------------------------------|------|-------|
|   | Dimethicone                            | 0.20 | 0.20  |
|   | Cyclohexasiloxane, cyclopentasiloxane  | 0.20 | 0.20  |
|   | Polycrylate crosspolymer-6             | 0.30 | 0.30  |
| C | Encapsulated boswellie acid derivative | 8.3  | 10.00 |

#### *Active Analysis of Boswellie Acid Derivative*

- Quantification of 3-*O*-acetyl-11-keto-boswellie acid (AKBA) level in the formulation was performed using Ultra Performance Liquid Chromatography (UPLC from Waters, USA). Briefly,
- 5 AKBA was extracted from the sample using 100% Methanol (Merck, Germany) with an ultrasonicator assist at 37 kHz. The extraction solution was filtered through a 0.22  $\mu$ m Polytetrafluoroethylene (PTFE) membrane (Agilent, USA) into UPLC amber vials prior to UPLC analysis. The UPLC system was equipped with a photodiode array detector (PDA) and a C18 Chromolith RP-18e octadecyl silane (ODS) separating column (Merck, Germany) with a mobile
- 10 phase of 0.5 % (w/w) acetic acid and 100 % (w/w) acetonitrile at mixing ratio of 1:10 as well as isocratic elution mode at flow rate of 1.00 mL/min throughout the analysis. Lastly, the AKBA peak was determined at a retention time of  $4.39 \pm 0.04$  minutes and an absorbance wavelength of 254 nm. An external calibration method was used for the quantification of AKBA, with the calibration standard range covering 10  $\mu$ g/mL to 150  $\mu$ g/mL.
- 15 The stability of the composition comprising an encapsulated boswellie acid derivative was assessed by analysis of the level of AKBA in the composition (%) and visual inspection before and after storing at 45 °C for 3 months.

#### **Results and Discussion**

- As shown in Table 4, both compositions with different dosages of encapsulated boswellie acid
- 20 derivative demonstrated excellent stability under accelerated temperature of 45 °C for 3 months, including the physical appearance and chemical stability of 3-*O*-acetyl-11-keto-boswellie acid (AKBA).

**Table 4** – Product stability of a composition comprising an encapsulated boswellic acid derivative

| No. | Product          | Active level of AKBA in composition (%) | Physical appearance |                    | Chemical stability of AKBA |                |
|-----|------------------|-----------------------------------------|---------------------|--------------------|----------------------------|----------------|
|     |                  |                                         | Fresh               | 3 months, 45°C     | Fresh                      | 3 months, 45°C |
| 1   | Formula 1 (V090) | 0.0382                                  | White opaque cream  | White opaque cream | 109.16 %                   | 113.86 %       |
| 2   | Formula 2 (V089) | 0.0465                                  | White opaque cream  | White opaque cream | 104.39 %                   | 103.23 %       |

**Conclusion**

The composition comprising an encapsulated boswellic acid derivative demonstrates good physical stability under elevated temperature (45 °C) for 3 months. Furthermore, the encapsulated boswellic acid derivative demonstrates excellent chemical stability under accelerated temperature (45 °C) for 3 months.

**Example 4 – Clinical evaluation of the composition comprising of an encapsulated Boswellic acid derivative on the skin microbiome profile and relief of subclinical symptoms of Sensitive**

**Skin**

**Objective**

To evaluate the product efficacy of selected Formulation 1 on skin barrier function, neuro-sensitivity and skin microbiome profile in panellists with sensitive skin and healthy skin.

**Methodology**

*Composition Preparation*

The composition was prepared as set out in Example 3.

*Clinical Study*

Briefly, 24 females were sub-divided into 2 groups, the sensitive skin group and the healthy skin group (as defined in the methodology section of Example 1). All panellists were instructed to use a standard facial cleanser for 3 continuous days prior to skin evaluation and microbial sampling for baseline measurement. Then, panellists were instructed to apply Formula 1 on the face region

twice daily for a duration of 4 weeks. Lastly, skin parameters of skin barrier function, skin neuro-sensitivity and microbial sampling were conducted to evaluate the impact of the product on changes of skin conditions and microbiome profile in both groups of sensitive skin and healthy skin as per the protocol described in the methodology section of Example 1.

## 5 Results and Discussion

As demonstrated in Figure 3, product treatment of Formula 1 significantly improved skin neuro-sensitivity by reducing the VAS score and improved skin barrier function by demonstrating reduction in TEWL ( $p$  value = 0.1328) in the sensitive skin group. On the contrary, no significant changes were found for the Formula 1 treatment among the healthy skin group on both clinical  
10 parameters. In association with the microbiome profile (Figure 4), Formula 1 significantly normalized the abundancy of bacteria belonging to the Firmicutes phylum, more specifically *Staphylococcus epidermidis* in the sensitive skin group and shifted the microbiome profile closer to that of healthy skin. Further, no significant changes of microbiome profile were found after Formula 1 treatment in the healthy skin group.

## 15 Conclusion

Formulation 1 comprising an encapsulated boswellic acid derivative improves skin neuro-sensitivity and skin barrier function among panelists with sensitive skin, whilst concomitantly shifting the skin microbiome profile closer to the healthy skin profile.

Formulation 1 comprising an encapsulated boswellic acid derivative normalizes the abundancy of  
20 bacteria belonging to the Firmicutes phylum, more specifically *Staphylococcus epidermidis*, among the panelists in the sensitive skin group.

Formulation 1 comprising an encapsulated boswellic acid derivative does not disrupt the skin microbiome profile of panelists with Healthy skin

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**The Claims Defining the Invention are as Follows:**

1. A topical composition comprising boswellic acid, one or more derivatives of boswellic acid, or a mixture thereof, wherein the boswellic acid, derivative of boswellic acid or mixture thereof is encapsulated in a nanoemulsion having a liquid lipid core and a negative surface charge.  
5
2. The composition of claim 1, wherein the size of each nanoemulsion is within the range of 50-200 nm.
3. The composition of claim 1 or claim 2, wherein the surface charge of the nanoemulsion is in the range of -50 to -90 mV.
- 10 4. The composition of any one of claims 1 to 3, wherein the boswellic acid, one or more derivatives of boswellic acid or a mixture thereof, is present in an amount of 0.001% to 2.0% by weight of the composition.
5. The topical composition of any one of claims 1 to 4, wherein the boswellic acid, one or more derivatives of boswellic acid or a mixture thereof comprises 3-O-acetyl-11-keto- $\beta$ -boswellic acid (AKBA).  
15
6. The topical composition of any one of claims 1 to 5, wherein the boswellic acid, one or more derivatives of boswellic acid or a mixture thereof is 3-O-acetyl-11-keto- $\beta$ -boswellic acid (AKBA).
7. The topical composition of any one of claims 1 to 6, wherein the boswellic acid, one or more derivatives of boswellic acid or a mixture thereof is obtained from or contained within a plant extract.  
20
8. The topical composition of claim 7 wherein the plant extract is an extract of *Boswellia serrata*.
9. The topical composition of claim 8 wherein the composition comprises *Boswellia serrata* extract.  
25
10. The topical composition of any one of claims 1 to 9 wherein the nanoemulsion comprising a liquid lipid core and a negative surface charge further comprises, in addition to the boswellic acid, one or more boswellic acid derivatives or a mixture thereof, at least one emulsifier, a stabilizer and at least one emollient.
- 30 11. The topical composition of claim 10 wherein the nanoemulsion comprises an emulsifier and a co-emulsifier.

12. The topical composition of claim 10 or 11 wherein the one or more emulsifier comprises glyceryl citrate/lactate/linoleate/oleate.
13. The topical composition of claim 11 or claim 12 wherein the co-emulsifier is diglyceryl monooleate.
- 5 14. The topical composition of any one of claims 10 to 13 wherein the stabilizer is pentylene glycol.
15. The topical composition of any one of claims 10 to 14 wherein the one or more emollients comprises C<sub>12-15</sub> alkyl benzoate.
16. The topical composition of any one of claims 1 to 15 further comprising one or more  
10 ingredients selected from vitamins and vitamin derivatives, cosmetic peptides, plant extracts, polyphenol compounds, preservatives, emollients, humectants, rheological modifiers, surfactants, foaming agents, sunscreen agents, sensorial additives or texturizers, color pigments, natural extracts, essential oils, macro or micro capsules containing vitamins and vitamin derivatives, peptides, plant extracts, sunscreens and essential oils.
- 15 17. The topical composition of any one of claims 1 to 16 wherein the composition is formulated as a leave-on skin toner, skin serum, skin ampoule, skin lotion and cream, skin cleanser, facial and eye make-up remover, shampoo, conditioner, hair tonic, leave on hair conditioner or hair vitamin, hair styling aid, shower gel/cream, toilet bar, antiperspirant, deodorant, depilatory, all color cosmetics not limited to lipstick and foundation, sunless  
20 tanner or sunscreen lotion.
18. A composition according to any one of claims 1 to 17, for use in alleviating a subclinical symptom of sensitive skin in a subject.
19. The composition of claim 18, wherein the subclinical symptom of sensitive skin in a  
25 subject is selected from one or both of impaired skin barrier function and skin neuro-sensitivity.
20. The composition of any one of claims 1 to 17, for use in normalising the skin microbiome profile of a subject with sensitive skin.
21. The composition of claim 20, wherein normalizing the skin microbiome profile reduces  
abundance of bacterial species belonging to the phylum Firmicutes.
- 30 22. The composition of claim 21 wherein the species of bacteria is selected from the group consisting of *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Staphylococcus hominis* or *Staphylococcus capitis*.

23. The composition of any one of claims 1 to 17, for use on a subject with healthy skin without causing disruption of the skin microbiome profile.
24. A method of treating a subclinical symptom of sensitive skin in a subject, comprising applying to the skin of said subject the composition of any one of claims 1 to 17.
- 5 25. Use of the composition of any one of claims 1 to 17, in the manufacture of a medicament for treating a subclinical symptom of sensitive skin.
26. A composition of any one of claims 1 to 17 for use in treating or preventing acne or acne prone skin, oily skin, oily scalp, dry skin, dry scalp, irritated skin, irritated scalp, aged skin, aged scalp, hair loss, hair greying, hyper-pigmented skin, dandruff, atopic dermatitis or seborrheic dermatitis.
- 10 27. A method of treating or preventing acne skin or acne prone skin, oily skin, oily scalp, dry skin, dry scalp, irritated skin, irritated scalp, aged skin, aged scalp, hair loss, hair greying, hyper-pigmented skin, dandruff, atopic dermatitis or seborrheic dermatitis, comprising applying to the skin of a subject the composition of any one of claims 1 to 17.
- 15 28. Use of a composition according to any one of claims 1 to 17 in the manufacture of a medicament for treating or preventing acne or acne prone skin, oily skin, oily scalp, dry skin, dry scalp, irritated skin, irritated scalp, hair loss, hair greying, aged skin, aged scalp, hyper-pigmented skin, dandruff, atopic dermatitis or seborrheic dermatitis.