Title: COSMETIC USE OF AN EXTRACT OF MIRABILIS JALAPA

Abstract: The present invention provides the use of an extract of Mirabilis Jalapa for non-therapeutic cosmetic treatment of the signs of a sensitive skin, a corresponding active ingredient and cosmetic composition. The extract is preferably obtained by alcoholic extraction from the aerial parts of the plant. The Mirabilis jalapa extract according to the invention can reduce the overproduction of Nerve Growth Factor (NGF) and TRPV1 receptor, thicken the epidermis and improve the barrier function of the stratum corneum.
COSMETIC USE OF AN EXTRACT OF MIRABILIS JALAPA

TECHNICAL FIELD

The invention relates to the use of a plant extract for a cosmetic treatment, as well as to a cosmetic active ingredient and a cosmetic composition containing it appropriate for said use.

BACKGROUND ART

The cosmetic industry is in increasing demand for new products, particularly in demand for new active ingredients that are derived from plants, because they can combine efficiency, limitation of the risks of irritation and allergy, reduction of side effects, biodegradability, with the possibilities of labelling/certifications and adequacy with a sustainable development and/or fair trade logic.

More particularly, the invention aims the use of a plant extract for a cosmetic treatment of sensitive skins (also called hyper-reactive skins).

The skin is a highly innervated organ since every 1 cm² of skin has on average 10 hairs and 12 nerve endings. Although the bodies of nerve cells are buried deep within ganglia, their long fibres, or axons, extend outwards towards the dermis and then perpendicularly towards the surface of the skin, passing through the epidermal cells. These sensitive nerve endings are unlike other cells in that they do not have a protective lipid-containing covering. Therefore, they are in direct contact with their surrounding environment, which is made up of epidermal keratinocytes. The keratinocytes play an important role in increasing or decreasing the activation level of the nerve ending to external stimuli. The nerve endings are intended to convey information of physicochemical nature, from the environment to the brain. This organization allows the individual to be notified immediately of changes in its environment. Once alerted, the nervous system activates a wide variety of defense systems the simplest of which to move away the danger.

The sensitive response is a mechanism allowing external stimuli to trigger in nerve cells a potential of action that propagates along the nerve fibers to the cell body where it is processed, this in turn generating a reaction. These stimuli can act directly on nerve endings by exciting them, or indirectly on neighboring keratinocytes that will in turn send signals in the direction of nerve endings to excite them.

At the molecular level, a family of receptors is particularly involved in the sensory response: the TRPV (Transient Receptor Potential Vanilloid). TRPV-1 are the most involved in the skin sensitization reaction. They are expressed in nerve cells, but also in keratinocytes.

In response to a stimulus, the TRPV-1 receptors are activated on the surface of nerve endings. This activation is the starting point of the sensitive response.

In the keratinocyte, TRPV-1 activation, in response to a stimulus will result in the release into the extracellular medium of the Nerve Growth Factor (NGF). This mediator will bind to the nerve endings and will trigger in the nerve cell the synthesis of TRPV 1, thus increasing the sensitivity of nerve cells to external stimuli.
In a sensitive skin, this mechanism is disturbed, gets out of control, causing epidermal NGF overproduction, which will itself cause an overproduction of TRPV1 receptors. And it is this overproduction and then the excitement of TRPV1 in excessive quantities which is causing the symptoms of sensitive skin.

Therefore, one objective of the cosmetic treatment of sensitive skin is then to lower the production by the keratinocyte of these two molecules (NGF and TRPV1).

TRPV4 receptor, found beside TRPV1, is also involved in skin sensitivity. TRPV4 is sensitive to osmotic pressure changes, mechanical stresses and temperatures around 33°C. Activation leads to calcium influx into the keratinocyte, which will promote its differentiation and the formation of the cornified envelope (stratum corneum). In addition, through its effect on calcium, TRPV4 strengthens the tight bonds between keratinocytes which is a key element in the effectiveness of the skin barrier. Unlike TRPV-1, TRPV-4 receptor is therefore positively involved in the treatment of sensitive skin and its expression is sought.

Furthermore, in the skin, the epidermis provides a protective barrier between the body and the environment. The keratinocytes form the bulk part of the epidermis; as they differentiate, they produce the stratum corneum, which is a complex assembly of cell bodies and semi-permeable lipids. The stratum corneum forms an effective barrier against aggressive agents, by making a buffer zone between them and the rest of the skin. The modification of the homeostasis of this layer thus leads to more or less serious unpleasant sensations and facilitates the penetration of irritants, who can more easily interact with keratinocytes and nerve cells according to the mechanism described above. This weakening of the barrier is observed in people with sensitive skin.

In the entire population, sensitive skin profile is far from being a rare case. It affects both sexes, women being however a little more affected than men. All skin types are affected: very oily to oily, combination, dry to very dry. This skin dysfunction is an important issue for the cosmetics industry, especially since it seems to be increasing.

Sensitive skin is described as more highly reactive than normal skin, with exaggerated reactions to external stimuli: hot, cold, wind, water, hygiene or care products, cloth friction or internal stimuli (menstrual cycle for example). People with sensitive skin reported frequent and unpleasant sensations such as itching and stinging.

One can be born with sensitive skin, as is often the case for people with light skin which is thin and prone to redness. One can also have a sensitive skin due to invasive, repetitive actions or a “too perfect” hygiene not allowing the regeneration of the stratum and break down the fine skin protection. Excessive sun, lasers and peels also contribute to irritate, aggress, and remove lipids of the protective layer constituting the stratum corneum.
Sensitive skins tend to have:
- Firstly a finer *stratum corneum* more damaged with reduced corneocyte surfaces, changes in intercellular lipids with less ceramides. These factors will lead to a greater permeability with regard to skin aggressive agents;
- Secondly, a greater density in nerve fibers which is accompanied by an increase in the basal rate of NGF and TRPV1 receptors in the upper part of the epidermis where the keratinocytes are in contact with the nerve endings.

It is therefore appropriate for this type of sensitive skin to provide an active ingredient able to:
- Strengthen the protective capacity of the epidermis, by thickening it and improving the *stratum corneum* barrier function; and
- reduce NGF formation and the expression of the keratinocytic TRPV1 receptors responsible for amplifying the sensitive response.

The present invention aims to meet this demand.

*Mirabilis jalapa* extracts from have already been proposed in cosmetics.

FR2818141 describes the use of an aqueous extract of *Mirabilis jalapa* in cosmetics as anti-aging, anti-wrinkle and to fight skin dryness.

JP63 192705 describes an extract of Peruvian Wonder to treat chapped skin.

JP03220129 describes an inhibitory effect of sebum production for an extract of *Mirabilis jalapa*.

*Mirabilis jalapa* is an ornamental plant also called "Marvel of Peru" or "Belle de Nuit". Native of Peru and implemented first in Spain, it is now possible to find in many parts of the world because it is both very popular and resistant.

**SUMMARY OF THE INVENTION**

The present invention provides the use of an extract of the *Mirabilis jalapa* plant for the non-therapeutical cosmetic topical treatment of the signs of a sensitive skin. The cosmetic treatment according to the invention improves comfort of sensitive skin.

The tests presented below show that an extract of *Mirabilis jalapa* can be used to prevent and/or correct an excessive sensitivity of the skin, by the decrease of the molecules that are at the origin (overproduction of Nerve Growth Factor (NGF) and TRPV1 receptor), by the thickening of the epidermis and the improvement of the barrier function of the *stratum corneum*. The extract from the *Mirabilis jalapa* changes people's perceptions about their sensitive skin (including stinging and itching) by acting on messengers that relay information about epidermal discomfort and quality.

« Extract » means according to the invention an extract of a plant or a part of a plant, said extract being obtained by a usual extraction method.

As examples, the usual extraction methods that can be used include maceration, simple decoction, lixiviation, extraction under reflux, supercritical fluid extraction (for example C02 supercritical), extraction using ultrasounds or microwaves, and counter flow extraction techniques. The extraction solvents may be selected from water, propylene glycol, butylene glycol, glycerin, caprylic/capric PEG-
6 glycerides, polyethylene glycol, methyl and/or ethyl ethers, diglycols, cyclic polyols, ethoxylated or propoxylated diglycols, alcohols (methanol, ethanol, propanol, butanol, isobutanol and isopropanol), ketones (in particular acetone), or any mixture of these solvents.

An extract obtained by *in vitro* plant culture is also encompassed by the present invention. Various techniques exist including the culture of dedifferentiated cells, tissue culture or organ or *in vitro* micropropagation by somatic embryogenesis or vegetative multiplication.

The preparation of an extract by plant cell culture has advantages compared to the agro-industrial way (growing plants in open fields and subsequent industrial extraction): plant materials obtained are free of toxic substances (herbicides etc.), reproducibility is improved, biodiversity is preserved, etc.

All parts of the plant, including aerial parts, such as leaves, stems, flowers or seeds, or else roots can be used according to the invention.

According to preferred features:

- The use is topical; and/or
- The *Mirabilis jalapa* extract is an extract of aerial parts of the plant; and/or
- The *Mirabilis jalapa* extract is obtained by alcoholic extraction, more preferably ethanolic extraction; and/or
- The *Mirabilis jalapa* extract is combined with a physiologically acceptable excipient; and/or
- The *Mirabilis jalapa* extract is a dry residue (obtained by removing the extraction solvent) and re-dissolved in said excipient comprising preferably at least a short hydrocarbon chain alcohol (C1, C2, C3, C4, C5 or C6); and/or
- The dry residue % is comprised between 0.00015 and 15% in weight of the total weight of the composition comprising said residue and excipient.

More preferably, the extract is prepared with the aerial parts of the plant obtained according to the following method:

- Crushing the aerial parts of the plant;
- Hot alcoholic extraction;
- Alcohol evaporation; and
- Dissolution of the dry residue in a cosmetic excipient (physiologically acceptable), for example a mixture of short hydrocarbon chain alcohols.

The present invention provides also a cosmetic active ingredient for a cosmetic use for the treatment of sensitive skin according to the invention, comprising an effective amount of an extract of *Mirabilis jalapa* obtained by alcoholic extraction, preferably an ethanolic extraction. More preferably the *Mirabilis jalapa* extract is obtained by hot alcoholic extraction, removing of alcohol to obtain a dry residue and dissolution of said residue in a physiologically acceptable excipient.

The present invention also provides a composition comprising, as active ingredient in the treatment of sensitive skins, an effective amount of an extract of *Mirabilis jalapa* according to the invention in a physiologically acceptable medium.
According to other preferred features of the invention, the *Mirabilis jalapa* extract can be used in association with one or more others active ingredients, to provide advantageously a wider range of cosmetic properties or to intensify the activity of the invention. The active ingredients may for example be selected from the lightening, pro-pigmenting, anti-redness, anti-spots, calming, UV sunscreens, moisturizers, humectants, exfoliating, smoothing, toning, anti-aging, anti-wrinkles and fine lines, improving mechanic and elastic properties, radiance of complexion, detoxifying actives, acting on cutaneous barrier, anti-acne, for the treatment of sensitive or reactive skins slimming, volumizing, acting on sebum production, mating, unifying, anti-inflammatory, anti-oxidant, anti-radical, anti-glycation, propigmenting or depigmenting, depilatories, anti-regrowth, or promoting hair growth, eye contours (dark circle, under eye bags), promoting blood circulation, peptides, vitamins, etc. These active ingredients can be obtained by synthesis, or from plant materials, such as extracts of plants or of plant cell culture products, or by fermentation.

More specifically, the *Mirabilis jalapa* extract can be combined with at least one of the compounds selected from the compounds of B3 vitamin, compounds as niacinamide or tocopherol, retinoids compounds, such as retinol, hexamidine, a-lipoic acid, resveratrol or DHEA, peptides, including N-acetyl-Tyr-Arg-O-hexadecyl ester, Pal-GVGAPG (SEQ ID NO: 1), Pal-KTTKS (SEQ ID NO: 2), Pal-GHK, Pal-KM02K and Pal-GQPR (SEQ ID NO: 3), which are widely used active ingredients in topical cosmetic or dermo-pharmaceutical compositions.

The CTFA International cosmetic ingredient dictionary & handbook (13th Ed. 2010) (published by the Cosmetic, Toiletry, and Fragrance Combination, Inc., Washington, D.C.) describes a non-limited wide variety of cosmetic and pharmaceutical ingredients conventionally used in the skin care industry that can be used as additional ingredients in the compositions of the present invention.

Further skin care and hair care active ingredients that are particularly useful combined with the composition can be found in SEDERMA commercial literature and on the website www.sederma.fr.

The following commercial actives can also be mentioned, as examples: betain, glycerol, Actimoi Bio 2™ (Active organics), AquaCacteen™ (Mibelle AG Cosmetics), Aquaphyline™ (Silab), AquaregulK™ (Solabia), Carciline™ (Greentech), Codivelane™ (Biotech Marine), Dermaflux™ (Arch Chemicals, Inc), Hydra'Flow™ (Sochibo), Hydromoist L™ (Symrise), RenovHyal™ (Soliance), Seamoss™ (Biotech Marine), Argireline™ (trade name of the acetyl hexapeptide-3 of Lipotec), spilanthol or an extract of Acmella oleracea known under the name Gatuline Expression™, an extract of Boswellia serrata known under the name Boswellin™, Deepaline PVB™ (Seppic), Syn-AKE™ (Pentapharm), Ameliox™, Bioxilift™ (Silab), Subliskin™ (Sederma), Venuceane™ (Sederma), Moist 24™ (Sederma), Vegesome Moist 24™ (Sederma), Essenskin™ (Sederma), Juvinity™ (Sederma), Revidrat™ (Sederma), Resistem™ (Sederma), Chronodyn™ (Sederma), Kombuchka™ (Sederma), Chromocare™ (Sederma), Calmosensine™ (Sederma), Glycokin factor S™ (Sederma), Biobustyl™ (Sederma), Idealift™ (Sederma), Ceramide 2™, Ceramide A2™ et Ceramide H03™ (Sederma), Odawhite™ (Sederma), Lumisphere™ (Sederma), Legance™ (Sederma),
Intenslim™ (Sederma), Zingerslim™ (Sederma), Prodizia™ (Sederma), Beautifeye™ (Sederma), Meiritage™ (Sederma), Senestem™ (Sederma), Sebuless™ (Sederma), Majestem™ (Sederma), or mixtures thereof.
Among other plant extracts which can be combined with the composition of the invention, there may more particularly be mentioned extracts of Ivy, in particular English Ivy (Hedera Helix), of Bupleurum chinensis, of Bupleurum Falcatum, of arnica (Arnica Montana L), of rosemary (Rosmarinus officinalis N), of marigold (Calendula officinalis), of sage (Salvia officinalis L), of ginseng (Panax ginseng), of Zingiber zerumbet sm., of ginko biloba, of St.-John's-Wort (Hypericum Perforatum), of butcher's-broom (Ruscus aculeatus L), of European meadowsweet (Filipendula ulmaria L), of big-flowered Jarva tea (Orthosiphon Staminucus Benth), of algae (Fucus Vesiculosus), of birch (Betula alba), of green tea, of cola nuts (Cola Nipida), of horse-chestnut, of bamboo, of Centella asiatica, of heather, of fucus, of willow, of mouse-ear, of escine, of canguzu, of chrysanthellum indicum, of the plants of the Armeniacea genus, Atractylodis Platycodon, Sinnomenum, Pharbitidis, Flemingia, of Coleus such as C. Forskohii, C. blumei, C. esquirolloi, C. scutellaroides, C. xanthantus and C. Barbatus, such as the extract of root of Coleus barbatus, extracts of Ballote, of Guioa, of Davallia, of Terminalia, of Barringtonia, of Trema, of antirobia, cecropia, argania, dioscoreae such as Dioscorea opposita or Mexican, extracts of Ammi visnaga, of Siegesbeckia, in particular Siegesbeckia orientalis, vegetable extracts of the family of Ericaceae, in particular bilberry extracts (Vaccinium angustifolium) or Arctostaphylos uva ursi, aloe vera, plant containing sterols (e.g., phytosterol), Manjistha (extracted from plants of the genus Rubia, particularly Rubia Cordifolia), and Guggal (extracted from plants of the genus Commiphora, particularly Commiphora Mukul), kola extract, chamomile, red clover extract, Piper methysticum extract (Kava Kava™ from SEDERMA), Bacopa monieri extract (Bacocalmine™ from SEDERMA) and sea whip extract, extracts of Glycyrrhiza glabra, of mulberry, of melaleuca (tea tree), of Larrea divaricata, of Rabdosia rubescens, of Euglena gracilis, of Fibraurea recisa Hirudinea, of Chaparral Sorghum, of sun flower extract, of Enantia chlorantha, of Mitracarpe of Spermacoecea genus, of Buchu barosma, of Lawsonia inermis L., of Adiantium Capillus-Veneris L., of Chelidonium majus, of Luffa cylindrica, of Japanese Mandarin (Citrus reticulata Blanco var. unshiu), of Camelia sinensis, of Imperata cylindrica, of Glaucium Flavum, of Cupressus Sempervirens, of Polygonatum multiflorum, of lovely hemsleya, of Sambucus Nigra, of Phaseolus lunatus, of Centaurium, of Macroystis Pyrifera, of Turnera Diffusa, of Anemarrhena asphodeloides, of Portulaca pilosa, of Humulus lupulus, of Coffea Arabica, of Ilex Paraguariensis, or of Globularia Cordifolia, of Albizzia julibrissin, of Oxydendron arboretum, of Zingimber Zerumbet Smith, of Astragalus membranaceus, of Bupleurum falcatum of Atractyloides macrocephalae, or of Plantago lanceolata.

The compositions of the present invention may include peptides, including, without limitation, the di-, tri-, tetra-, penta-and hexapeptides and their derivatives. According to a particular embodiment, the
concentration of the additional peptide, in the composition, ranges from $1 \times 10^{-2}\%$ and 20%, preferably from $1 \times 10^{-3}\%$ and 10%, preferably between $1 \times 10^{-3}\%$ and 5% by weight.

According to the present invention, the term "peptide" refers to peptides containing 10 amino acids or less, their derivatives, isomers and complexes with other species such as a metal ion (e.g. copper, zinc, manganese, magnesium, and others). The term "peptides" refers to both natural peptides and synthetic peptides. It also refers to compositions that contain peptides and which are found in nature, and/or are commercially available.

Suitable dipeptides for use herein include but are not limited to Carnosine (beta-AH), YR, VW, NF, DF, KT, KC, CK, KP, KK or TT. Suitable tripeptides for use herein include, but are not limited to RKR, HGG, GHK, GKH, GGH, GHG, KFK, GKH, KPK, KMOK, KM02K or KAvaK. Suitable tetrapeptides for use herein include but are not limited to RSRK (SEQ ID NO: 4), GQPR (SEQ ID NO: 5) or KTFK (SEQ ID NO: 6). Suitable pentapeptides include, but are not limited to KTTKS (SEQ ID NO: 7). Suitable hexapeptides include but are not limited to GKTTKS (SEQ ID NO: 8) and VGVAPG (SEQ ID NO: 9).

Other suitable peptides for use herein include, but are not limited to: lipophilic derivatives of peptides, preferably palmitoyl derivatives, and metal complexes as aforementioned (e.g. copper complex of the tripeptide HGG). Preferred dipeptide include for example N-Palmitoyl-beta-Ala-His, N-Acetyl-Tyr-Arg-hexadecylester (Calmosensine™, Idealift™ from Sederma). Preferred tripeptide derivatives include for example N-Palmitoyl-Gly-Lys-His, and Pal-Gly-His-Ly, (Pal-GKH and Pal-GHK from Sederma), the copper derivative of HGG (Lamin™ from Sigma), Lipospondin (N-Elaidoyl-KFK) and its analogs of conservative substitution, N-Acetyl-RKR-NH2 (Peptide CK+), N-Biot-GHK (from Sederma), Pal-KM02K (Matrixyl Synthe6™ from Sederma) and derivatives thereof. Suitable tetrapeptide derivatives for use according to the present invention include, but are not limited to, N-Pal-GQPR (SEQ ID NO: 3) (from Sederma), suitable pentapeptide derivatives for use herein include, but are not limited to, Pal-KTTKS (SEQ ID NO: 2) (available as Matrixyl™ from Sederma), Pal-YGGF-X (SEQ ID NO: 10) with X Met or Leu or mixtures thereof. Suitable hexapeptide derivatives for use herein include, but are not limited to, Pal-VGVAPG (SEQ ID NO: 1) and derivatives thereof.

The mixture of Pal-GHK and Pal-GQPR (SEQ ID NO: 3) (Matrixyl™ 3000, Sederma) can also be mentioned.

The preferred compositions commercially available containing a tripeptide or a derivative include Biopeptide-CL™, Maxilip™, Biobustyl™, Procapil™ and Matrixylsyn®Synthe6™ of Sederma. The compositions commercially available preferred sources of tetrapeptides include Rigin™, Eyeliss™, Matrixyl™ Reloaded and Matrixyl 3000™ which contain between 50 and 500 ppm of Pal-GQPR (SEQ ID NO: 3) and an excipient, proposed by Sederma.

The following marketed peptides can be mentioned as well as additional active ingredients:
- Vialox™ (INCI name = Pentapeptide-3 (synthetic peptide comprising alanine, arginine, isoleucine, glycine and proline)), Syn-ake™ (β-Ala-Pro-Dab-NH-Bzl) or Syn-Coll™ (Pal-Lys-Val-Lys-OH) marketed by Pentapharm;
- Argireline™ (Ac-Glu-Glu-Met-Gln-Arg-Arg-NH₂ (INCI name = Acetyl hexapeptide-3) (SEQ ID NO: 11), Leuphasyl™ (Tyr-D-Ala-Gly-Phe-Leu) (SEQ ID NO: 12), Aldenine™ (Gly-His-Lys), Trylagen™ (INCI name = Pseudoalteromonas Ferment Extract, Hydro lyzed Wheat Protein, Hydro lyzed Soy Protein, Tripeptide-10 Citrulline (reaction product of Citrulline and Tripeptide-10 (synthetic peptide constituted of aspartic acid, isoleucine and lysine)), Tripeptide-1), Eyeseryl™ (Ac-p-Ala-His-Ser-His)(SEQ ID NO: 13), Serilesine™ (Ser-Ile-Lys-Val-Ala-Val) (SEQ ID No 14) or Decorinyl™ (INCI name: Tripeptide-10 Citrulline = reaction product of Citrulline and Tripeptide-10 (synthetic peptide constituted of aspartic acid, isoleucine and lysine) marketed by Lipotec;
- Collaxy™ (Gly-Pro-Gln-Gly-Pro-Gln (SEQ ID NO 15)) or Quintescine™ (Cys-Gly) marketed by Vincience;
- Cytokino™LS (casein hydrolysate) marketed by Les Laboratoires Serobiologiques/Cognis;
- Kollaren™ (Gly-His-Lys), IP2000™ (Pal-Val-Tyr-Val) or Meliprene™ (INCI name = Monofluoroheptapeptide-1: reaction product of acetic acie and a synthetic peptide comprising arginine, glycine, glutamic acid, histidine, norleucine, p-fluorophenylalanine and tryptophan) marketed by l'Institut Europeen de Biologie Cellulaire;
- Neutrazen™ (Pal-His-D-Phe-Arg-NH₂) marketed by Innovations; or
- BONT-L-Peptide™ (INCI name = Palmitoyl Hexapeptide-19: reaction product of palmitic acid and Hexapeptide-19 (synthetic peptide constituted of asparagine, aspartic acid, lysine and methionine), Timp-Peptide™ (INCI name = Acetyl Hexapeptide-20: reaction product obtained by acetylation of Hexapeptide-20 (synthetic peptide constituted of alanine, glycine, lysine, valine and proline) or ECM Moduline™ (INCI name = Palmitoyl Tripeptide-28: reaction product of palmitic acid and Tripeptide-28 (synthetic peptide constituted of arginine, lysine and phenylalanine) marketed by Infinitec Activos.

**DETAILED DESCRIPTION**

The present invention will be better understood and other advantages will be apparent from the following details and non-limiting examples.

**Composition preparation**

"Physiologically acceptable medium" means according to the present invention, without limitation, an aqueous or hydro-alcoholic solution, a water-in-oil emulsion, an oil-in-water emulsion, a micro-emulsion, an aqueous gel, an anhydrous gel, a serum, a dispersion of vesicles, or a powder.

"Physiologically acceptable" means that the compositions are suitable for topical or transdermal use, in contact with mucous membranes, appendages (nails, hair and hairs), scalp and skin of mammals,
particularly human, compositions which may be ingested or injected into the skin, without risk of toxicity, incompatibility, instability, allergic response, and others.

This "physiologically acceptable medium" forms what is commonly called the excipient of the composition.

For the use according to the invention, the effective amount of the *Mirabilis jalapa* extract, that is to say its dosage, depends on various factors, such as the age, the condition of the skin of the patient, etc. An effective amount means a non-toxic amount enough to achieve the desired effect.

For the use in the treatment of sensitive skins according to the invention, the extract of *Mirabilis jalapa*, to be present in an effective amount, is generally present in an amount ranging from 0.00015 % to 1.5 % of dry residue. The one skilled in the art is capable of adjusting the active content depending on the specific destination and the desired effect.

All percentages and ratios used herein are by weight of the total composition and all measurements are made at 25°C unless it is otherwise specified.

The choice of the excipient of the composition is made according to the constraints in link with the *Mirabilis jalapa* extract (its stability, solubility, etc.), and if necessary according to the galenic form envisaged afterwards for the composition.

The *Mirabilis jalapa* extract can be incorporated in a composition by means of usual physiologically acceptable solubilizers, such as for example: ethanol, propanol, isopropanol, propylene glycol, glycerin, butylene glycol, 1,3 propane diol, or polyethylene glycol or any combination thereof. It can be interesting to solubilize the agent with emulsifiers. A powder support can also be used.

Preferably, a short hydrocarbon chain alcohol is used (at most 6 carbons).

The compositions of the present invention are generally prepared by conventional methods well known to one skilled in the art for making topical or oral compositions and compositions for injection. Such methods may involve a mixture of ingredients in one or more steps to obtain a uniform state, with or without heating, cooling, etc.

All galenic forms that can contain the *Mirabilis jalapa* extract according to the invention can be used, i.e., creams, lotions, milk or cream ointments, gels, emulsions, dispersions, solutions, suspensions, cleansers, foundations, anhydrous preparation (sticks for example lip balm, body and bath oils), shower and bath gels, shampoos and hair care lotions, milks or creams for skin and hair cares, cleansing lotions or milks, sunscreen lotions, milks or creams, artificial tanning lotions, milks or creams, pre-shave, shaving or after-shave creams, foams, gels or lotions, make-up, lipsticks, mascaras or nail varnishes, skin "essences," serums, adhesive or absorbent materials, transdermal patches, emollient powders, lotions, milks or creams, sprays, oils for the body and the bath, foundation tint bases, pomade, emulsion, colloid, compact or solid suspension, pencil, sprayable or brossable formulation, blush, red, eyeliner, lip liner, lip gloss, facial or body powder, styling foams or gels, nail conditioner, lip balms, skin conditioners, moisturizers, hair sprays, soaps, exfoliation agent, astringents, depilatories, permanent waving solutions, antidandruff formulations, anti-sweat and
antiperspirant compositions, such as sticks, roll-on deodorant, deodorizing agent, nose sprays etc. These compositions can also be presented in the form of lipsticks intended to apply color or to protect the lips from cracking, or of make-up products for the eyes or tints and tint bases for the face.

Compositions in accordance with the invention include cosmetics, personal care products and pharmaceutical preparations. A composition in the form of foam or in the form of compositions for aerosol also including a propellant agent under pressure can be envisaged.

Compositions according to the invention may also be for orodental use, for example, toothpaste. In that case, the compositions may contain the usual adjuvants and additives for compositions for oral use and, in particular, surfactants, thickening agents, moisturizing agents, polishing agents such as silica, various active substances such as fluorides, particularly sodium fluoride, and, possibly, sweetening agents such as saccharin sodium.

The *Mirabilis jalapa* extract within the scope of the present invention may be used in the form of solution, dispersion, emulsion, paste, or powder, individually or as a premix or in vehicles individually or as a premix in vectors such as macro-, micro-, or nano-capsules, macro-, micro- or , nano-spheres, liposomes, oleosomes or chylomicrons, macro-, micro-, or nanoparticles or macro-, micro- or nano-sponges, , micro- or nano-emulsions or adsorbed on organic polymer powders, talcs, bentonites, spores or exines, and other inorganic or organic supports.

The *Mirabilis jalapa* extract according to the present invention may be used in any form whatsoever, in a form bound to or incorporated in or absorbed in or adsorbed on macro-, micro-, and nanoparticles, or macro-, micro-, and nano-capsules, for the treatment of textiles, natural or synthetic fibers, wools, and any materials that may be used for clothing or underwear for day or night intended to come into contact with the skin, handkerchiefs or cloths, to exert their cosmetic effect via this skin/textile contact and to permit continuous topical delivery.

**Cosmetic treatment method**

The present invention also provides a topical treatment method for the treatment of sensitive skins, comprising the topical application to the skin of a subject in need thereof of an effective amount of a composition according to the invention comprising an extract of *Mirabilis jalapa* as recited above.

« Topical treatment » means according to the invention, an application that is intended to act where it is applied: skin, mucosa and/or appendages.

Improvements of the appearance and general state of sensitive skin can be obtained by topical application on a regular basis, for example daily or bi-daily.

The practitioner will appreciate the topical cosmetic treatment that will include a composition comprising the extract of *Mirabilis jalapa*; this treatment can be achieved for example by applying topically the composition described in the present invention, according to a method usually used to apply such a composition. The topical composition is preferably applied once a day for a period of at least one week, but it can be applied for periods of 2, 4, 8 or 12 weeks.
Preferably, the topical composition is applied to the face and neck, but can be applied to any skin part requiring a treatment, where the composition remains on the skin area to be treated, and preferably is not removed or flushed from the skin.

For example, for a face cosmetic treatment, the European Cosmetics Directive has set a standard amount for applying a cream of 2.72mg/cm²/day/person and for a body lotion of 0.5mg/cm²/day/person.

It is also to be understood that, as used herein, the terms treating and treatment include and encompass the reduction, improvement, progress, relief, and/or elimination of undesirable effects of a sensitive skin.

The compositions of the present invention and the methods can be used for the treatment of dermatological conditions of skin in numerous areas of skin, including without limitation face, forehead, lips, neck, chest, breasts, arms, hands, body, legs, knees, feet, back, buttocks, or abdomen or else.

One of the major advantages of the present invention resides in the ability whenever necessary or desirable to be able to apply local selective "gentle" treatments through this topical, non-invasive method of application. An application can be realised very locally using a syringe or micro-canula.

It is also possible, however, to consider a composition containing the ingredient according to the invention intended to be injected subcutaneously.

According to other specific features, the cosmetic treatment method according to the invention can be combined with one or more other treatment methods targeting the skin such as lumino-therapy, heat or aromatherapy treatments.

According to the invention, devices with several compartments or kits may be proposed to apply the method described above which may include for example and non-restrictively, a first compartment containing the *Mirabilis jalapa* extract of the invention, and in a second compartment a composition containing another active ingredient and/or excipient, the compositions contained in the said first and second compartments in this case being considered to be a combination composition for simultaneous, separate or stepwise use in time, particularly in one of the treatment methods recited above.

**A) Example of preparation of an extract of *Mirabilis jalapa* that can be used according to the invention**

A hot extraction under reflux of the aerial parts (crushed leaves and stems) of *Mirabilis jalapa* is performed using an alcohol or a mixture of alcohols, preferably ethanol. After filtration and removal of alcohol, the dry residue is dissolved in a cosmetic excipient (physiologically acceptable) type of short-chain alcohols (such as butylene glycol); preferably 0.5% of the dry residue in a mixture of diols. The extract thus obtained (with 0.5% dry residue) can be used as an active ingredient in a galenic formulation. It is this ingredient that is used in below *in vitro* and *in vivo* evaluations as well as in the galenic formulations (paragraph C).
B) \textit{In vitro evaluations}

The interest of the cosmetic treatment of sensitive skin of the invention with an extract of \textit{Mirabilis jalapa} is illustrated by the \textit{in vitro} tests given below.

1. \textit{Moderation of the factors that cause sensitive skin}
   a. \textit{NGF synthesis by keratinocytes in stress situation.}

   Too much NGF production is in part responsible for sensitive skin. Released by the keratinocyte, it will induce at the nerve ending an overproduction of TRPV1 receptors. Activation of these many receptors by external stimuli triggers the symptoms of sensitive skin (stinging, itching ....). It is therefore advantageous to limit the production of NGF.

Protocol

Human keratinocytes (HK) are cultured in the presence of TNF-a, an agent who stimulates the production of NGF, and of the extract of \textit{Mirabilis jalapa} (cf. above section A) for 24 h. After this contact, the concentration of NGF produced by the cells was evaluated by ELISA in the culture media and reduced to the number of cells.

Results

\textbf{Table 1:} Modulation of the NGF production by HK treated with TNF-a - effect of the extract of \textit{Mirabilis jalapa} according to the invention

<table>
<thead>
<tr>
<th>TNF-α</th>
<th>Variation of the number of cells (%)</th>
<th>NGF (pg/mL/10⁶ cells)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reference</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α + 0.6%</td>
<td>-4.6%, \textit{dhs}</td>
<td>1.062 ± 0.171</td>
<td>-32%; (p &lt; 0.05)</td>
</tr>
<tr>
<td>\textit{Mirabilis jalapa} extract</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α + 1.2%</td>
<td>-5.7%, \textit{dhs}</td>
<td>0.792 ± 0.223</td>
<td>-49%; (p &lt; 0.01)</td>
</tr>
<tr>
<td>\textit{Mirabilis jalapa} extract</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TNF-a increases the release of NGF. The application of the \textit{Mirabilis jalapa} extract according to the invention together with TNF-a reduces this release in a dose-dependent manner up to -49.2% \((p < 0.01)\).

Therefore the extract of \textit{Mirabilis jalapa} according to the invention decreases the release of NGF at the level of the keratinocyte.

b. \textit{TRPV1 synthesis by skin explant in stress situation.}

TRPV1 is the key receptor for sensitive skin. In excessive amount it will lead to a disproportionate sensitive response, too strong, causing the symptoms of sensitive skin. Furthermore in the keratinocyte its activation triggers the release of NGF. Limiting the production of TRPV1 is therefore always sought.

Protocol

Skin explants (female, 28 years, breast) were kept alive in their culture medium. They were then placed in contact with Substance P, a pain mediator synthetized by neurons, and received a cream comprising 3% of the extract of \textit{Mirabilis jalapa} (as described below in the paragraph C), or a placebo.
cream (same cream without the active ingredient of the invention) for 24 hours (n=4). After this contact the skins were prepared and cut, the skin sections thus obtained were immuno-stained with an antibody directed against TRPV1. 40 photos per case (10 per sample) were used to measure the fluorescence intensities and quantify effects.

5 Results

Table 2: Modulation of the expression of TRPV1 by explants treated with Substance P; effect of the Mirabilis jalapa extract of the invention

<table>
<thead>
<tr>
<th>Placebo cream</th>
<th>TRPV1 (AFU)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>18.8 ± 1.42</td>
<td>Reference</td>
</tr>
<tr>
<td>Cream comprising 3% of the Mirabilis jalapa extract</td>
<td>16.7 ± 1.65</td>
<td>-11.2%, p&lt;0.01</td>
</tr>
</tbody>
</table>

AFU: arbitrary fluorescence unit.

The Mirabilis jalapa extract according to the invention reduces the expression of stress-related TRPV1. The result of this effect is lesser sensitive to stress.

c. TRPV1 synthesis by keratinocytes in stress situation.

Protocol

Normal human keratinocytes in culture were placed in the presence of the extract of Mirabilis jalapa for 24h and H$_2$O$_2$ based oxidative stress is applied and the residual effect on TRPV1 is evaluated by ELISA after 24 hours. The result is reduced to the cell number.

Results

Table 3: Modulation of the TRPV1 production by HK treated with H$_2$O$_2$; effect of the Mirabilis jalapa extract of the invention

<table>
<thead>
<tr>
<th>Variation of the number of cells (%)</th>
<th>TRPV1 (ng/mL/10$^6$ cells)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>+17%, dhs</td>
<td>3.90 ± 0.10</td>
</tr>
<tr>
<td>H$_2$O$_2$</td>
<td>Reference</td>
<td>5.20 ± 0.40</td>
</tr>
<tr>
<td>H$_2$O$_2$ + 1.2% of the extract of Mirabilis jalapa</td>
<td>-1%, dhs</td>
<td>3.90 ± 0.20</td>
</tr>
</tbody>
</table>

The Mirabilis jalapa extract according to the invention can reduce the production of TRPV-1 caused by oxidative stress.

2. Improvement of the skin barrier function and stratum corneum

a) Production of TRPV4 by skin explants in stress situation.

The TRPV4 receptor is, as the TRPV1 receptor, involved in cutaneous sensitivity. But unlike TRPV1, its activation will have beneficial effects for sensitive skin. By causing a release of calcium in the keratinocyte, it will promote differentiation and therefore participate in the thickening of the horny layer. The increase of the production of this receptor will therefore be an important factor.
**Protocol**
Same as above for TRPV1.

**Results**

Table 4: Modulation of TRPV4 expression by explants treated with Substance P; effect of the *Mirabilis jalapa* extract of the invention.

<table>
<thead>
<tr>
<th></th>
<th>TRPV4 (AFU)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo cream</td>
<td>14.0 ± 2.07</td>
<td>Reference</td>
</tr>
<tr>
<td>Cream with 3% of the extract of <em>Mirabilis jalapa</em></td>
<td>16.3 ± 2.74</td>
<td>+16.4%, p&lt;0.01</td>
</tr>
</tbody>
</table>

AFU: arbitrary fluorescence unit.

An increase of the TRPV4 synthesis of +17% (p<0.01) on skin explants treated with Substance P is thus obtained thanks to the action of the *Mirabilis jalapa* extract of the invention. This reflects a positive effect of the extract of *Mirabis jalapa* on the thickening of the stratum corneum.

b) **Tight junctions in a skin explant: occludin measurement.**

Tight junctions between keratinocytes are a key element in the effectiveness of the skin barrier. Occludin is a key protein of these tight junctions. The effect of the *Mirabis jalapa* extract is evaluated on the synthesis of this molecule.

**Protocol**

Skin explants (female, 31 years, breast) were kept alive in their culture medium. They were then placed in contact with a cream comprising 3% of the extract of *Mirabilis jalapa* (as described below in the paragraph C), or a placebo cream (same cream without the active ingredient of the invention) for 6 days (n=4). After this contact the skins were prepared and cut, the skin sections thus obtained were immuno-stained with an antibody directed against occludin. 40 photos per case (10 per sample) were used to measure the fluorescence intensities and quantify effects.

**Results**

Table 5: Modulation of occludin formation in explants; effect of the *Mirabilis jalapa* extract of the invention.

<table>
<thead>
<tr>
<th></th>
<th>Occludin (AFU)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo cream</td>
<td>12.7 ± 3.34</td>
<td>Reference</td>
</tr>
<tr>
<td>Cream with 3% of the extract of <em>Mirabilis jalapa</em></td>
<td>15.6 ± 3.64</td>
<td>+22.8%, p&lt;0.01</td>
</tr>
</tbody>
</table>

AFU: arbitrary fluorescence unit.

The *Mirabilis jalapa* extract according to the invention promotes the synthesis of one of the key protein of the tight junction, the occluding, on a skin explant, which helps to strengthen the cohesion of the epidermis.
c) **Tight junctions in a culture HK: occludin measurement.**

**Protocol** Normal human keratinocytes are cultured in the presence of the *Mirabilis jalapa* extract for 72h and then the cells were fixed and immuno-stained with an antibody directed against occludin. The result is reduced to the cell number.

**Results**

Table 6: Modulation of occludin production by HK (n = 4); effect of the *Mirabilis jalapa* extract the invention.

<table>
<thead>
<tr>
<th>Variation of the number of cells (%)</th>
<th>Occludine (AFU/106 cells)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>Reference</td>
<td>Reference</td>
</tr>
<tr>
<td>0.6% of the <em>Mirabilis jalapa</em> extract</td>
<td>-19%*</td>
<td>0.115 ± 0.052</td>
</tr>
<tr>
<td>1.2% of the extract of <em>Mirabilis jalapa</em></td>
<td>-22%*</td>
<td>0.143 ± 0.097</td>
</tr>
</tbody>
</table>

*No toxicity was observed compared to control; the decrease in the number of cells comes from the formation of differentiated cells (i.e. non proliferative) and sometimes without cores, this reflects the positive effect of the *Mirabilis jalapa* extract on the formation of the barrier.*

The *Mirabilis jalapa* extract according to the invention stimulates the synthesis of a key protein of tight junctions, the occludin, in a culture of HK.

**d) Ceramide synthesis on explants**

The stratum corneum forms a barrier against harmful external agents. In this perspective, the lipid layer, formed of 50% of ceramides, is essential and particularly effective. This layer contributes to the improvement of the barrier in sensitive skin. The effect of the *Mirabilis jalapa* extract on the ceramide synthesis is therefore aimed.

**Protocol**

Skin explants (female, 31 years, breast) were kept alive in their culture medium. They were then placed in contact with a cream comprising 3% of the extract of *Mirabilis jalapa* (as described below in the paragraph C), or a placebo cream (same cream without the active ingredient of the invention) for 6 days (n=4). After this contact the skins were prepared and cut, the skin sections thus obtained were immuno-stained with an antibody directed against a ceramide precursor. 40 photos per case (10 per sample) were used to measure the fluorescence intensities and quantify effects.

**Results**

Table 7: Modulation of ceramide synthesis in explants; effect of the extract of the invention.

<table>
<thead>
<tr>
<th>Ceramides (AFU)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo cream</td>
<td>Reference</td>
</tr>
<tr>
<td>14.2 ± 2.98</td>
<td></td>
</tr>
<tr>
<td><strong>Cream with 3% of <em>Mirabilis jalapa</em> extract</strong></td>
<td>17.2 ± 4.92</td>
</tr>
</tbody>
</table>

AFU: arbitrary fluorescence unit.
The *Mirabilis jalapa* extract according to the invention promotes the synthesis of ceramides.

e) Ceramide synthesis on subjects having a sensitive skin (ex-vivo).

Protocol

Adhesives were applied to the skin of volunteers with sensitive skin (N=20) in order to collect their corneocytes (stripping), at T0 and after 2 months of application of a cream containing 3% of the *Mirabilis jalapa* extract (as described in below paragraph C) or a placebo cream (same cream without the active ingredient of the invention). The immunolabeling of a precursor of ceramides was performed on these adhesives and photographs were made to assess the labeling levels (12 photos/adhesives/voluntary).

Results

Table 8: Modulation of ceramide synthesis on subjects with sensitive skin; effect of the *Mirabilis jalapa* extract of the invention.

<table>
<thead>
<tr>
<th>Ceramides</th>
<th>Placebo</th>
<th>3% of the extract of <em>Mirabilis jalapa</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>T 2 months</td>
<td>T0</td>
</tr>
<tr>
<td>Mean</td>
<td>1.960 ± 1.300</td>
<td>1.824 ± 0.900</td>
</tr>
<tr>
<td>Variation (%)</td>
<td>Significance vs. T0</td>
<td>-7%, dns</td>
</tr>
<tr>
<td>Variation (%)</td>
<td>Significance vs. placebo</td>
<td>Ref. T0</td>
</tr>
</tbody>
</table>

The results show that the *Mirabilis jalapa* extract promotes the ceramide synthesis in this ex vivo system.

f) Transglutaminase activity on keratinocytes

Transglutaminase is strongly induced upon differentiation of the epidermis in order to bond together the proteins produced by the keratinocytes of the granular layer and which thus form the *stratum corneum*. The increase in transglutaminase activity may be followed using a fluorescent probe. The probe is used as a substrate for the transglutaminase and the transglutaminase links the probe to the protein cells.

Protocol

Normal human keratinocytes were cultured in the presence of the *Mirabilis jalapa* extract during 21 days. After this contact, fluorescence fixed to the cells was measured and compared with the control layer. The results were harmonized by a parallel count of the number of cells.
Results

Table 9: Modulation of the transglutaminase activity HK (n=6); effect of the Mirabilis jalapa extract of the invention.

<table>
<thead>
<tr>
<th>Negative control</th>
<th>Transglutaminase (UFA/106 cells)</th>
<th>Variation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2% of the extract of Mirabilis jalapa</td>
<td>34751 ± 4968</td>
<td>+40%, p&lt;0.01</td>
</tr>
<tr>
<td>0.6% of the extract of Mirabilis jalapa</td>
<td>39229 ± 2717</td>
<td>+58%, p&lt;0.01</td>
</tr>
</tbody>
</table>

The results show that the Mirabilis jalapa extract of the invention promotes the global activity of the transglutaminase in the cells of keratinocytes in a dose dependent manner.

C) Examples of galenic formulations

As mentioned above, obviously different cosmetic formulations including the active ingredient of the invention may be implemented. A cream formula is given below by way of example.

Additional active ingredients, in support and/or in addition to the action of the ingredient of the invention, may be added in the appropriate phase of the formulations according to their hydrophobic or hydrophilic nature. These ingredients can be of any class according to their function(s), site of application (body, face, neck, chest, hands, hair, eyelashes, eyebrows, body hairs, etc.), the final desired effect and the targeted consumer, for example anti-aging, anti-wrinkle, moisturizing, anti-dark circles, firming, anti-glycation, slimming, soothing, myo-relaxant, anti-redness, anti-stretch marks, etc.

Cream form

<table>
<thead>
<tr>
<th>Phase A: aqueous</th>
<th>%</th>
<th>INCI name</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O QsplOO</td>
<td>0.30</td>
<td>Carbomer</td>
</tr>
<tr>
<td>Optasense G83™</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phase B: oily, comprising the surfactants</th>
<th>%</th>
<th>INCI name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brij S2-SS-(RB)™</td>
<td>0.40</td>
<td>Steareth-2</td>
</tr>
<tr>
<td>Brij SIO-SO-(RB)™</td>
<td>1.20</td>
<td>Steareth-10</td>
</tr>
<tr>
<td>Crodacos CES-PA-(RB)™</td>
<td>4.00</td>
<td>Cetearyl Alcohol &amp; Dicetyl Phosphate &amp; Ceteth-10 Phosphate</td>
</tr>
<tr>
<td>Crodacol CS90-PATM</td>
<td>1.50</td>
<td>Cetearyl Alcohol</td>
</tr>
<tr>
<td>Laurocapram TM</td>
<td>2.50</td>
<td>Laurocapram</td>
</tr>
<tr>
<td>BRB CM 56™</td>
<td>2.00</td>
<td>Cyclopentasiloxane &amp; Cyclohexasiloxane</td>
</tr>
<tr>
<td>Crodamol OSU-LQ-(RB)™</td>
<td>4.00</td>
<td>Diethylhexyl Succinate</td>
</tr>
<tr>
<td>Crodamol GTCC-LQ-(MV)™</td>
<td>3.00</td>
<td>Capryl/Capric Triglyceride</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Phase C: humectant and preservative</th>
<th>%</th>
<th>INCI name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycerin</td>
<td>4.00</td>
<td>Glycerin</td>
</tr>
<tr>
<td>Octanediol</td>
<td>0.50</td>
<td>Caprylyl Glycol</td>
</tr>
</tbody>
</table>
**Phase D:** preservative
Phenoxyethanol qs Phenoxyethanol

**Phase E:** antifungal
Potassium sorbate qs Potassium Sorbate

**Phase F:** pH adjustment

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
<th>Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>H₂O</td>
<td>4.00</td>
<td>Water</td>
</tr>
<tr>
<td>NaOH 30%</td>
<td>0.40</td>
<td>Sodium Hydroxide</td>
</tr>
</tbody>
</table>

**Phase G**
Active ingredient according to the invention 3.00

**Phase H**
Perfume 0.10 Fragrance

---


Examples of ingredients marketed by Sederma that can be added to this cream formula:

- **CENTECCELL™**: active ingredient against rednesses comprising an extract of *Centella asiatica*, that protects blood capillaries and extracellular matrix against oxidative attacks and against collagenase; 2% of this active can be added at the end of the formulation before phase H.
- **NG Birch Sap™**: raw sap from birch sapwood; skin toning and moisturizing.
- **MATRIXYL™5000**: peptide-based anti-wrinkle ingredient (WO2005/048968) comprising two matrikines Pal-GHK and Pal-GQPR, which in synergy helps repairing skin damages caused by aging.
- **MATRIXYL svnthe6™**: peptide-based anti-wrinkle ingredient (WO2010/082175) which helps repair skin damage caused by aging.
- **PRODIZIA™**: active ingredient (WO2013/046137) fighting the signs cutaneous fatigue caused by glycation and glycoxidation. 3% of this ingredient can for example be added to the phase F.

The following galenic formulations can be mentioned as way of examples of other particularly interesting formulation for the implementation of cosmetic treatment according to the invention:

**Mask form**, with the following additional ingredient marketed by Sederma as examples:

- **SEBULESS™**: purifying sebo-regulator ingredient comprising a *Syringa vulgaris* extract mattifies and refreshes complexion, fades the inflammatory blemishes.
- **YEAST WALLS™**: active ingredient based on cell membranes with high enzymatic activity having anti-seborrhea activity, an effect on the texture and the complexion of the skin and flexibility.
- **AQUALANCE™**: osmo-protector moisturising active ingredient (WO2009/104118) comprising homarine and erythritol.
**Serum form (fluid emulsion),** with the following additional ingredient marketed by Sederma as examples:

- RESISTEM™: anti-aging active ingredient (WO 2012/104774) helping the skin to build its own anti-aging defense system, based on an extract obtained by cell culture of *Globularia cordifolia* plant.
- MARUCELL™: active ingredient against pollution and antioxidant comprising an extract of Marrubium vulgare.
- NG UNSAPONIFIABLE SHEA BUTTER™: active ingredient with nourishing and protective properties for the treatment of skin damaged by the environment.
- VENUCEANE™: active ingredient (WO2002/066668) comprising a *Thermus thermophilus* biotechnological extract that prevents visible signs of photo-aging (spots, wrinkles, dryness ...), protects cell structures from damages caused by UV and strengthens skin integrity.

**Cleaning lotion form,** with the following additional ingredient marketed by Sederma as examples:

- ECODERMINE™: active ingredient including an association of lactitol and xylitol, aimed to fight skin problems due to microbial imbalance (acne-prone skin, dryness, itching) by preserving the natural mechanism of skin defense.
- HAIRSPA™: Scalp moisturisation and soothing active ingredient comprising lactitol and xylitol in glycerin, acts on skin microflore balance to fight against scalp discomfort (dryness, itching, dandruff, and irritations).

**Milk form,** with the following additional ingredient marketed by Sederma as examples:

- LEONTOCELL™: anti-wrinkle active comprising an extract of *Leontopodium alpinum* (Edelweiss).
- LEGANCE™: anti-aging active (WO2013/105047) corresponding to a *Zingiber zerumbet Smith* extract obtained by C0₂ supercritical in a water-soluble excipient and titrated in zerumbone ingredient. It is a global anti-aging ingredient for legs. It improves their appearance and comfort by reducing water retention, improving microcirculation and refining adipose tissue.
- INTENSLIM™: slimming active ingredient (WO2013/105048) corresponding to a synergistic combination of extracts obtained by *Globularia cordifolia* plant cell culture, *Zingiber zerumbet Smith* titrated in zerumbone and vegetable caffeine obtained by supercritical C0₂ extraction.

**Gel form (example for eye contour, a particularly sensitive area),** with the following additional ingredient marketed by Sederma as examples:

- EYELISS™: active ingredient (WO2003/068141) that helps prevent against the appearance of bags under the eyes. It combines three components: hesperidin methyl chalcone reducing capillary permeability, Valyl-Tryptophan (VW) dipeptide which promotes lymphatic circulation and Pal-GQPR lipopeptide that improves firmness, elasticity and reduces inflammation.
• BEAUTIFEYE™: active ingredient corresponding to an association of an *Albizia julibrissin* extract and darutoside extracted from *Siegesbeckia orientalis*, demonstrates a lifting action on sagging upper eyelids.

D) *In vivo evaluations*

The evaluation of the efficacy of the treatment of sensitive skins of the extract according to the invention was conducted on the face and forearm through the series of clinical tests detailed below. A cream prepared according to the above paragraph C was used for these tests.

- **Study 1**: on sensitive skins by a stinging test;
- **Study 2**: on discomfort felt by volunteers with sensitive (reactive) skins (self-evaluation);
- **Study 3**: on the barrier effect (epidermis thickness, corneocyte size and epidermis regeneration).

Principle: a total of 30 women, mean age 39 years (23 - 54 years) stating having sensitive skin. In the 21 volunteers were selected for the 1st study.

30 volunteers filled out a self-evaluation questionnaire for the 2nd study.

24 volunteers were selected for the 3rd study.

A second study on cutaneous sensitivity (Study 2a) was also realised.

Principle for this study 2a: a panel of 22 women mean age 35 years (19 - 50 years) with sensitive skin (validated by a dermatologist) was included.

The effect of the extract of the invention on the decrease of the cutaneous sensitivity was thus evaluated by:

- A measurement of skin sensitivity by a stinging test (Study 1); and
- Two evaluations of perceived effect through questionnaires. (Studies 2 and 2a).

The effect of the extract of the invention on skin barrier improvement (Study 3) was evaluated by:

- A measurement of corneocyte size by analysing images following stripping;
- A measurement of epidermal thickness through confocal microscopy; and
- A measurement of the skin barrier using a Vapometer™.

The study synopsis can be summarized by the following diagram:

<table>
<thead>
<tr>
<th>TO</th>
<th>T 1 month</th>
<th>T 2 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity (Stinging test)</td>
<td></td>
<td>Sensitivity (Stinging test)</td>
</tr>
<tr>
<td>Skin barrier (Vapometer™)</td>
<td></td>
<td>Skin barrier (Vapometer™)</td>
</tr>
<tr>
<td>Epidermis thickness (Vivascope™)</td>
<td></td>
<td>Epidermis thickness (Vivascope™)</td>
</tr>
<tr>
<td>Corneocytes size (Stripping)</td>
<td>Corneocytes size (Stripping)</td>
<td></td>
</tr>
<tr>
<td>Perceived effect (Self-evaluation)</td>
<td></td>
<td>Perceived effect (Self-evaluation)</td>
</tr>
<tr>
<td>(Studies 2 and 2a)</td>
<td></td>
<td>(Studies 2 and 2a)</td>
</tr>
</tbody>
</table>
1. **Skin sensitivity study - decrease of cutaneous sensitivity**

As seen before, skin sensitivity is mainly felt. This is what happens when a trigger causes stinging, itching and/or tightness etc.

The study of the reduction of the sensitivity with the *Mirabilis jalapa* extract of the invention had 3 phases: by causing an unpleasant sensation with the stinging test (Study 1) and using a self-evaluation by the volunteer under normal use (Study 2 and 2a).

a) **Perceived sensitivity measured by the stinging test**

Specific inclusion criteria: volunteers needed to have a sufficient level of skin sensitivity as estimated with a specific questionnaire and a positive stinging test. Some parameters were controlled during the test (volunteers were requested to maintain hormonal consistency prior to and during the test, to pre-apply the provided moisturiser and to refrain from taking medication that interfere with the effects of the effects).

**Study types and duration:** this was a single-blind study during which a cream comprising 3% of the *Mirabilis jalapa* extract of the invention was applied to one side of the face and to the same-side forearm while a placebo cream was applied to the other side. Both creams were massaged into the skin twice daily for 2 months.

Statistical testing was performed using the Student's t test or, if needed, a Wilcoxon signed-rank test. In both cases, bilateral tests were performed on paired series. For the self-evaluation, a Chi^2_ test was used.

**Principle:** the stinging test is used to diagnose sensitive skin and study the soothing effects of cosmetic products. This test consists in the standardized application of a lactic acid solution on the nasolabial folds and cheeks to cause stinging of varying intensity, depending on the volunteer. This stinging is caused by the acidity of the applied product, which activates the TRPV1 receptors. The advantage of this activation over activation with capsaicin is that it is temporary and moderate.

A stinging test was performed at T0 and then repeated 2 months after application of the cream comprising 3% of the extract of *Mirabilis jalapa* or its placebo. Right after applying the acid, volunteers rated the sensations felt every minute for 15 minutes on a 6-point scale from 0=nothing to 5=distinct stinging + strong burning sensations.

The results of this stinging test demonstrate a 30% decrease of the unpleasantness felt versus T0 and a clear and significant advantage in using the cream comprising 3% of the extract of Mirabilis jalapa over using the placebo (-6.8%; p<0.01). The intensity of the perceived unpleasant sensation caused by the acid decreases with the extract of the invention from "distinct stinging" to "slight stinging", or even "slight sensation".

b) **Perceived sensitivity measured by a self-evaluation questionnaire (Study 2)**

A self-evaluation questionnaire was completed by 30 volunteers who had applied the cream comprising 3% of the *Mirabilis jalapa* extract of the invention or placebo for 2 months according to
the recommendations for use. The volunteers could give a positive, negative or indifferent opinion of the two products after the application period.

Table 10: perceived sensitivity after 2 months of applying of the cream according to the invention or placebo cream (% positive opinion, n=30 volunteers)

<table>
<thead>
<tr>
<th></th>
<th>According to the invention</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin with less tightness</td>
<td>77%**</td>
<td>63%</td>
</tr>
<tr>
<td>Skin feels comfortable all day</td>
<td>83%**</td>
<td>67%*</td>
</tr>
<tr>
<td>Skin is less sensitive</td>
<td>67%*</td>
<td>50%</td>
</tr>
</tbody>
</table>

** Very significant variation versus negative + indifferent opinions; p<0.01 (Chi² test)
* Significant variation versus negative + indifferent opinions; p<0.01 (Chi² test)

The results of this self-evaluation performed after using both creams demonstrates a distinct benefit with the cream comprising the extract of Mirabilis jalapa according to the invention versus the placebo cream. The users therefore found that their skin felt significantly more comfortable (83%), less sensitive (67%) and with less tightness (77%).

c  Perceived sensitivity by a questionnaire (Study 2a)

The efficacy of the extract of Mirabilis jalapa of the invention was also determined on a panel of 22 women with sensitive skin caused by one or more factors. The study methodology, which was designed in collaboration with a neurosciences and sensitive skin specialist, involved the use of a questionnaire designed specifically for these sensitive skin types. The questionnaire targeted relevant criteria, such as stinging and itching. Each criterion was graded on a scale of 0 to 10.

This was a double-blind study of the face; the cream comprising 3% of the extract of Mirabilis jalapa or a placebo cream was applied and massaged in twice daily for 21 days. The evaluation was performed at T0 and T21 days.

The results demonstrate that the cream comprising the extract of the invention has a significant soothing effect. As for the placebo, the 2 traditional types of discomfort for people with reactive skin did not vary from T0 to T21 (-22% and -13%, all non-significant). In contrast, the use of the cream according to the invention significantly reduced stinging by 86% and itching by 80% (p<0.05 and p<0.01 respectively).

2. Study of skin barrier improvement

Having a deficient skin barrier is one of the factors contributing to sensitive skin. This may be related to a decrease in epidermal thickness, to corneocyte size, or to a poor regenerative capacity following stress. The effect of the ingredient according to the invention on these 3 aspects were studied.

a) Epidermal thickness measured by confocal microscopy

A thinner epidermis is associated with a more sensitive skin. The thickness of the epidermis can be measured non-invasively by using confocal laser microscopy (Vivascope® 3000; Mavig, Germany), which is used to see through the skin and study healthy skin or skin ageing.
A special light is emitted through the skin to provide an image produced as the light is reflected differently by the structures it hits (keratin, melanin, collagen). This makes it possible to generate a clear, real time image of an ultrafine layer of the skin at a controlled depth that would be equivalent to a biopsy in real time.

For this study, regularly-spaced vertical image acquisitions were performed to reconstruct the entire epidermis. The measurement of epidermal thickness (between the stratum corneum and the basal stratum) was performed on several areas of a given acquisition to enhance measurement accuracy.

Table 11: Modulation of epidermal thickness after applying a cream comprising 3% of the Mirabilis jalapa extract or a placebo cream (N=22 n=22 volunteers, measurements in um).

<table>
<thead>
<tr>
<th></th>
<th>Cream according to the invention</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>T2months</td>
<td>T0</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>58.2 ± 14.8</td>
<td>64.9 ± 12.8</td>
</tr>
<tr>
<td>Variation vs. T0 (µm)</td>
<td>-</td>
<td>+6.7µm</td>
</tr>
<tr>
<td>Variation vs. T0 (%)</td>
<td>-</td>
<td>+11.5%</td>
</tr>
<tr>
<td>Significance</td>
<td>p=0.07</td>
<td>dns</td>
</tr>
<tr>
<td>% variation; significance vs. placebo</td>
<td>+13.6%; p&lt;0.05</td>
<td></td>
</tr>
</tbody>
</table>

The thicknesses thus calculated concur with those of the literature. The study of the results shows that there is an undeniable improvement in skin thickness after applying the cream of the invention. After the second month of the application of the cream of the invention, the epidermis is restructured, leading to a +14% increase in thickness (approximately +8 µm) versus placebo (significant at p<0.05). This 8-µm difference is similar to what is found in the literature when distinguishing normal skin from sensitive skin.

b) Corneocyte size measured on stripping

At T0 and T1 month, corneocytes were collected in a standardised way from the skin of volunteers using strips. These strips were then fluorescently labelled so that their surface could be visualised and measured using a microscope. Approximately 150 corneocytes were measured per volunteer and per case.

Table 12: Modulation of corneocyte size after applying a cream according to the invention or a placebo cream (n=24 volunteers, measurements in µm2).

<table>
<thead>
<tr>
<th></th>
<th>Cream of the invention</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>T0</td>
<td>T1month</td>
<td>T0</td>
</tr>
<tr>
<td>Mean</td>
<td>952 ± 41</td>
<td>982 ± 43</td>
</tr>
<tr>
<td>Variation vs. T0</td>
<td>-</td>
<td>+30µm²</td>
</tr>
<tr>
<td>% variation vs. T0 (%)</td>
<td>-</td>
<td>+3.2%</td>
</tr>
<tr>
<td>Significance</td>
<td>p&lt;0.01</td>
<td>dns</td>
</tr>
</tbody>
</table>
Variation; +2.8%
Significance vs. placebo

Physiological scale (*) Variation; +10%
Significance vs. placebo

These results demonstrate that corneocyte surface area increases by a mean +30.7 µm² following the application of the cream of the invention for 1 month. This increase in size is much more pronounced \((p<0.05)\) when compared with the stagnation observed after applying a placebo.

(*) In order to better understand this result, this increase can be compared on a physiological scale ranging from the smallest \((750 \, \mu m^2 = 0\%)\) to the largest corneocyte \((1050 \, \mu m^2 = 100\%)\). The variation after on month of application of the cream according to the invention would be +10%.

c) **Regeneration capacity following a stress**

TransEpidermal Water Loss (or TEWL) is a very good indication of the quality of the skin barrier and its ability to regenerate that can be evaluated after causing slight damage to the skin barrier with adhesive strips.

At T0, basal TEWL was measured using a VapoMeter™ (Delfin Technologies, Finland) and a series of controlled stripping was performed to cause a slight break in the homeostasis of the skin barrier. After stabilising the signal, the increase in TEWL due to this break was measured. After applying a cream according to the invention comprising 3% of *Mirabilis jalapa* or a placebo cream for 2 months the protocol used at T0 was repeated. This enabled an evaluation of any improvement in resistance to be performed. The test was performed on the forearm, since the facial skin of the volunteers was too sensitive for this test.

Table 13: Variation of the increase of TEWL after stripping - effect of applying a cream according to the invention or a placebo cream (N=21 volunteers, measurements in g/m²/h).

<table>
<thead>
<tr>
<th>Ingredient according to the invention</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td></td>
</tr>
<tr>
<td>T0</td>
<td>6.6 ± 5.7</td>
</tr>
<tr>
<td>T2months</td>
<td>3.6 ± 3.5</td>
</tr>
<tr>
<td>T0</td>
<td>6.3 ± 6.8</td>
</tr>
<tr>
<td>T2months</td>
<td>4.5 ± 2.8</td>
</tr>
<tr>
<td><strong>Variation vs. T0 (g/m²/h)</strong></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-5.6</td>
</tr>
<tr>
<td>-</td>
<td>-3.9</td>
</tr>
<tr>
<td><strong>Variation vs. T0 (%)</strong></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>-44.8%</td>
</tr>
<tr>
<td>-</td>
<td>-28.9%</td>
</tr>
<tr>
<td><strong>Significance</strong></td>
<td></td>
</tr>
<tr>
<td>p=0.05</td>
<td>dns</td>
</tr>
<tr>
<td><strong>Variation; Significance vs. placebo</strong></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>+15.9%</td>
</tr>
<tr>
<td>p=0.05</td>
<td></td>
</tr>
</tbody>
</table>

These results demonstrate that applying a cream according to the invention greatly improves the regeneration of the damaged barrier. The barrier was 45% more resistant than at T0. The cream of the invention enhances the action by 16% \((p<0.05)\) compared with that of the placebo cream.
1. Use of an extract of *Mirabilis jalapa* for a non-therapeutical cosmetic topical treatment of the signs of a sensitive skin.

2. Use according to claim 1, wherein the extract of *Mirabilis jalapa* reduces the surproduction of the Nerve Growth Factor (NGF) and the TRPV1 receptor, thickens epidermis and approves the barrier function of the *stratum corneum*.

3. Use according to claim 1 or 2, wherein the extract of *Mirabilis jalapa* is an extract from the aerial parts of the plant.

4. Use according to anyone of claims 1 to 3, wherein the extract of *Mirabilis jalapa* is obtained from alcoholic extraction.

5. Use according to claim 4, wherein the extract of *Mirabilis jalapa* is obtained by ethanolic extraction.

6. Use according to anyone of claims 1 to 5, wherein the extract is combined with a physiologically acceptable excipient.

7. Use according to claim 6, wherein the extract is a dry residue dissolved in said excipient.

8. Use according to claim 7, wherein the dry residue % is comprised between 0.00015 and 15% in weight of the total weight of a composition comprising said residue and excipient.

9. Use according to claim 7 or 8, wherein the excipient comprises at least a short hydrocarbon chain alcohol.

10. Use according to anyone of claims 1 to 9, wherein the extract is combined with one or more active ingredients selected from lightening, propigmenting, anti-redness, anti-spots, calming, UV sunscreens, moisturizers, humectants, exfoliating, smoothing, toning, anti-aging, anti-wrinkles and fine lines actives, actives improving the mechanical and elastic properties, complexion radiance, detoxifying, anti-hair regrowth, anti-acne, acting on sebum secretion, mattifying, unifying, anti-inflammatory, anti-oxidant, anti-radical, anti-glycation, eye contours (anti-dark circles and under eye bags), promoting blood circulation, peptides and vitamins actives.

11. Use according to anyone of claims 1 to 10, wherein the extract of *Mirabilis jalapa* is used in a form bound to or incorporated in or absorbed in or adsorbed on macro-, micro-, and nanoparticles, or macro-, micro-, and nanocapsules, for the treatment of textiles, natural or synthetic fibers, wools, and any materials that may be used for clothing or underwear for day or night intended to come into contact with the skin, handkerchiefs or cloths, to exert their cosmetic effect via this skin/textile contact and to permit continuous topical delivery.

12. Cosmetic active ingredient for a cosmetic use according to anyone of claims 1 to 11, comprising an effective amount of an alcoholic extract of *Mirabilis jalapa*. 
13. Ingredient according to claim 13, wherein the extract of *Mirabilis jalapa* is obtained by hot alcoholic extraction, alcohol elimination to obtain a dry residue and dissolution of said residue in a physiologically acceptable excipient.

14. Cosmetic composition for a cosmetic use according to anyone of claims 1 to 11, comprising at least one cosmetic ingredient according to claim 13 or 13 in a physiologically acceptable excipient.
A. CLASSIFICATION OF SUBJECT MATTER

INV. A61Q19/00 A61K8/97

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, EMBASE, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
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<td>wo 02/47653 A2 (SEDERMA S.A [FR]; LINTNER KARL [FR]) 20 June 2002 (2002-06-20) cited in the application the whole document</td>
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<td>DATABASE GNPD [Online] MINTEL; 1 September 2011 (2011-09-01), Somang Cosmetics: &quot;Emulsion&quot;, XP002732391, Database accession no. 1627455</td>
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Further documents are listed in the continuation of Box C. See patent family annex.

Date of the actual completion of the international search: 26 May 2015

Date of mailing of the international search report: 19/06/2015

Authorized officer: Estanol, Inma
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<td>Y BENECKE H ET AL: &quot;Neuropeptides and their receptors as a molecular explanation for sensory skin; Neuropeptide und ihre Rezeptoren als molekulare Grundlage der empfindlichen Haut&quot;. DER HAUTARZT; ZEITSCHRIFT FÜR DERMATOLOGIE, VENEROLOGIE UND VERWANDTE GEBIETE, SPRINGER, BERLIN, DE, vol. 62, no. 12, 10 December 2011 (2011-12-10); pages 893-899, XP019989Q25, ISSN: 1432-1173, the whole document</td>
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<td>Y WALKER CRISTIANI I B ET AL: &quot;Antinociceptive effect of Mirabilis jalapa on acute and chronic pain models in mice&quot;, JOURNAL OF ETHNOPHARMACOLOGY, vol. 149, no. 3, 1 October 2013 (2013-10-01); pages 685-693, XP002732392, ISSN: 0378-8741, the whole document</td>
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