Extraits végétaux : innovations cosmétiques et contraintes réglementaires

Dr Laurent Sousselier

Unitis board scientific advisor

UNITIS est une association professionnelle européenne réunissant des sociétés produisant des ingrédients cosmétiques naturels.
Cette association sans but lucratif a été fondée en 2002.

UNITIS regroupe une quarantaine de PME afin de protéger leurs intérêts en termes de **qualité et de sécurité des produits** face à la pression des clients dans un environnement réglementaire toujours plus complexe.

Les membres d'UNITIS innovent

Comment lutter contre le stress oxydant en dermocosmétique ? Les membres d'Unitis proposent des extraits originaux et les objectivent grâce à de nombreux tests originaux.

Nous ne citerons que 4 exemples présentés par:

- GREENTECH
- ID BIO
- LETICC
- PROVITAL GROUP

PROTECTION

SOLIBERINE®

GLOBAL PHOTO-PROTECTOR

BIOTECHNOLOGIES

GREENTECH

SOLIBERINE®

TARGETS DIFFERENT HARMFUL EFFECTS OF LIGHT RAYS, REGARDLESS OF THEIR ORIGIN

UV, IR and I

- Maintains the skin's

- Youth
- Quality
- Radiance

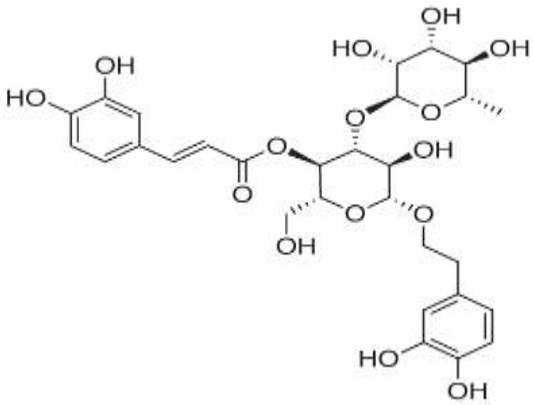
- It prevents from darkspots, redness and premature wrinkles.
- The skin is hydrated and supple, the cutaneous relief is smooth, the complexion is luminous.

BIOTECHNOLOGIES
GREENTECH

SOLIBERINE®

Buddleja officinalis flowers

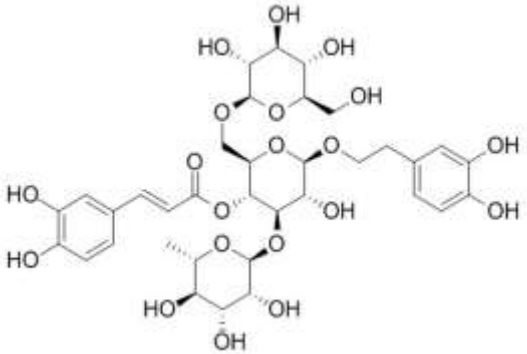
- From the sacred mountains of Chinese province of Sichuan, called celestial country
- Named also butterflybush because its strong perfume attracts butterflies
- Adapted to high levels of solar radiation exposure



- *Buddleja officinalis has selected* for its **exceptional richness in phenylpropanoids**

To Develop Phyto-bioactive agent, highly concentrated it in verbascoside and echinacoside

•a **powerful** natural active ingredient
•at **maximum concentration** above that is found in nature, obtained through a **high-tech process**

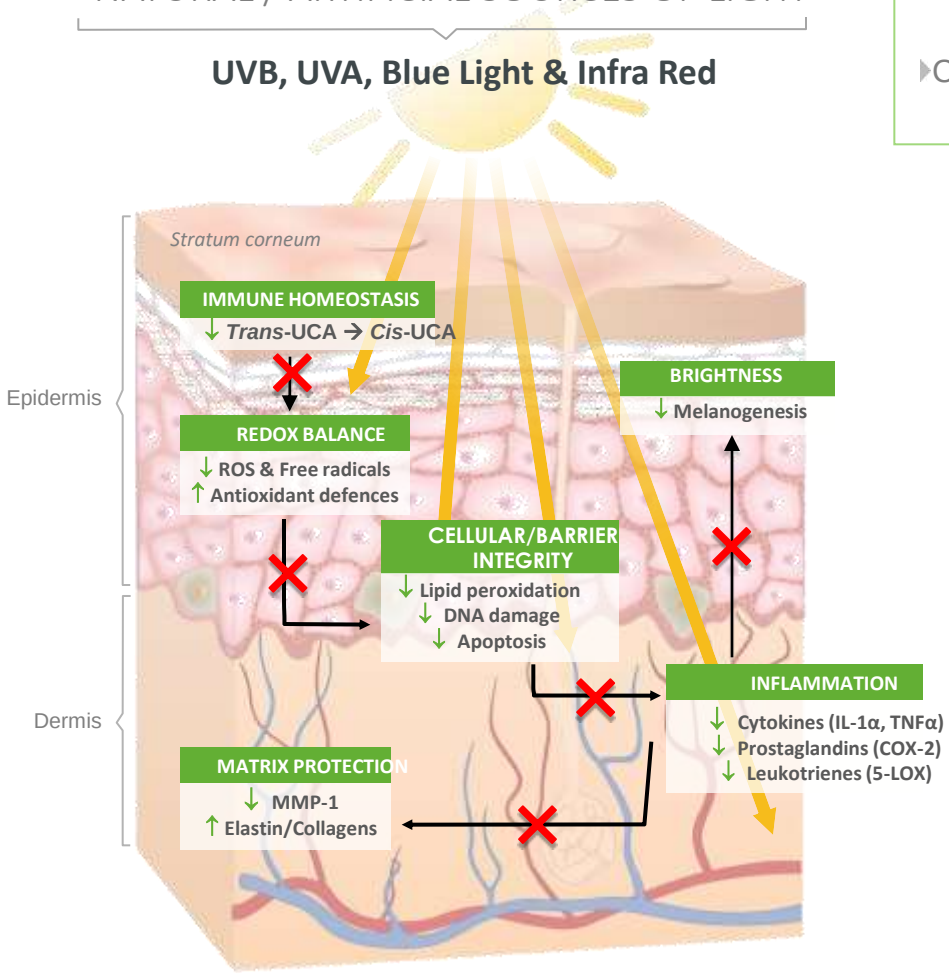


SOLIBERINE®

- **50% of free radicals formed during sun exposition are due to IR and blue light**

NATURAL / ARTIFICIAL SOURCES OF LIGHT

UVB, UVA, Blue Light & Infra Red



Recommendation use level: 0.5 to 2%

- INCI Name: Buddleja Officinalis Flower Extract
- PRESERVATIVE: None
- China Compliant with the INCI name "Buddleja Officinalis Extract "

SOLIBERINE®

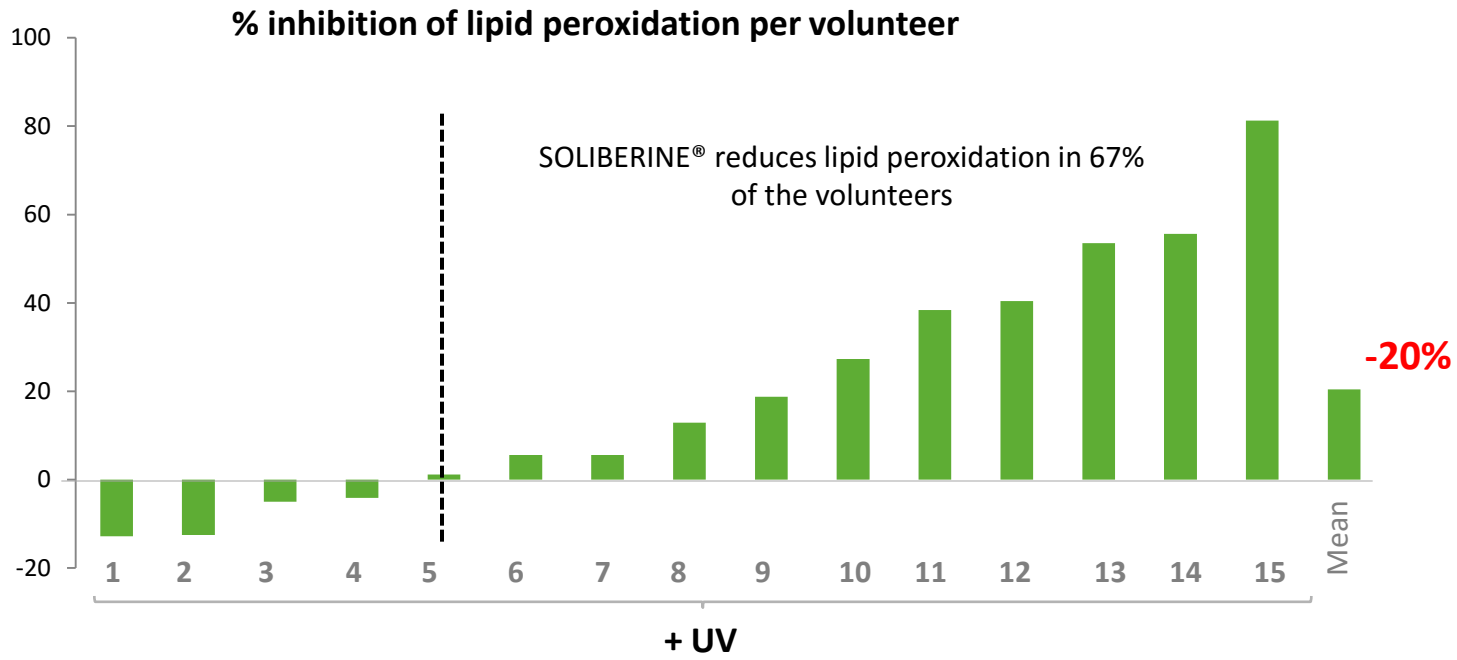
GLOBAL PHOTOPROTECTION

- ↘ SKIN DRYNESS
- ↘ ERYTHEMA
- ↗ BRIGHTENING

- ↘ AGE SPOTS
- ↘ PHOTOAGING
- ↘ PHOTOCARCINOGENESIS

SOLIBERINE®

SOLIBERINE® PROTECTS AGAINST UV-INDUCED LIPID PEROXIDATION

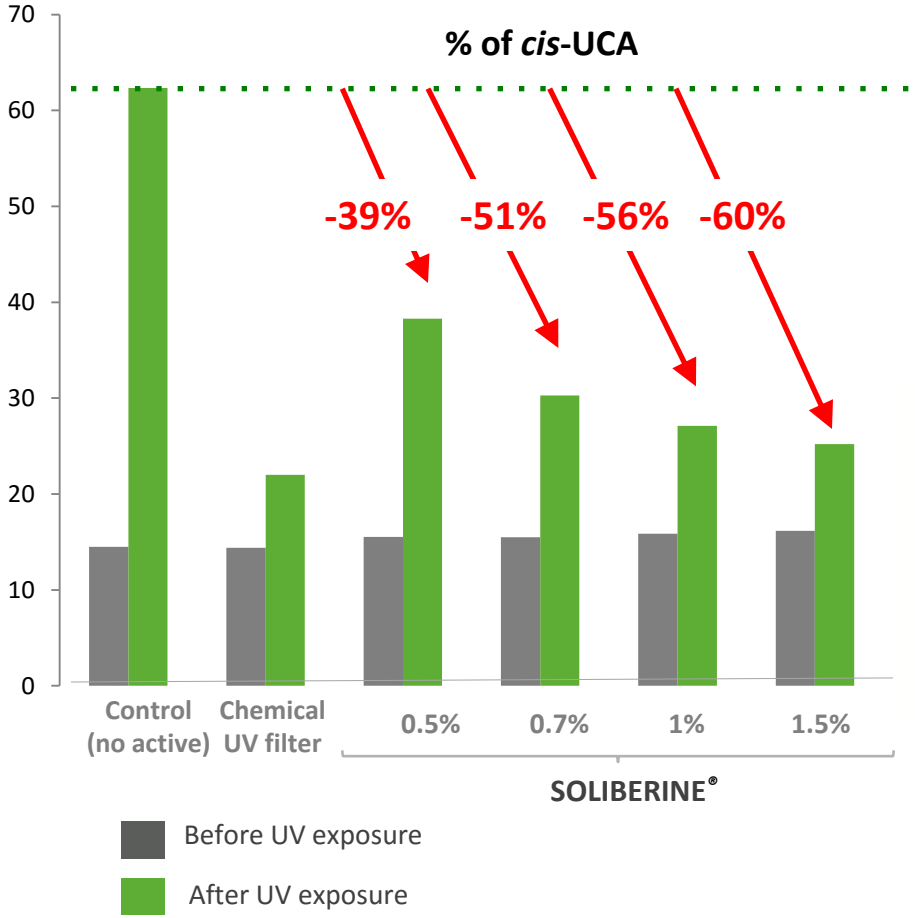


- Lipid isolated from *Stratum corneum* of SOLIBERINE® 2%-treated area, presented an inhibition of UV-induced lipid peroxidation by 20%, compared to placebo treated area

SOLIBERINE® preserves cellular integrity and thus, epidermal homeostasis is maintained. Skin's barrier function is optimized.

SOLIBERINE®

SOLIBERINE® INHIBITS *TRANS*-UROCANIC ACID CONVERSION TO *CIS*-UROCANIC ACID



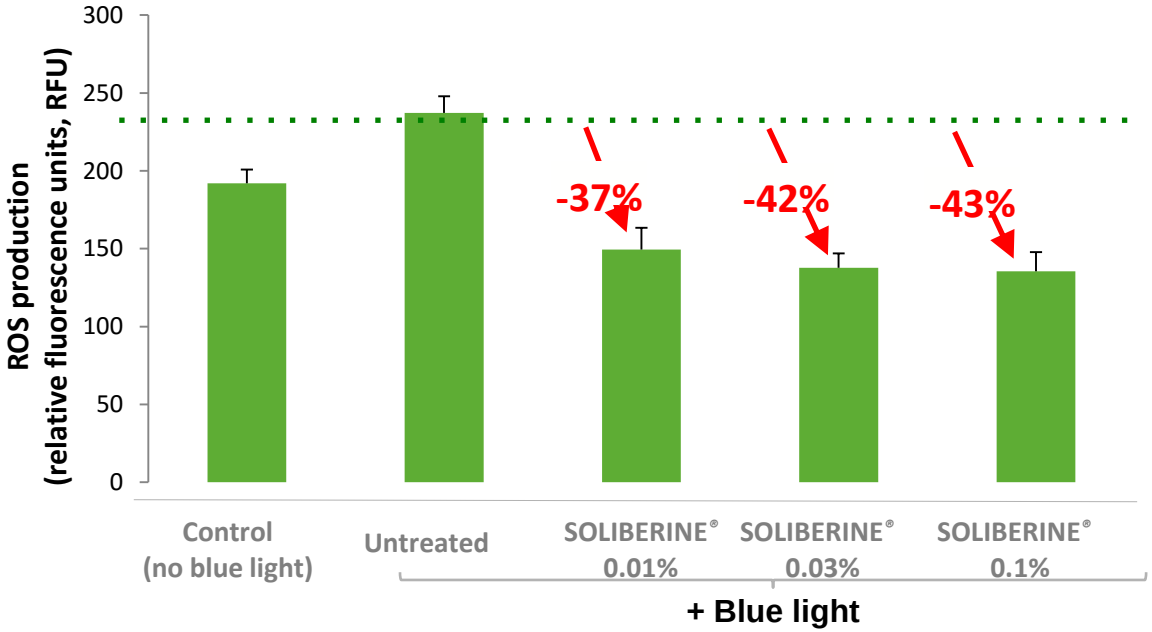
Urocanic acid is extracted from corneocytes obtained by tape stripping.
The solution is treated by UVs.
Cis and trans UCA are assayed by HPLC.

- SOLIBERINE® reduces in a dose-dependent manner the conversion of *trans*-UCA to *cis*-UCA, an immunosuppressive mediator

➡ **SOLIBERINE® maintains the immune homeostasis of the skin**

SOLIBERINE®

SOLIBERINE® REDUCES BLUE LIGHT-INDUCED ROS PRODUCTION



Keratinocytes are seeded on a 96 well plate and treated with Soliberine for24h.
They are exposed to Blue light (420 nm) for 20' and immediately treated with the solution enabling to titrate the ROS content. After1h, ROS are titrated by spectrofluorimetry.

- SOLIBERINE® significantly reduces ROS production induced by blue light

SOLIBERINE® protects from oxidative damages induced by blue light

SOLIBERINE®

COSMETIC APPLICATION

- Photoprotection
- Photoaging
- Filter booster
- Sensitive and reactive skin
- Redness
- Anti-darkening

BIOTECHNOLOGIES GREENTECH

ID bio

Your manufacturer of custom-made cosmetic ingredients

Cell'intact®, une solution efficace contre les effets cutanés du stress oxydatif et de la pollution

Adebiotech, UNITIS

ID bio - ESTER Technopole - 6 allée Skylab - 87068 LIMOGES Cedex - France
www.idbio.eu

La plante : sarrasin

De la plante à l'actif

Cultivé en France

Selon les normes de l'agriculture biologique

Ain (01)

Les grains sont moulus pour être transformés en farine dans un moulin 100 % conforme aux normes bio

ID bio, Limoges, Haute-Vienne

Cet actif cosmétique est dérivé d'un bio-procédé enzymatique 100% naturel

Composition indicative

- Acides aminés essentiels
- Polyphénols
- Flavonoïdes
- Oligo-éléments
- Minéraux
- Vitamines (P, B1, B2, B3, B5 et B6)

A. Tests *in vitro*

B. Tests *ex vivo*

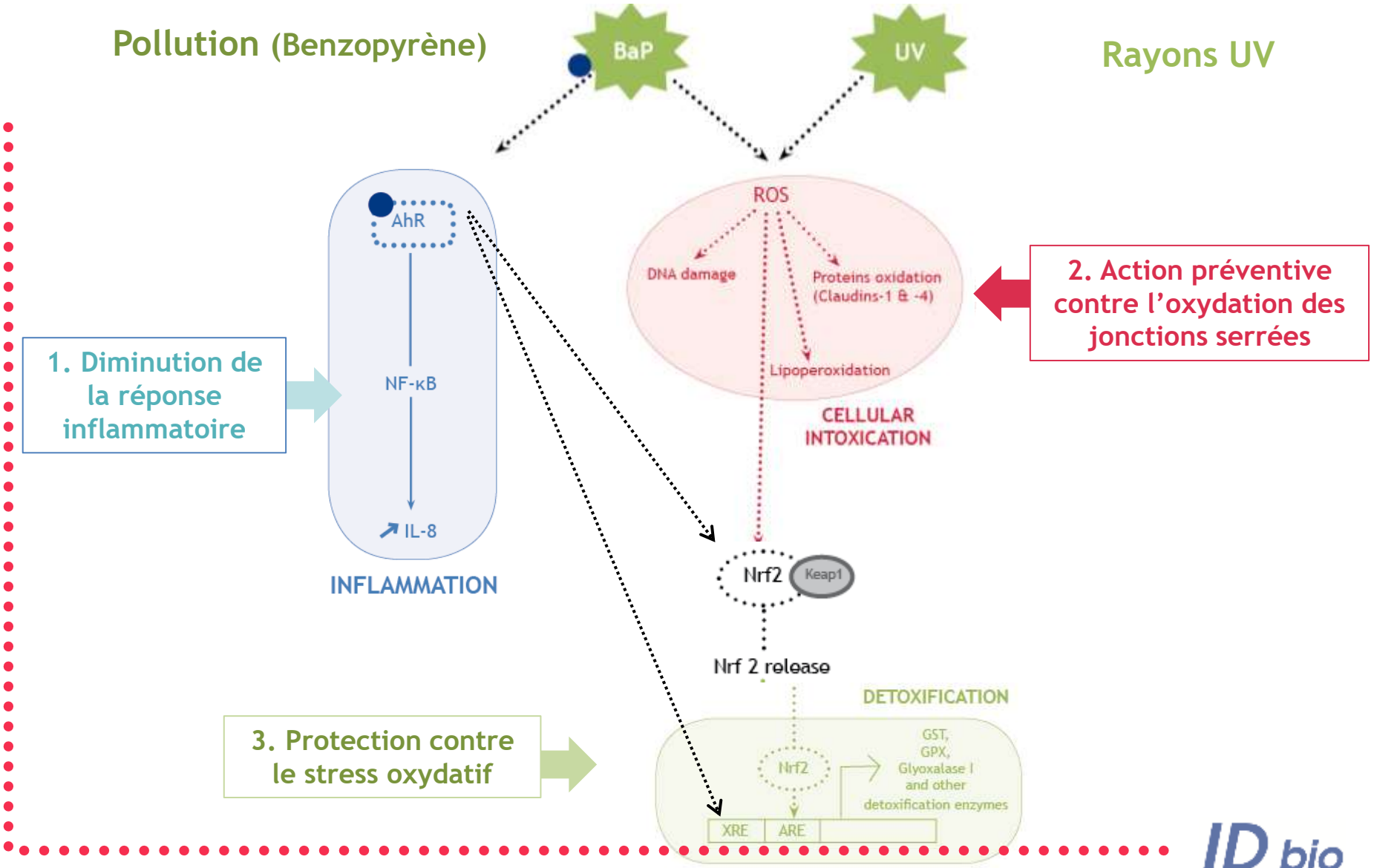
Pollution et stress oxydant

La pollution :

- Affaiblit le système immunitaire de la peau
- Génère des radicaux libres & un stress oxydatif
- Provoque des dommages de l'ADN et l'oxydation des protéines et des lipides
- Conduit à une perte d'homéostasie cutanée

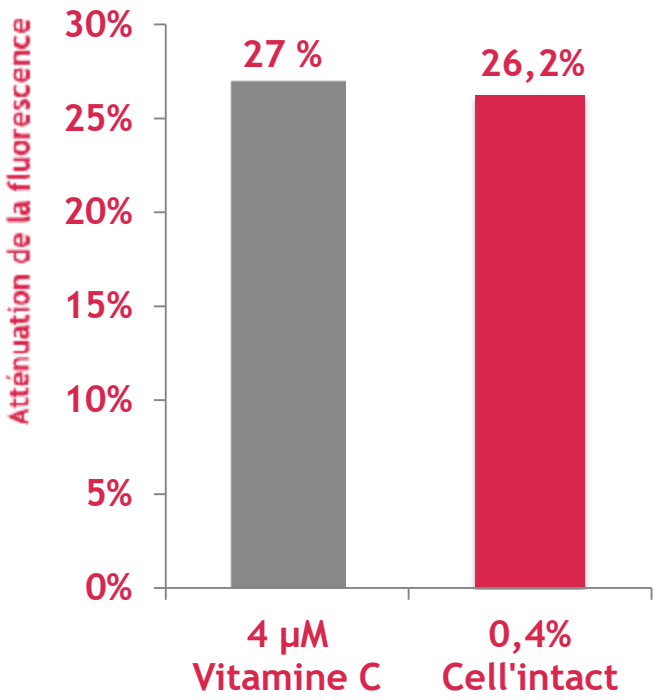
Cette détérioration de la peau fait apparaître diverses imperfections : irritations, rougeurs, perte de fermeté, rides ...

Cell'intact ® : mécanismes d'action

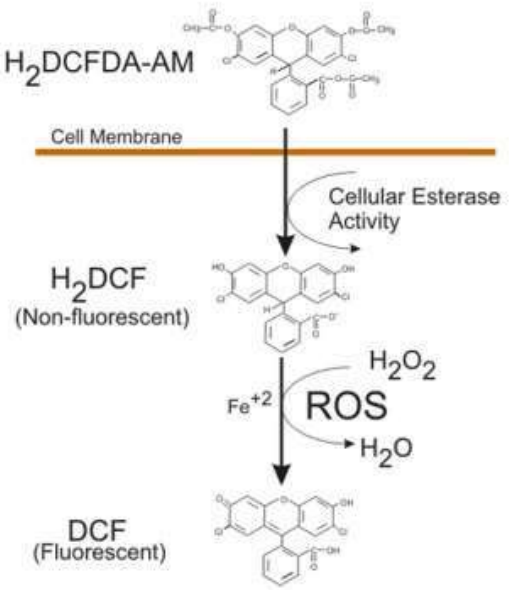


Résulat *in vitro*

% inhibition de l'oxydation créée par t-BuOOH



Une culture de kératinocytes est marquée par une sonde qui devient fluorescente sous l'action d'espèces réactive de l'oxygène: ROS



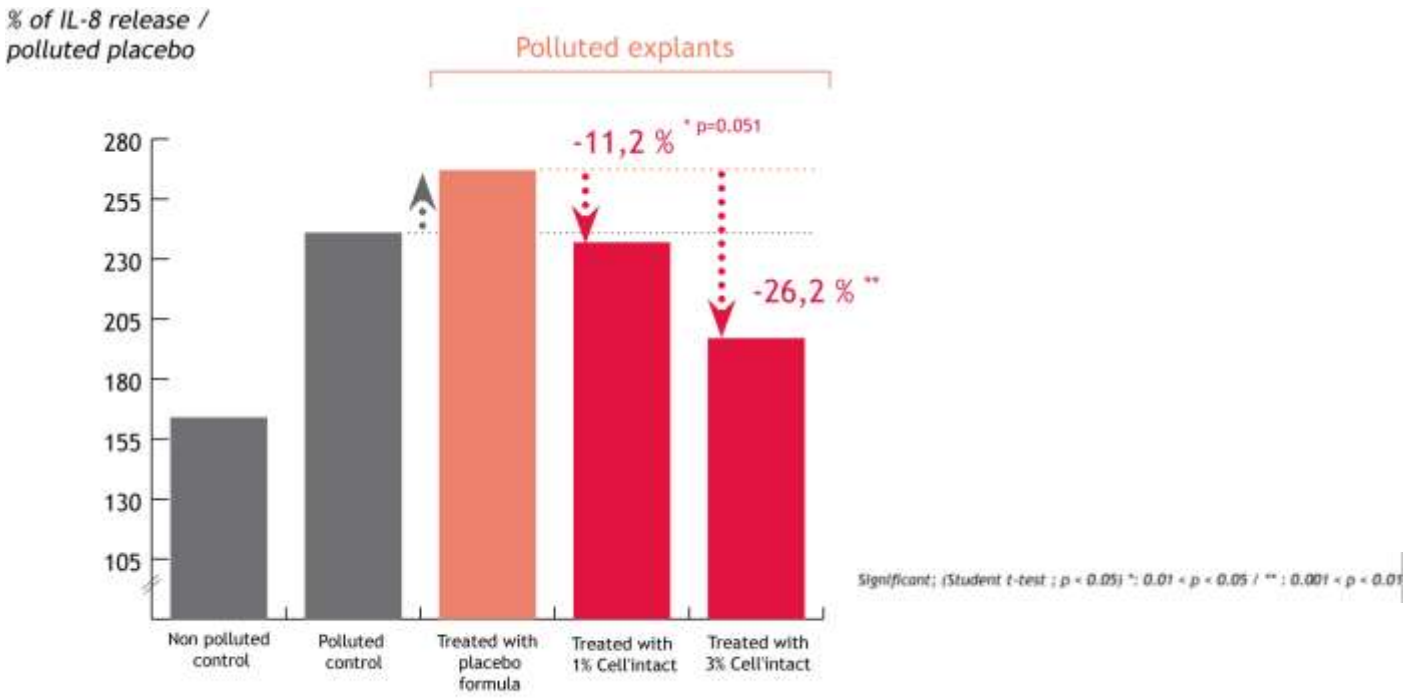
Cell'intact® (0,4%) a un effet contre le stress oxydatif comparable à la vitamine C (4 µM)

ID bio

Résultats : Etudes antipollution *ex vivo*

1.Diminution de la réponse pro-inflammatoire

Tests sur explant *Interleukine-8* *(IL-8)*



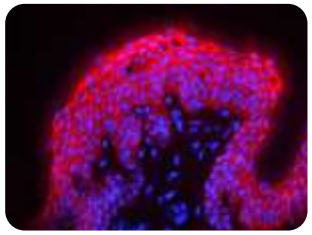
A 1 et 3% de concentration, Cell'intact® protège significativement la peau de la réponse pro-inflammatoire induite par la pollution

2. Préservation de l'oxydation de protéines clefs

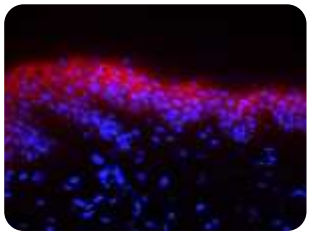
Tests sur explant : immunomarquage des claudines-1 et -4 (CLDN1 & 4), Protéines de la jonction serrée

Claudine-1

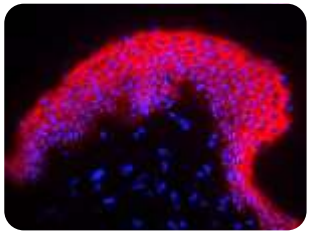
Explant non pollué



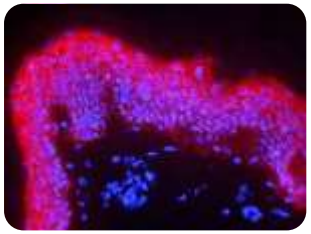
Explant pollué



Explant pollué avec 1% Cell'intact®

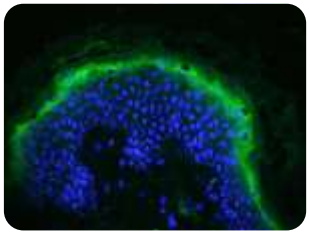


Explant pollué avec 3% Cell'intact®

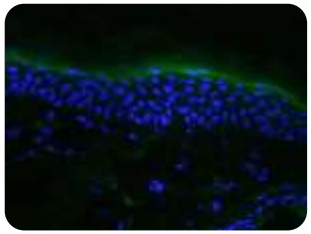


Claudine-4

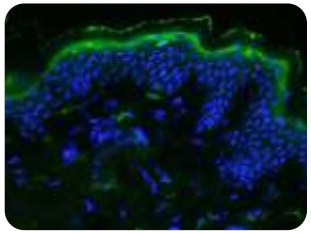
Explant non pollué



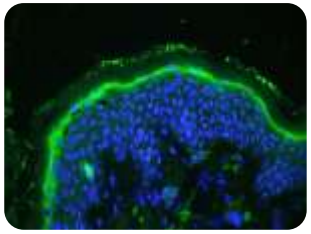
Explant pollué



Explant pollué Avec 1% Cell'intact®



Explant pollué Avec 3% Cell'intact®

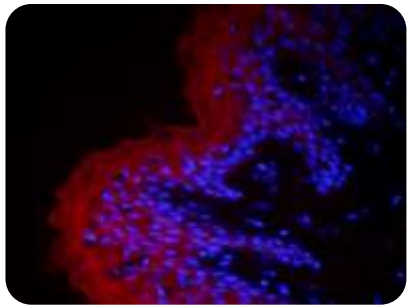


Prévention de la dégradation des claudines 1 et 4
Maintien de l'intégrité des tissus, de l'homéostasie de la cohésion cellulaire
Protection contre le vieillissement prématuré dû à l'oxydation des protéines

3. Etude sur la glyoxalase I

Marquage de la glyoxalase I, enzyme surexprimée en cas de stress oxydatif

Explants irradiés
+ placebo



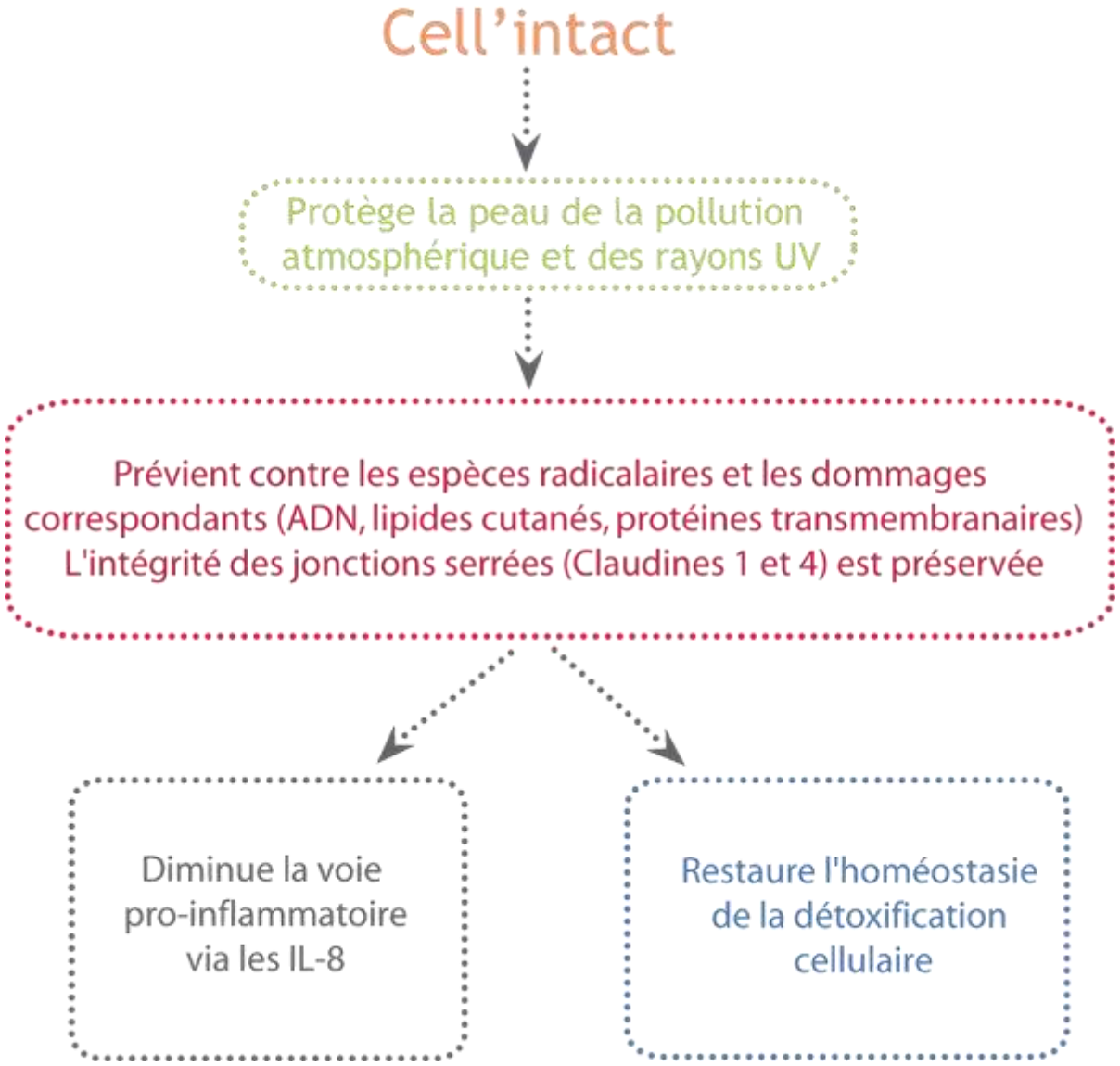
Sans protection :
la glyoxalase I est surexprimée

Explants irradiés
+ 4 % Cell'intact ®

Avec protection (Cell'intact) :
la glyoxalase I est normalement exprimée

Cell'intact ® crée un bouclier antiradicalaire en amont, évitant la surexpression de la glyoxalase I

Résumé : Cell'intact® & le stress oxydatif



ID bio

PRODHYDERM® XPP CE

Concentré de xylopolyphénols

Le chêne centenaire, source de protection pour votre peau.

Chêne

Bois de chêne

Cellules de chêne

EXTRAIT HYDRO-GLYCÉRINÉ DE BOIS DE CHÊNE AUX PROPRIÉTÉS PROTECTRICES DE LA PEAU CONTRE LE VIEILLISSEMENT CUTANÉ INDUIT

PRODHYDERM® XPP CE

Botanique & Composition

Pérennité

Quercus Robur.
Famille des Fagaceaes.
Issu des Forêts du Limousin.
Peut atteindre jusqu'à 50m de hauteur et vivre centenaire.

Robustesse

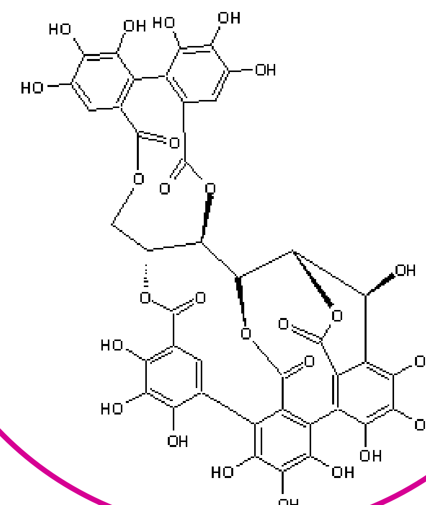
Cœur de chêne

Compositon

Polyphénols sous forme d'ellagitanins

Vescalagine

Exemple d'ellagitanin



Chêne

PRODHYDERM® XPP CE

Efficacité (1) – in vitro

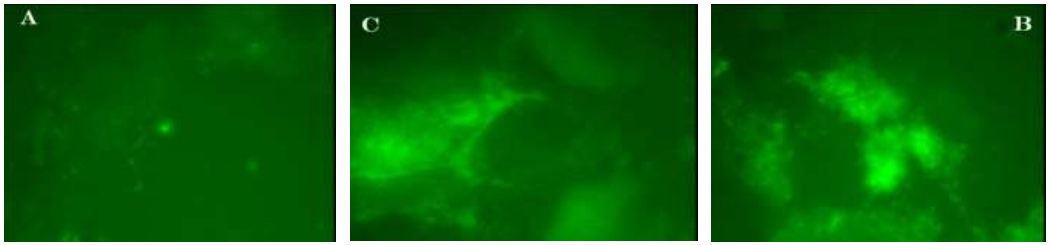
EFFET PROTECTEUR VIS-À-VIS D'UN STRESS OXYDATIF GENERE PAR LES UV-B SUR KERATYNOCYTES HUMAINS

Etude réalisée sur kératinocytes humains HaCaT cultivés en milieu DMEM
24 h - 37°C - atmosphère air-CO_2 (95%-5%).

Les cultures sont ensuite rincées, exposées aux UVB (20 mJ/cm^2) puis incubées 24 h avec le produit à tester.

Le test de « Stress Oxydatif » est basé sur la mesure du degré d'oxydation intracellulaire à l'aide d'une sonde spécifique DCFH-DA (2',7'-dichloro-dihydrofluorescein diacétate).

La sonde pénètre par diffusion passive à l'intérieur de la cellule. Après clivage des groupement acétates par les estérases intracellulaires, le DCF s'accumule au niveau du cytosol.
L'oxydation intracellulaire du DCFH par différentes espèces réactives de l'oxygène conduit à la formation d'un produit fluorescent.
La mesure de l'intensité de fluorescence permet d'évaluer le degré d'oxydation des cellules soumises à un stress oxydatif.



Negative test (UV-) | PRODHYDERM® XPP CE 20µg/ml | Positive test (UV+)

Test 1: Fluorescence
Représentation de l'efficacité du PRODHYDERM® XPP CE sur les effets du stress oxydatif subit par les cellules de la peau. **16% d'efficacité testé à 20 µg/ml**

PRODHYDERM® XPP CE

Efficacité (2) – in vitro

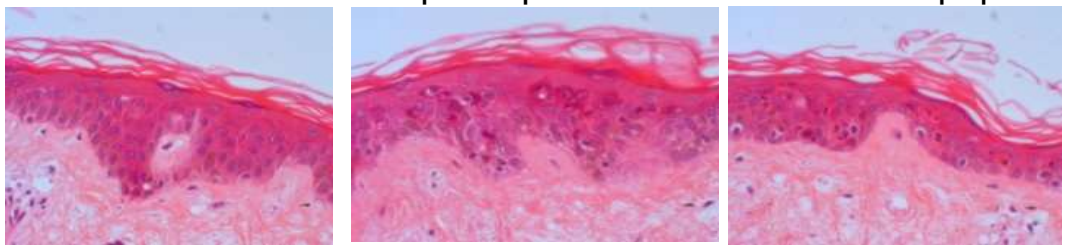
PREVENTION DE L'APPARITION DE SUN BURN CELLS GENEREE PAR LES UV-B SUR PEAU HUMAINE

Etude réalisée sur fragment de peau recueilli après opération de plastie abdominale et placé en milieu de culture fibroblastes (MCF).
24 h - 37°C - atmosphère air-CO_2 (95%-5%).

Les cultures sont ensuite rincées, exposées aux UVB (1 J/cm^2) puis incubées 24 h avec le produit à tester.

Le test de «Sun Burn Cells» est basé sur la mesure par dénombrement des cellules en apoptose (mort cellulaire).

Le disque de peau est fixé par une solution de formaldéhyde puis une inclusion est réalisée dans la paraffine.
Les coupes histologiques sont colorées à l'hémalun-éosine-safran.
Les «Sun burn cells » sont observées et comptées par observation microscopique.



Negative test (UV-) | PRODHYDERM® XPP CE | Positive test (UV+)

Test 2: Sun burn cells

Coupe de peau représentant l'efficacité du PRODHYDERM® XPP CE contre les effets des UVB sur la production de cellules apoptiques. **12% d'efficacité testé à 2,5% et 67 % d'efficacité testé à 5%.**

PRODHYDERM® XPP CE

Efficacité clinique (3) – in vivo

EVALUATION DE L'EFFET ANTIOXYDANT APRES EXPOSITION AUX UV-B SUR VOLONTAIRES HUMAINS

Etude réalisée sur 29 volontaires, d'âge moyen 47 ± 6 ans et de phototype II à IV.

Le produit est formulé dans une émulsion utilisée également en parallèle comme contrôle placébo.
3 zones sont délimitées sur le dos du volontaire : Z1 : Non traitée, Z2 : émulsion placébo,
Z3: émulsion placébo + Produit à 4%. Les émulsions sont administrées 1 fois à J_0 et à 0,2 mg/cm^2.

Après exposition des 3 zones aux UV_B, des mesures spectro-colorimétriques sont réalisées sur les différentes zones à J_2.

Z1 :Non traitée

Z2 : Emulsion placébo + PRODHYDERM® XPP CE 4%

Z3 : Emulsion placébo

Test 3: Exposition aux UVB sur volontaires

La différence de pigmentation de la peau entre la Zone 2 traitée et la Zone 1 non traitée est significative (p = 0,0247).

La Zone 2 montre une **diminution de 16% de l'érythème** vis-à-vis de la Zone 3 placébo.

PRODHYDERM® XPP CE

Issus d'une démarche éco-responsable, Les polyphénols de coeur de bois de chêne sont des molécules actives et efficaces contre la lutte du vieillissement cutané.

Action

Photo-vieillissement

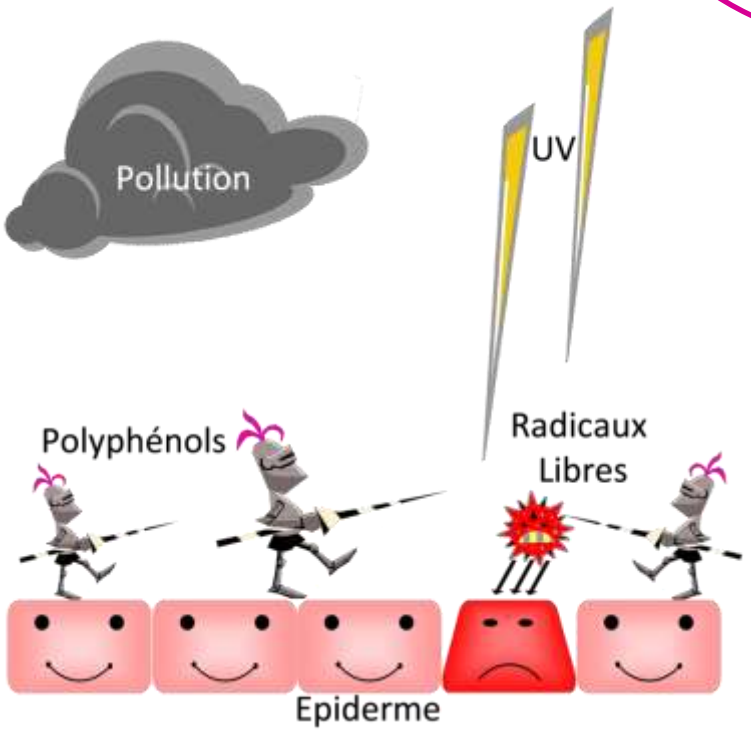
Le rayonnement UV est la cause de nombreux dommages sur la peau.
Le PRODHYDERM® XPP CE diminue l'apparition des cellules apoptiques. La dégénérescence cutanée est ralentie.

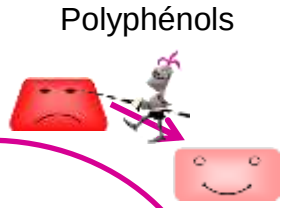
Radicaux libres
Stress oxydatif

Le stress oxydatif est une des cause de l'apparition des radicaux libres. Le PRODHYDERM® XPP CE lutte efficacement contre les effets néfastes de l'environnement et fait barrière contre les radicaux libres.

Anti ride

Grâce à son rôle protecteur, le PRODHYDERM® XPP CE est un actif essentiel dans la lutte contre les effets du vieillissement cutané.





PROVITAL GROUP

For a beautiful life from cells to the skin

CHINA
IECIC LISTED

SUN CARE

Lingostem™

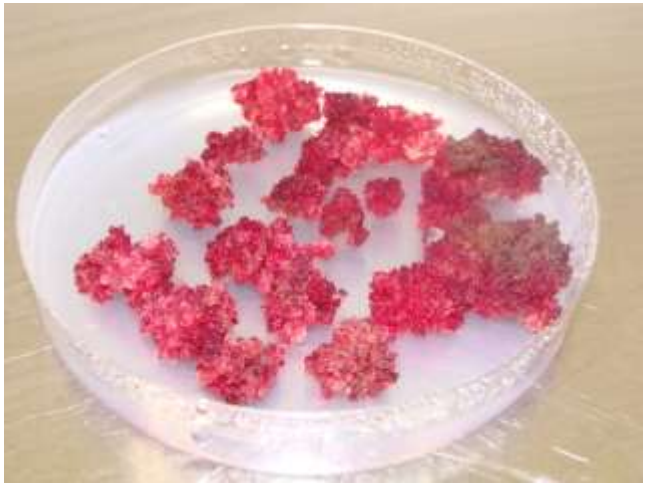
PROVITAL GROUP
For a beautiful life from cells to the skin

» Lingonberry stem cells

›Lingonberry (**Airelle rouge**) is considered a“superfruit”, rich in vitamin C and E (tocopherol), with photoprotective properties.

›Lingostem™ are plant stem cells obtained from Lingonberry (Vaccinium vitis-idaea) enriched in **polyphenols** by an exclusive elicitation process.

›Lingostem™ shows protective and repairing properties against the damaging effects of sun radiation.



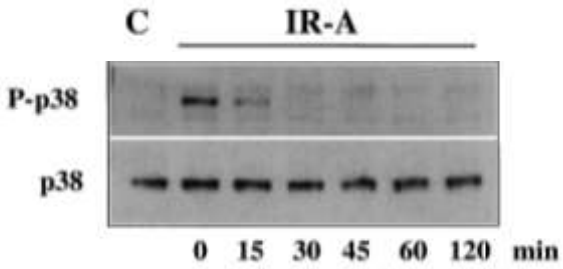
SUN CARE

Cell Protect Solar

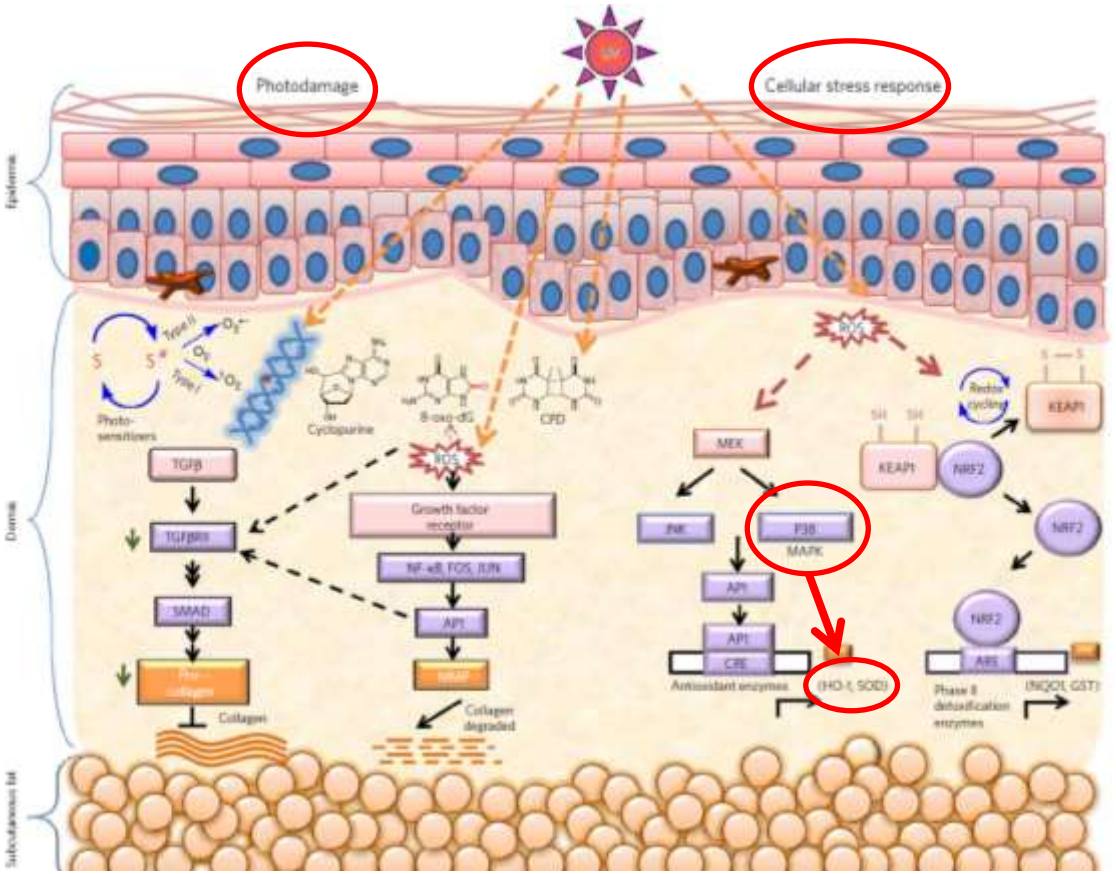
PROVITAL GROUP
For a beautiful life from cells to the skin

Enhancement of the cellular antioxidant response

p38, protein of the cellular signalling pathway that activates the expression of antioxidant enzymes, like **superoxide dismutase (SOD)**



Schieke *et al.* 2002

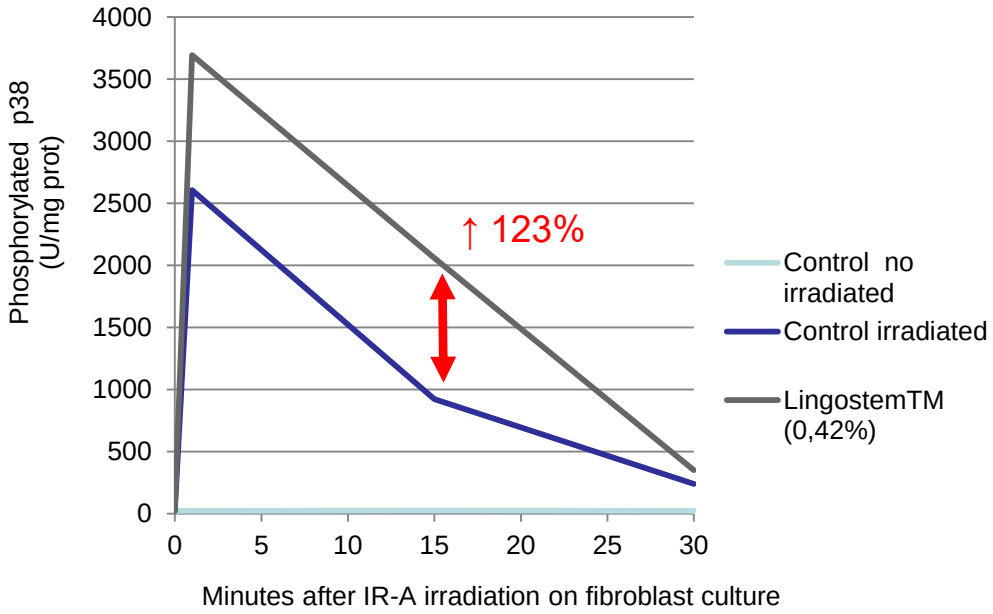


As a response to oxidative stress, p38 protein is activated by phosphorylation. It then triggers the biochemical process that promotes the synthesis of antioxidant enzymes such as SOD.

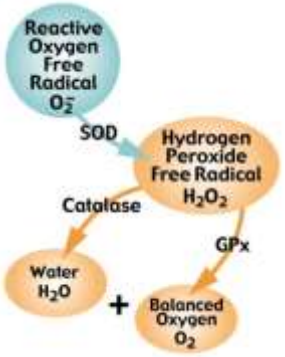
After serving its function, p38 is dephosphorylated to return to its inactive state.

» *In vitro* efficacy: protection against IRA radiation

› **Antioxidant response activation**: **p38,** protein of the cellular signalling pathway that activates the expression of antioxidant enzymes, like **superoxide dismutase (SOD)**.



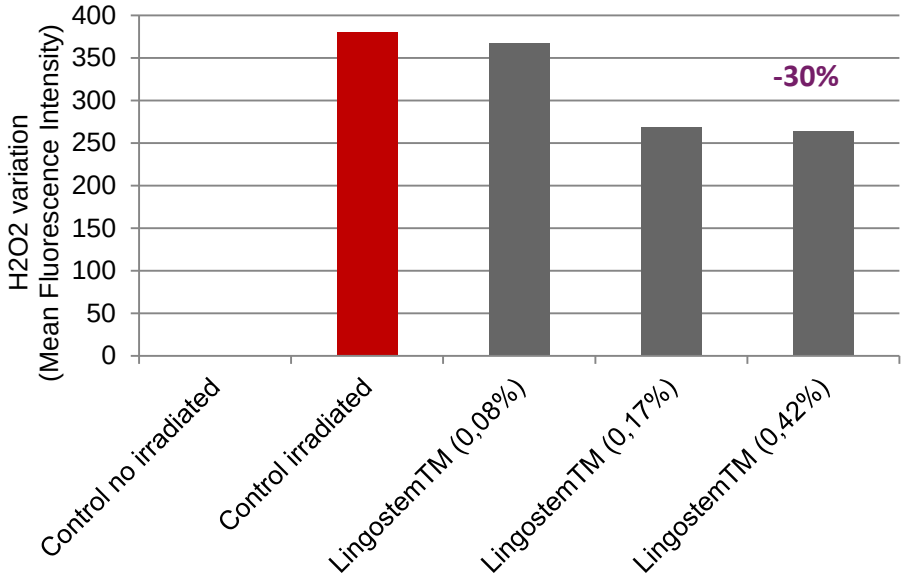
% SOD		
Control	Irradiated Control	IRA + Lingostem™ (0,08%)
100	238	290



Lingostem™ facilitates the rapid and transient phosphorylation of p38 by IR-A enhancing the natural cellular defenses against oxidative stress.

» *In vitro* efficacy: protection against IRA radiation

› **Oxidative stress:** Assessment of **hydrogen peroxide** (H_2O_2) variation after IRA treatment of a fibroblast culture.



Lingostem™ decreases oxidative stress caused by IR-A radiation, thus reducing oxidative damage.

» *In vivo* efficacy

- **Measurement of repairing activity :**

  32 volunteers, between 40 and 60 years old with photo-aged skin signs. Active formula (1.5% Lingostem™) on one half of the face and a forearm, and placebo in the other half of the face and on the other forearm. Two daily applications, 28 days, from September 22th to October 20th in Portugal, **after maximum sun exposure season**. Measurements at D0 and D28.

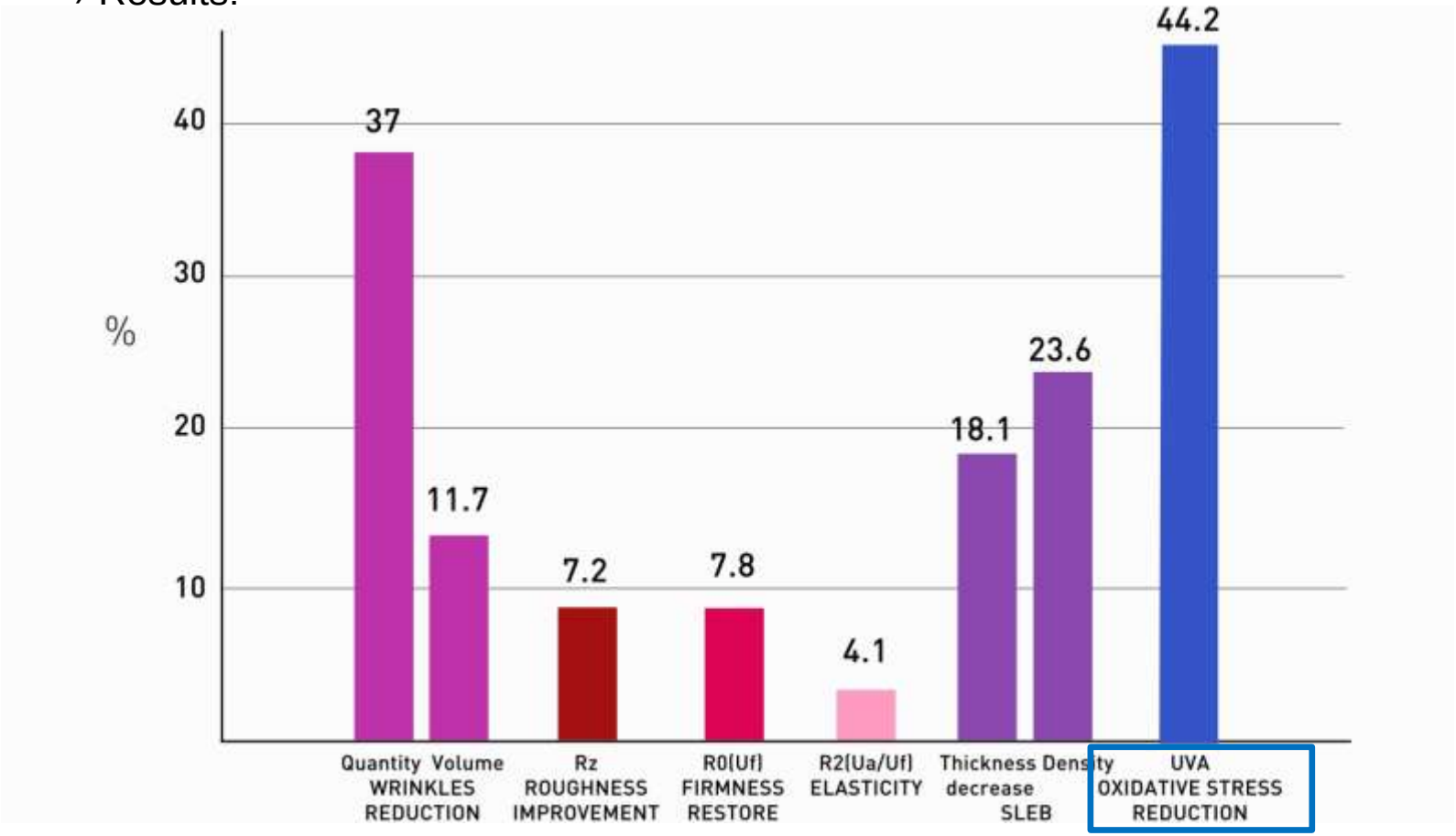
  UVA oxidative stress reduction is measured through assessing, by colorimetry, the improvement of the skin's antioxidant capacity to prevent the UVA-induced oxidative discoloration of beta-carotene in the skin.

  A 44.2% reduction is observed. These results reveal an increased antioxidant capacity of the skin treated with Lingostem™.

Lingostem™

» *In vivo* efficacy. Repairing activity

› Results:



Contraintes réglementaires

Comment mettre sur le marché ces innovations en respectant les contraintes réglementaires?

- Règlement cosmétique
- Arrêt des tests animaux

→ **PROJET NCS TOX**

NCS TOX PROJECT

PRESENTATION

Predictive Database and Guidelines
to determine the Toxicological Profile of
Natural Complex Substances - Plant Extracts

General Background

- New regulations demand complete safety profile of cosmetic ingredients for ensuring **better consumer safety**.
- In particular in Europe where annex 1 of European cosmetic regulation mandates full safety profile of ingredients.
- Plant extracts: NCS are barely covered by REACh and due to the ban of animal testing and the unavailability of valid alternative methods, a new approach is needed for determining if an extract is “safe for use”.
- UNITIS has developed a unique methodology to assess the safety profile of extracts. It has been **validated by US based CIR**.
- All the tools used are approved for REACh and their use is accepted by SCCS.

UNITIS: The Calendula Exercise

- 2001: the CIR* collected information about Calendula extracts for use in cosmetics and stated:

> **CONCLUSION**
>
> The CIR Expert Panel concludes that the available data are insufficient to support the safety of Calendula Officinalis Extract and Calendula Officinalis for use in cosmetic products.

- 2004: Unitis drafted a Report on the toxicological profile of Calendula extracts, according to a new methodology adapted to NCS, based on chemical profile of the plant and toxicological data collection.
- 2006: CIR considered that this Report obtained without any new tests was appropriate enough to reopen the file of Calendula extracts.
- 2009: CIR amended safety assessment of Calendula extracts

> On the basis of the data presented in this report, the CIR Expert Panel concluded that Calendula Officinalis Extract, Calendula Officinalis Flower, Calendula Officinalis Flower Extract, Calendula Officinalis Flower Oil, and Calendula Officinalis Seed Oil are safe for use in cosmetics in the practices of use and concentration given in this tentative amended safety assessment.[1]

*CIR: *Cosmetic Ingredient Review, Washington DC*

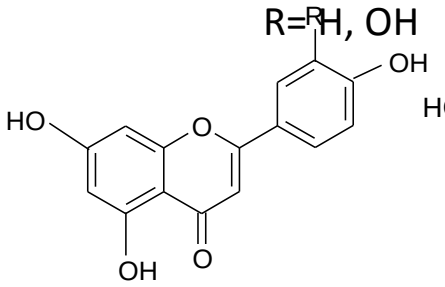
The Project: PRINCIPLE.

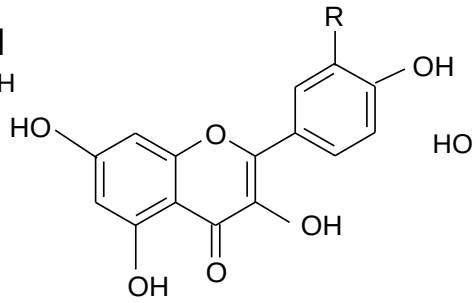
- There are few biosynthetic pathways in botanicals, the same families and components may be found in many different plants, in different combinations and at various concentrations.
- So instead of testing thousands of plant extracts it is wiser and cheaper to gather all available data (literature and company tests) on plant constituents and collect them in a data base after validation.
- Should an unknown compound be detected, the use of in silico models (QSARs) built in the database will give the tox profile of this constituent (Read across).
- Companies will only need to determine the composition of their extract to access, through using the platform, the toxicological data of their constituents and allow them to establish the safety profile of their extract.

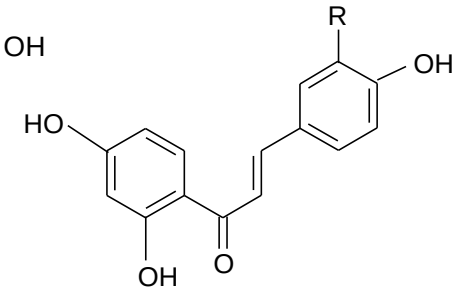
The Project : SOME DEFINITIONS

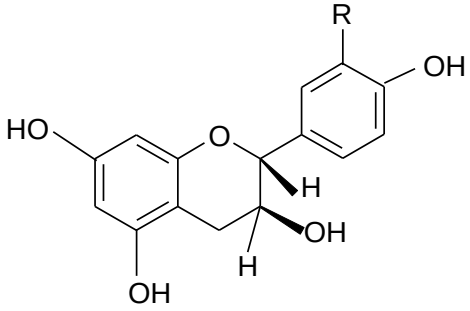
- **Chemical classes: ~ 28 - Lipids, Glucids, Coumarins, Quinones, Tannins, Triterpenes, Lignans, EOs, Oleoresins, Flavonoids, …**
- **Molecular groups of flavonoids:**

Flavones, **Flavonols,** **Chalcones,** **Flavan-3-ols, …**

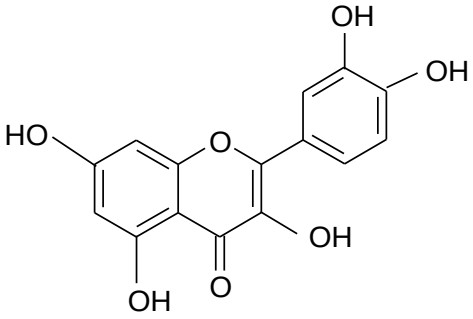




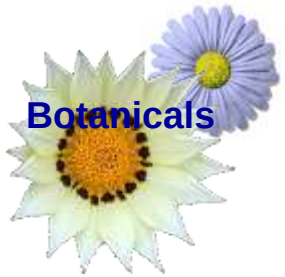




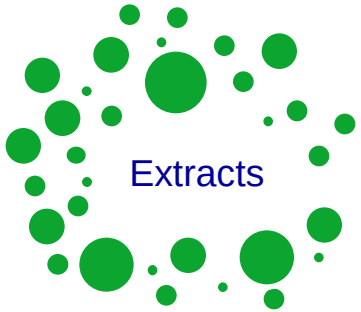
- **Critical compound of flavonols: Quercetin ?**



The Project: OBJECTIVE



DATABASE on molecular groups, critical compounds in botanicals



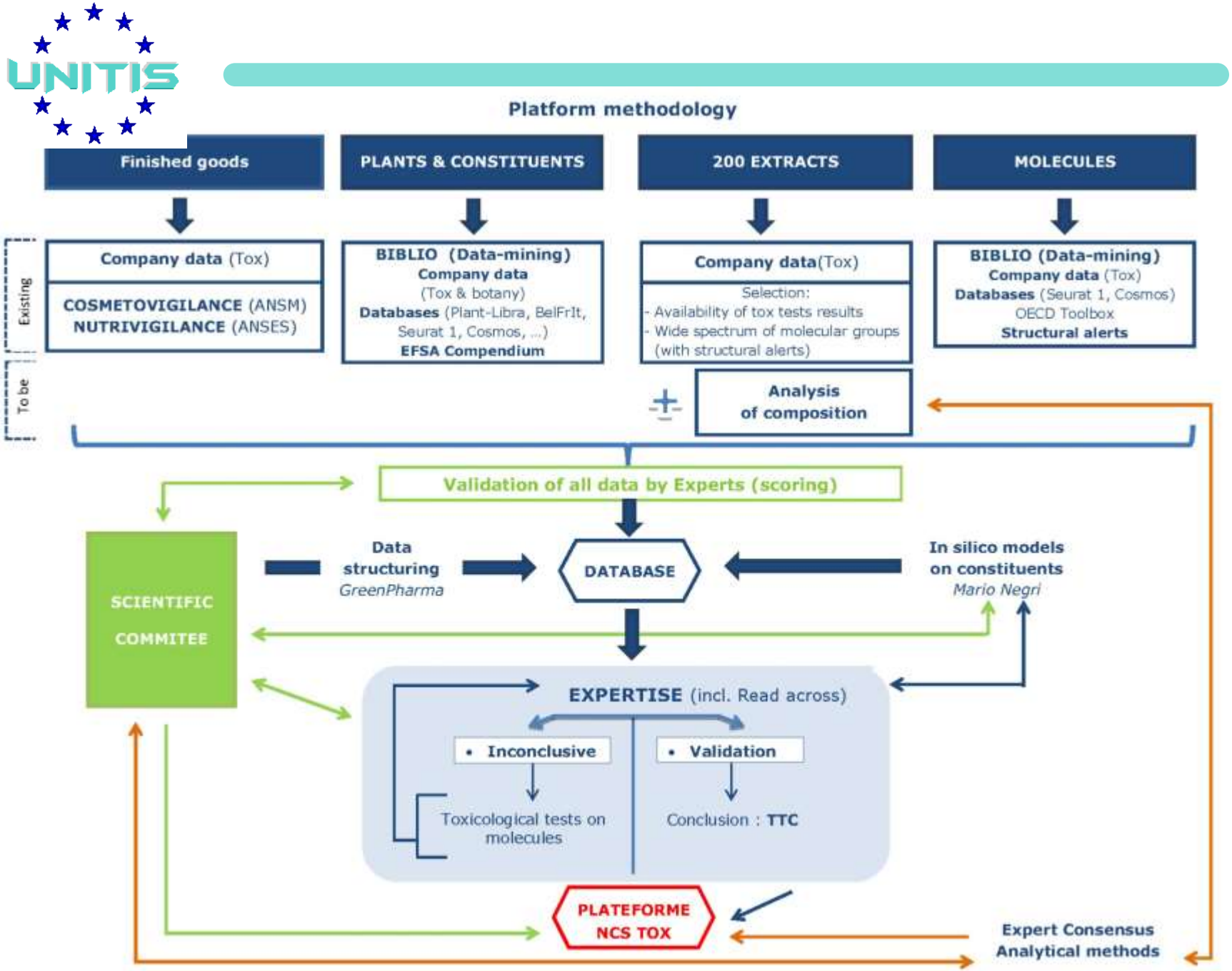
DOCUMENTED USER's GUIDELINES

1 **LISTING & BIBLIOGRAPHY** of molecular groups, critical compounds

2 **ANALYTICAL METHODS** for identification / quantification of molecular groups, critical compounds

3 **BIBLIOGRAPHY OF TOXICOLOGICAL DATA / *IN SILICO* MODELS** for identification of potential danger of molecular groups and critical compounds

4 **TOXICOLOGICAL PROFILE** of molecular groups, critical compounds



The Project: EXPECTED GENERAL RESULTS

The UNITIS NCS TOX PROJECT is due to make available to all links of the supply chains, particularly to SMEs, a predictive Database and related Guidelines in which they will find:

- Toxicological data and profiles concerning these molecular groups and critical compounds present in botanical extracts, separated in 2 categories:
  - **molecular groups with demonstrated safety,**
  - **molecular groups and compounds with potential danger according to their level.**
- Analytical methods enabling to identify/quantify the molecular groups and critical compounds contained in a plant extract, existing or new.
- Information on molecular groups and critical compounds at risk enabling to decide additional tests so as to complete the safety evaluation of the extract, e.g.: when the level of critical compounds is higher than the TTC.

The Project: EXPECTED RESULTS for the SUPPLY CHAINS

The UNITIS NCS TOX PROJECT will:

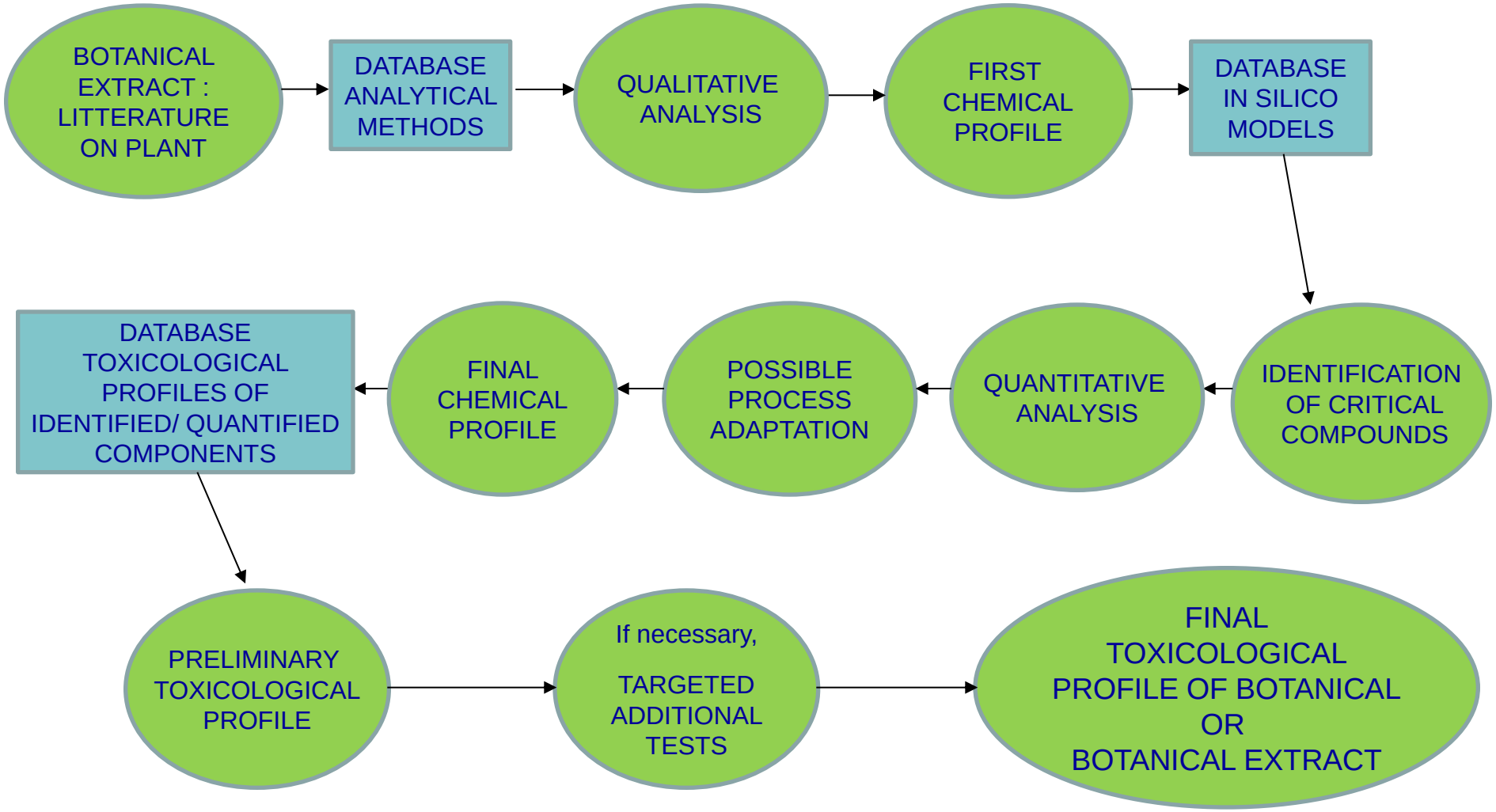
- Help the industries to prepare their safety files - for instance, the cosmetic product safety report - while continuing to formulate innovative products using NCS.

- Enable plant extracts manufacturers to:
  - **Analyze the chemical profile of the plant extract,**
  - **Predict the safety of the NCS they are developing,**
  - **Adapt their processes to the suppression of possible toxic molecular groups or critical compounds,**
  - **Document accurately the industries on the toxicological profile of the NCS they are supplying.**

Moreover,

- It will enable to pursue innovation in the field of natural ingredients when the ban of animal testing and the insufficient availability of alternative methods block any future development.

The Project: EXPECTED RESULTS for INDIVIDUAL COMPANIES



THE INNOVATIVE PROJECT 1/3

- Predictive *in silico* models

  Adapt and/or develop new *in silico* models for the various toxicological end points enabling to run quantitative structure-activity relationship (QSAR) or to perform grouping and read-across within the various molecular groups, thus enabling to predict the possible presence of critical compounds in molecular groups and **to determine their TTC**.

- Toxicological tests

  In order to validate the *in silico* models, selected toxicological tests may be performed, preferably through in vitro or alternative methods.

- Final toxicological profiles

  The threshold of toxicological concern (TTC) will be used to support a global “weight of evidence” of safe use of most compounds and molecular groups present in plant extracts.

THE INNOVATIVE PROJECT 2/3

- Database creation

  The knowledge developed during the project will be included into a Database which will be open to academics and professionals in the supply chain of products using NCSs.

  The database will be set up as predictive.

  It will be designed “user friendly” in order to be readily accessible by SMEs.

- Demonstration

  The database will be presented in particular to European SMEs and academic research centers through scientific publications and seminars.

  Written guidelines will be developed so as to explain the easiest way to use it.

  An example will be given on how to establish the safety profile of a plant extract.

THE INNOVATIVE PROJECT 3/3

- Dissemination

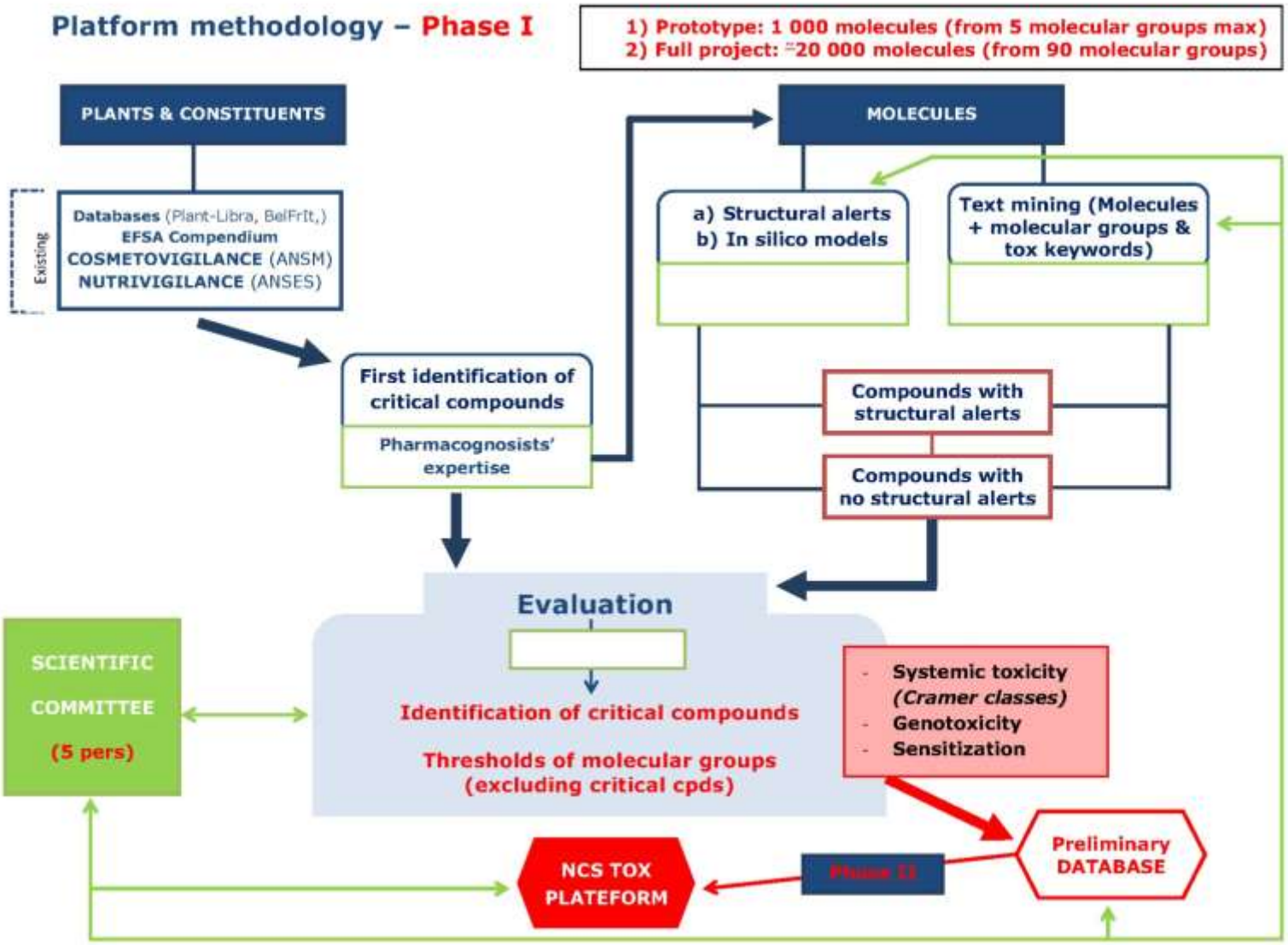
  The final Database and related Guidelines resulting from the UNITIS NCS TOX Project will be made accessible to all interested parties:

  - **Entities having actively participated to the development of the project through a preferential symbolic access fee only dedicated to maintenance of the database, with the aim to compensate partially their involvement and investment.**

  - **Other entities in return of a standard consultation fee dedicated to cover the cost of management and updating of the database.**

During phase I, the use of the database will be restricted to the companies which have financed this phase.

THE PROJECT : first phase



The Project: why participating?

- Extract users: for obtaining the necessary data for establishing the safety profiles required in annex 1
- Savings on the budget for toxicity testing
- Maintain the capacity to innovate with new extracts (even of low volumes) without excessive costs for toxicity testing
- Allows to anticipate requests from EU authorities concerning safety profiles before finalization of the database
- Improve the company image: participation to a Research programme for assessing the safety of plant extracts

Globally: excellent return on investment

+ savings on tox budget!

The project: key assets

The Unitis NCS TOX project enables to:

- **offer an alternative approach to the animal testing ban and cope with the insufficiency of validated alternative methods for key endpoints**
- **improve the knowledge of NCS**
- **continue to develop new products based on plant extracts**
- **reformulate existing products while improving their safety profile**
- **reduce the toxicity studies budget of companies**
- **answer to consumer looking for natural products**
- **improve consumer safety**


תודה
Dankie
Gracias
Спасибо
شكراً
Merci
Takk
Köszönjük
Terima kasih
Grazie
Dziękujemy
Dėkojame
Ďakujeme
Vielen Dank
Paldies
Kiitos
Täname teid
谢谢
Thank You
Tak
感謝您
Obrigado
Teşekkür Ederiz
감사합니다
Σας ευχαριστούμε
ขอบคุณ
Bedankt
Děkujeme vám
ありがとうございます
Tack