

# SPRING SNOW<sup>TM</sup> ODYCEA

# THE NEURO-SOOTHING AND ANTI DARK SPOT SHIELD



# SPRING SNOW<sup>TM</sup>

- Reinforcement of the skin barrier against the side effects of the exposome - Neuro-soothing for skin comfort - Shield against hyperpigmentation and dark spots
- INCI: Glycerin (and) Aqua/water (and) Hydrolyzed prunus domestica
- Water soluble
- Patent pending
- Vegan
- COSMOS, Natural Origin Index 1
- Preservative free
- Local sourcing: France
- Recommended dosage: 1 to 3%
- China compliant



# SPRING SNOW

*Prunus domestica variety spinosa* has dark grey-brown bark and spines that gives it its name of black thorn

It growths on the Brittany coast.

In early spring it shines white as snow, the bush entirely covered with countless flowers.

It is one of the first, if not the first shrub to bloom in spring.

The white flowers appear before the leaves, so that its dark silhouette seems dressed in snow.

WATCH THE VIDEO





Local and sustainable sourcing of Prunus domestica variety spinosa, Selected for its powerful adaptation capacity





#### Morphological adaptation



#### DARK BARK & THORNS

Black bark is a way for the plant to protect itself from sun damage and ultraviolet radiation. The dark color of the bark helps to absorb heat from the sun, which helps the plant maintain a constant internal temperature.

The short twigs are often transformed into thorns. Thorns helps to protect the plant from damage caused by wind, heavy rain or other environmental disturbances



### 1. Morphological adaptatic

#### DARK BARK & THORNS

2. Biochemical adaptation



## BIO FLAVOINOIDS:

- QUERCETINE DERIVATIVES
- CHLOROGENIC-ACID DERIVATIVES
- KAEMPFEROL DERIVATIVES

AMINO ACIDS





## A unique variety of a combination of bioactive molecules known for their multiple properties and benefits

Spring Snow is standardized in total polyphenols expressed in Eq. Rutine (quercetine-3-rutinoside)

Family	Compound	Content (µg⁄g liquid ; n=3)
Phenolic acids	Neoclorogenic acid (3-CQA)	40.3 ± 0.4
	Chlorogenic acid (5-CQA)	8.7 ± 0.2
	Cryptochlorogenic acid (4-CQA)	21.7 ± 0.1
Phenolic acid glycerols	Hydroxybenzoic acid glycerol	
	Protocatechuic acid glycerol	12.1 ± 0.2
Glycerol hydroxycinnamate	Caffeoyl glycerol	11.1 ± 0.5
Flavonol glycoside	Kaempferol-O-rhamnoside	51.0 ± 0.6
	Kaempferol-O-pentoside	$34.2 \pm 0.4$
	Kaempferol-O-hexoside-O- rhamnoside	27.1 ± 0.7
	Kaempferol di-rhamnoside	23.0 ± 0.7
	Kaempferol-O-pentoside-O-rhamnoside	28.2 ± 0.4
	Quercetin-O-rhamnoside-O-hexoside	40.9 ± 0.2
	Kaempferol di-pentoside	
	Quercetin-O-pentoside	26.3 ± 0.4
	Queretin-O-rhamnoside	26.4 ± 0.3
Family	Compound	Content (%)
Amino Acids	Glutamic Acid (Natural Moisturizing Factor	6.99% Total,
	component, precursor of PCA)	87.11% of glutamic acid

## SKIN IS CHALLENGED BY INTERNAL AND EXTERNAL STRESS

LACK OF SLEEP SEDENTARI **EMOTIONS POOR DIET** LIFESTYLE **IRRITANTS** ALLERGENS POLLUTION **CLIMATE CHANGE SOLAR RADIATION CIGARETTE SMOKE** 

- **Exposome** is defined as the totality of exposures to which an individual is subjected from conception to death. It includes both external and internal factors (see picture)
- COVID-19 pandemic has led to unprecedented hazards to mental health, characterized by high rates of symptoms of anxiety (6.33% to 50.9%), depression (14.6% to 48.3%), post-traumatic stress disorder (7% to 53.8%), psychological distress (34.43% to 38%), and stress (8.1% to 81.9%).
- Stress conditions exert their effects to skin mainly through the hypothalamic-pituitary-adrenal (HPA) axis.
- 92% of the population live in places where **air pollution** exceeds WHO limits, which can contribute to diseases including **skin disorders**.
  - Skin sensitivity
  - Dark spots / Uneven skin tone
  - Dull skin
  - Premature aging
  - Inflammatory disorders: Acne, psoriasis, atopic dermatitis

Xiong J, et al. J Affect Disord. 2020 Dec 1;277:55-64.

https://www.who.int/news/item/27-09-2016-who-releases-country-estimates-on-air-pollution-exposure-and-health-impact



# THE VICIOUS CIRCLE OF CHALLENGED SKIN



- External and internal factors weakens the skin barrier.
- Fragile barrier leads to **penetration of exogenous factors** inducing **neurogenic inflammation and hypersensitivity of nerve fibers**.
- Neurogenic inflammation promote itch, pain and discomfort sensation, leading to stress and anxiety.
- Inflammatory response can further weaken the barrier and induce **pigmentation disorders**.
- Pigmentary disorders can also lead to psychological disturbances, including stress, anxiety and depression.

## SKIN PERCEIVES STRESS AND STRESS IMPACTS SKIN (SKIN-BRAIN AXIS)



# MECHANISMS BEHIND THE VICIOUS CIRCLE OF CHALLENGED SKIN



External and internal stressors **diruspt the skin barrier**, detected as peripheral lesions.

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Fragile barrier increases the **penetration of exogenous components** (allergens, pollutants, microbial antigens, irritants...) activating inflammatory response.

Epidermal Keratinocytes participate in inflammation by producing pro-inflammatory cytokines (IL-8) and growth factors such as the polypeptide Nerve Growth Factor (NGF), a proinflammatory member of the neurotrophic factor family.

# MECHANISMS BEHIND THE VICIOUS CIRCLE OF CHALLENGED SKIN



NGF binds to TrkA on the peripheral nociceptors and activates pro-nociceptive receptors such as TRPV1.

Capsaicin, the active molecule of chili pepper *via* the activation of TRPV1, is responsible for pain, itching and burning sensations.

Activation of TRPV1 channels on the sensory nerves stimulates the **release of calcitonin** gene-related peptide (CGRP).

**CGRP impacts skin cells** contributing to a number of skin conditions, **including skin hyperpigmentation**.



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CGRP can also upregulate the IL-8/IL-8RA system of keratinocytes and fibroblasts maintaining the inflammatory response and the skin barrier defects.

# TRPV1, A PAIN-MEDIATING ION CHANNEL, ACTIVATED BY PHYSICAL AND CHEMICAL FACTORS



- Sensory neurons detect noxious heat and pain thanks to nociceptive receptors like TRPV1 reacting to stimuli (such as heat, acidic pH and capsaicin, the pungent compound in chili peppers) that may be harmful by sending pain signals to the brain.
- Nociception (also nocioception, from Latin nocere to harm or hurt) is the sensory nervous system's process of encoding noxious stimuli.

Bagood MD, Isseroff RR. Int J Mol Sci. 2021 Jun 7;22(11):6135.





# SPRING SNOW

Demonstration of efficacy In vitro assays

# GLOBAL STRATEGY / APPROACH FOR NEURO-SOOTHING EFFECTS AND SKIN COMFORT

## In vitro evaluation



#### In vitro studies, 2D and 3D models

- Effects of Spring Snow on Skin barrier repair / Skin barrier reinforcement
- Effects of Spring Snow on Hydration level of the superficial layers of the skin
- Protection against deleterious impacts of irritant (SDS, capsaicin), pollutants-induced inflammation and neurogenic inflammation
- Prevention of pollutants-induced hyperpigmentation

# 1 – SKIN BARRIER REPAIR / SKIN BARRIER REINFORCEMENT In vitro evaluation

## Proteomic analysis of epidermal differentiation and barrier markers on 2D cultured keratinocytes



The effects of **SPRING SNOW** were evaluated on key markers involved in epidermal differentiation and skin barrier. Calcium, at high concentration, was used as positive control of epidermal differentiation

- Involucrin (IVL) is an important cornified envelope constituent.
- Transglutaminase-1 (TGM1) catalyses crosslinking of structural proteins, including involucrin, at the cell periphery during the cornification process.
- Claudin-4 (CLDN4) is a component of tight junctions that are located in the *stratum granulosum* of epidermis where it contributes to the maintenance of skin barrier function.

# 1 – SKIN BARRIER REPAIR / SKIN BARRIER REINFORCEMENT In vitro evaluation

### Increase of differentiation and barrier markers Involucrin, Transglutaminase-1 and Claudin-4



# 1 – PROTECTION AGAINST DELETERIOUS IMPACTS OF UV RADIATION In vitro evaluation

### Transcriptomic analysis of key genes coding for differentiation markers on UVA-irradiated 2D cultured keratinocytes



- UV radiation stress causes alterations in expression of differentiation-associated proteins and involved in skin barrier, including keratins 1 and 10, Desmocollin 1, Desmoglein 1, involucrin, transglutaminase... leading to alteration of *stratum corne*um and skin barrier function.
- UV exposure also disrupts the epidermal permeability barrier and cell-cell communication by altering the arrangement of the tight junction proteins occludin and claudins-1 and -4.
- The effect of SPRING SNOW 3% was evaluated on several genes coding for these key proteins involved in the maintenance of the skin barrier, in stressed condition by UVA.

## WHAT IS A FOLD CHANGE OR FC

A fold change describes the difference of two values.

For transcriptomic analysis, the Fold change indicates whether a gene is up-regulated or down-regulated and how many times.

Fold Change > 0 for gene A means that gene A is more expressed (= up-regulated) in samples 1 and 2 compared to control. Fold Change < 0 for gene A means that gene A is less expressed (= down-regulated) in sample 3 compared to control.



Gene A Expression

In sample 1, the gene is expressed 2 times more than in control.

In sample 2, the gene is expressed 4 times more than in control.

In sample 3, the gene is expressed 2 times less than in control.

## 1 – PROTECTION AGAINST DELETERIOUS IMPACTS OF UV RADIATION

## In vitro evaluation

Activation of epidermal differentiation markers in UVAirradiated 2D cultured keratinocytes





In stressed UVA condition, SPRING SNOW increases the expression of key genes coding for proteins involved *in stratum corneum* desquamation, filaggrin proteolysis (NMF production), lipid biosynthesis and cornified envelope formation.

SPRING SNOW, despite UV stress, reinforces main components involved in skin barrier.

# 1 – PROTECTION AGAINST DELETERIOUS IMPACTS OF UV RADIATION

## In vitro evaluation

Activation of tight junction and (corneo)desmosome markers in UVAirradiated 2D cultured keratinocytes





In stressed UVA condition, SPRING SNOW increases the expression of key genes coding for proteins involved in **epidermal permeability barrier and cell-cell communication**.

SPRING SNOW, despite UV stress, reinforces main components involved in skin barrier.

Does it maintain stratum corneum hydration?

# 2 – HYDRATION OF THE SUPERFICIAL LAYERS OF THE SKIN

## In vitro evaluation

## Corneometer analysis of stratum corneum hydration on 3D skin explants



The effect of topical application of **SPRING SNOW 3%** was evaluated on Skin Hydration using normal human skin explants and Corneometer<sup>®</sup>.

# 2 – HYDRATION OF THE SUPERFICIAL LAYERS OF THE SKIN In vitro evaluation

#### Improves the hydration of the stratum corneum in a Short-term and Long-term manner



\*\*\*p<0,001 *vs* control

## 3 – PROTECTION AGAINST UV-INDUCED NEUROGENIC INFLAMMATION

## In vitro evaluation

Inhibition of Nerve Growth Factor in UVA-irradiated 2D cultured keratinocytes



\*\*p<0,01*vs* control



According to the literature, disrupted skin barrier demonstrates an increase in TEWL, decrease in hydration and **increase of NGF**.

The restoration of the barrier immediately following its disruption by acetone treatment inhibited the increase in NGF mRNA levels

In stressed UV condition, SPRING SNOW decreases the expression of the gene coding for the Nerve Growth Factor.

SPRING SNOW, modulates neurogenic inflammatory response induced by UV.

# 3 – PROTECTION AGAINST SDS-INDUCED NEUROGENIC INFLAMMATION

## In vitro evaluation

## Analysis of NGF protein expression on SDS-damaged 3D skin explants



SDS (2.5%), is a skin irritant is used to alter skin barrier and to induce inflammatory state.

The effect of **topical application of SPRING SNOW 3%** was evaluated on protein expression of NGF in skin whose barrier is altered by SDS.

# 3 – PROTECTION AGAINST SDS-INDUCED NEUROGENIC INFLAMMATION

## In vitro evaluation

### Inhibition of NGF expression in SDS-damaged 3D skin explants



Disturbance of skin barrier by SDS (2.5%), a skin irritant, leads to an increase of NGF expression (+91%), demonstrating a neurogenic inflammatory response.

SPRING SNOW helps to resolve SDS-induced neurogenic inflammation by inhibiting NGF overproduction (-89%).

The reinforcement of the barrier by SPRING SNOW demonstrated previously, may be responsible for the inhibition of the overproduction of NGF expression.

#### % of positive surface for NGF in the granular layer



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# 3 – PROTECTION AGAINST CAPSAICIN-INDUCED NEUROGENIC INFLAMMATION In vitro evaluation

#### Analysis of capsaicin induced-CGRP release in co-culture of neurons and keratinocytes

Sensory neurons derived from hiPS cells (human induced Pluripotent Stem cells).



TRPV1 activation by capsaicin at 10 µM induces CGRP release by sensory neurons and induction of neurogenic inflammation, responsible for skin discomfort sensations, like burning, itching, tingling.

The effect SPRING SNOW 0,1% was evaluated on capsaicin induced-CGRP release using a model of co-culture of sensory neurons and keratinocytes.

# 3 – PROTECTION AGAINST CAPSAICIN-INDUCED NEUROGENIC INFLAMMATION

## In vitro evaluation

## Inhibition of capsaicin induced-CGRP release in co-culture of neurons and keratinocytes

Sensory neurons derived from hiPS cells (human induced Pluripotent Stem cells).



Endogenous release of Calcitonin gene-related peptide (CGRP) is induced by capsaicin through activation of the transient receptor potential vanilloid 1 (TRPV1) channel.

CGRP is a potent vasodilating neuropeptide with a pivotal role in skin sensitivity and discomfort.

Capsaicin, leads to an increase of CGRP release (+31%), demonstrating an activation of TRPV1 and neurogenic inflammatory response.

SPRING SNOW helps to limit capsaicin-induced neurogenic inflammation by inhibiting CGRP release (-22%).



CGRP release by sensory neurons (pg/ml)

\*\*\*p<0,001*vs* control or *vs* capsaicin

# 3 – PROTECTION AGAINST CAPSAICIN-INDUCED NEUROGENIC INFLAMMATION

## In vitro evaluation

## Inhibition of capsaicin induced-IL-8 release in co-culture of neurons and keratinocytes

Sensory neurons derived from hiPS cells (human induced Pluripotent Stem cells).



CGRP induces cytokine synthesis and release, like IL-8, responsible for neurogenic inflammation and neuronal cytotoxicity and cell death.

Wang JJ *et al,* 2018

Capsaicin, leads to an increase of IL-8 release (+40%).

SPRING SNOW helps to limit capsaicin-induced neurogenic inflammation and preserves neurons viability by inhibiting IL-8 release (-32%).



\*\*\*p<0.001*vs* control or *vs* capsaicin

# 3 – PROTECTION AGAINST POLLUTANTS-INDUCED INFLAMMATION In vitro evaluation

### Analysis of IL-8 protein expression and/or release by pollutants-stressed 2D cultured keratinocytes



Particle pollution, also called particulate matter (PM), penetrates the barrier-disrupted skin, causing inflammation. PM causes inflammation in the skin through increased IL-8, MMP-1, ROS production.

The effect of SPRING SNOW 1% was evaluated on IL-8 release by normal human epidermal keratinocytes treated with pollutants from urban dust 1649b.

Urban dust 1649b consists mainly of polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyl congeners, pesticides, and dioxins, all of which are the main components of urban Particulate matter. Kim M, et al. Exp Dermatol. 2019 Jul;28(7):809-815.

# 3 – PROTECTION AGAINST POLLUTANTS-INDUCED INFLAMMATION In vitro evaluation

### Inhibition of IL-8 protein expression and/or release by pollutants-stressed 2D cultured keratinocytes



\*\*\*p<0,001 vs control or vs pollutants

Pollutants strongly increase the expression and/ or release of IL-8 (+459%), demonstrating an induction of inflammatory response.

SPRING SNOW prevents pollutants-induced IL-8 expression and/ or release (-57%) demonstrating its anti-inflammatory and anti-pollution properties.

# 3 – PROTECTION AGAINST POLLUTANTS-INDUCED OXIDATIVE STRESS In vitro evaluation

#### Analysis of Heme Oxygenase-1 protein expression on pollutants-stressed 3D skin explants



A large variety of environmental factors such as solar radiations, environmental pollution, increase the production of reactive oxygen species (ROS) in the skin.

The effect of topical application of SPRING SNOW 3% was evaluated on protein expression of Heme-oxygenase-1, an antioxidant enzyme activated in conditions of stress, like pollution.

Pollutant mixture used here with Pollubox® system, consists of heavy metals, hydrocarbons and particulate matter. Pollubox®, was built to allow a controlled nebulization of pollutants on the cultured human skin explants. Patatian A, et al. Toxicol Lett. 2021 Sep 15;348:85-96.

# 3 – PROTECTION AGAINST POLLUTANTS-INDUCED OXIDATIVE STRESS

## In vitro evaluation

## Inhibition of HO-1 expression in pollutants-stressed 3D skin explants



Pollutants increase the expression of HO-1 (+27%), demonstrating a state of oxidative stress (overproduction of Reactive Oxygen species, ROS).

SPRING SNOW prevents pollutants-induced HO-1 expression (-27%) and consequently counteracts oxidative stress and inflammation.

What about the fast oxidation of melanin induced by pollutants and responsible for hyperpigmentation?



# 4 – PROTECTION AGAINST POLLUTANTS-INDUCED HYPERPIGMENTATION

In vitro evaluation

### Quantification of oxidized melanin deposits with ImageJ



#### 3) Analyse particles

Count of particles that correspond to visible melanin deposits

Average particles calculated using 7 images for each condition: Control, Pollutants, Pollutants + Spring Snow 3%

# 4 – PROTECTION AGAINST POLLUTANTS-INDUCED HYPERPIGMENTATION

## In vitro evaluation



#### Inhibition of melanin oxidation and browning induced by pollutants

Pollutants increase the melanin oxidation and browning responsible for the appearance of



Number of dark particles corresponding to visible melanin deposits



\*p<0.05 vs control, \*\*p<0.01 vs pollutants

SPRING SNOW limits melanin oxidation and browning induced by pollutants.

SPRING SNOW reduces pollutants-induced hyperpigmentation for brighter skin and even complexion.

Melanin synthesis was analysed using Fontana-Masson staining. No variation in melanin synthesis was observed. We conclude that these differences are due to melanin oxidation.

Irreversible darkening or persistent pigmentation are observed after UVA irradiation known as Meirowsky phenomenon, a photooxidation of melanin precursors.





# SPRING SNOW

Demonstration of efficacy In vivo studies

## GLOBAL STRATEGY / APPROACH FOR NEURO-SOOTHING EFFECTS AND SKIN COMFORT

## In vivo evaluation



#### **Clinical studies**

Assessment of Skin Microcirculation with TiVi700 imager in SDS-damaged skin

Assessment of skin barrier with TEWL measurement in SDS-damaged skin

Assessment of soothing (hands) and brigthening effects

# SOOTHING AND REPAIRING EFFECTS FOLLOWING SKIN IRRITATION

#### **CLINICAL STUDY 1**

Tested at 3% versus placebo

- 23 volunteers
- Mean age: 40±3 years
- Dry skin (cutaneous hydration rate ≤50 A.U. using Corneometer®)
- Forearms
- Measurement after 2 (TEWL and TiVi), 5 (TEWL) or 6 (TiVi) applications



Induction of skin barrier defects and irritation using SLS patches on forearms

Irritation and barrier damage due to the application of the SLS patch is measured by monitoring skin microcirculation with the TiVi700<sup>®</sup> and TEWL with Tewameter<sup>®</sup>, respectively<sup>.</sup>.

A decrease in microcirculation, demonstrates a reduction in irritation and a soothing effect. A decrease in TEWL shows a strengthened and better repaired skin barrier.

## 1 – SOOTHING EFFECT FOLLOWING SKIN IRRITATION

In vivo evaluation of Soothing effect

# Reduction of SLS-induced irritation, demonstrating a soothing effect







On Day 2 and Day 6, SPRING SNOW was more efficient than placebo in reducing skin microcirculation, demonstrating a more powerful soothing effect.

SPRING SNOW Soothing effect at Day 6: 84% of the volunteers against only 68% for the placebo cream.

# 2 – REPAIRING EFFECTS FOLLOWING SKIN IRRITATION

In vivo evaluation of skin barrier repair and reinforcement

### Reduction of SLS-induced barrier damage, demonstrating a barrier repairing effect



SPRING SNOW cream: 65% of the volunteers had a reduction in TEWL of more than 50% compared to D0, at Day 2.

At Day 2 and Day 5, SPRING SNOW showed a better skin barrier repair effect than placebo.

\*p<0,05 vs placebo

# SOOTHING / BRIGHTENING EFFECT

#### **CLINICAL STUDY 2**

Tested at 3% versus placebo

- 26 volunteers
- Mean age: 53±1 years
- Phototype III or IV
- Hands, Measurements of the skin color (skin redness, a\* parameter) using Spectrophotometer®
- Cheeks, Measurements of the melanin index (MI) using  $\mathsf{Mexameter}^{\circledast}$
- 56 days of application, twice a day

Hands, Measurements of the melanin index and of the skin color using Spectrophotometer<sup>®</sup>
Cheeks, Measurements of the melanin index (MI) using Mexameter<sup>®</sup>
PLACEBO
PLACEBO
SPRING SNOW 3%

A decrease in a\* parameter demonstrates a reduction in skin redness and soothing effect.

A decrease in melanin index shows a brightening effect.

# – SOOTHING EFFECT ON HANDS

In vivo evaluation of Soothing effect on hands



SPRING SNOW Soothing effect at Day 56 in hands: 65% of the volunteers against only 46% for the placebo cream.

# 2 – BRIGTHENING EFFECT ON CHEEKS

In vivo evaluation of brightening effect

Reduction of melanin index (MI) parameter measured with  $Mexameter^{\circledast}$ 



\*p<0.05, \*\*\*p<0.001 *vs* D0



Volunteer 17

After 56 days of application, SPRING SNOW was twice more efficient in reducing melanin index than placebo, demonstrating a brightening effect.

SPRING SNOW brightening effect at Day 56 in cheeks: 81% of the volunteers against only 69% for the placebo cream.

## THE VIRTUOUS CIRCLE CREATED BY SPRING SNOW



- Reinforces the barrier function in stressful situations.
- Enhances hydration.
- Limits the hypersensitivity of nerve fibers and neurogenic inflammation for better skin comfort by inhibiting the expression of NGF and the release of CGRP.
- Relieves and soothes the skin against the deleterious effects of pollutants.
- **Prevents the oxidation and browning of melanin** induced by stressors including pollution and responsible for the appearance of dark spots.
- Accelerates the recovery of the skin and soothes.
- Brightens and evens out the complexion.
- Skin comfort and beauty of the complexion reinforces the well-being.



## THE VIRTUOUS CIRCLE CREATED BY SPRING SNOW



# COSMETIC CLAIMS



## NEURO-SOOTHER

- Neuro-soother for skin comfort
- Resets the skin' pain threshold
- Regulates hyperinnervation
- Rescues stressed skin
- Helps to calm reactivity and reduce redness
- Feel good
- Promotes feelings of wellbeing
- Skin looks fresh and well-rested

### SKIN RADIANCE

- Youthful radiance
- Brigthening
- Skin-tone evening
- Illuminating
- Radiance-boosting

## SKIN BARRIER REPAIR

- Builds and strengthens the skin barrier
- Repairs damaged skin and protects it against environmental irritants

## INTENSIVE & LONG-LASTING HYDRATION

- Protects against moisture loss
- Provides intensive and long-lasting hydration boost

Fast results (clinical study 2 days)