Soliance - Pongamia Extract
Natural protection against UV radiation

Givaudan

engage your senses

Summary

Sun care skin damages

Skin is subject to aging. It may be chronological or due to extrinsic factors. Both of them contribute to a loss of homeostasis and lead to physiological dysfunctions. The most upper layer of skin, the epidermis, is naturally more exposed to environment as UV, that causes irreversible damages.

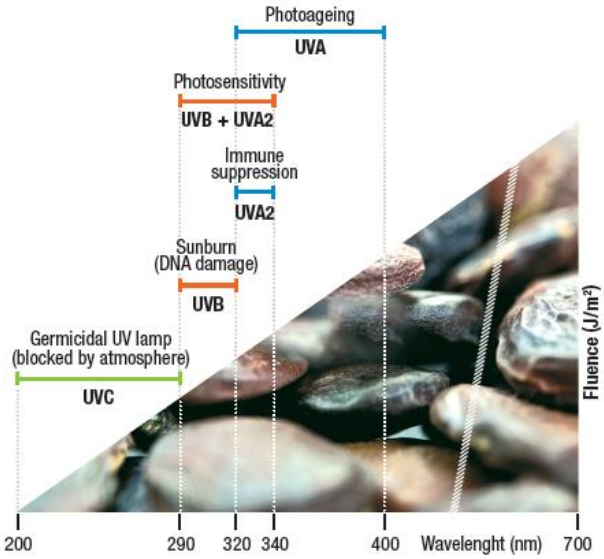
Three types of UV exist; they are classified according to their wavelength.

Depending on the type of UV, different reactions are observed.

UVA, the longer ones, represents 95% of the UV radiation reaching the skin surface. It contributes to skin ageing, wrinkling and may also enhance the development of skin cancers.

Medium-wavelengths: UVB acts on superficial skin layers. They are responsible for skin ageing and can induce skin cancer.

The short UV radiation: UVC are the most dangerous one, but as they are filtered by our atmosphere, they are less able to reach the skin.



Mechanism of action

Pongamia Oil is a:

- Powerful natural antioxidant for down regulation of stress proteins
- Natural and stable UVA absorber
- Skin matrix proteins UV damages protector

It contains 90% of Pongamol.

Production process

Pongamia Glabra seeds expressed → Seed oil solvent extraction → Crude extract crystallization → Crude Pongamol solvent washed & dried → **Pongamol**

Tests

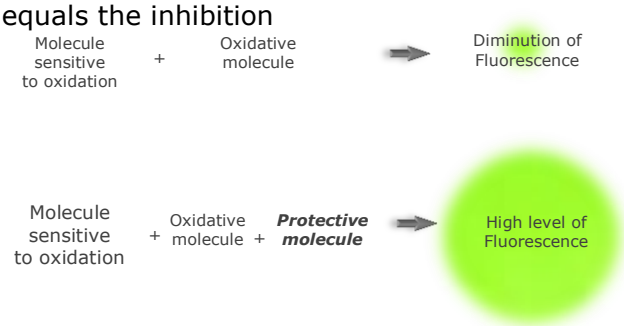
Antioxidant power
In tubo test

Oxidative stress is a physiological condition where there is an imbalance between concentration of ROS and antioxidants. However, excessive ROS accumulation will lead to cellular injury, such as damage to DNA, proteins, and lipid membranes. The cellular damage caused by ROS has been implicated in the development of many disease states.

Under normal physiological conditions, cellular ROS generation is counteracted by the action of cellular antioxidant enzymes and other redox molecules. Because of their potential harmful effects, excessive ROS must be promptly eliminated from the cells by this variety of antioxidant defense mechanisms. Antioxidants include both hydrophilic and lipophilic molecules for metabolizing ROS. Although the products of ROS are extensively used to monitor the effects of oxidative stress, it is also important to evaluate the antioxidant capacity of biological fluids, cells, and extracts. In order to measure the antioxidant power of Pongamia Extract, we performed an Oxygen Radical Absorbance Capacity (ORAC) test.

Protocol

The ORAC Activity Assay is based on the oxidation of a fluorescent probe by peroxyl radicals by way of a hydrogen atom transfer (HAT) process. Peroxyl radicals are produced by a free radical initiator, which quenches the fluorescent probe over time. Antioxidants present in the assay work to block the peroxyl radical oxidation of the fluorescent probe until the antioxidant activity in the sample is depleted. The remaining peroxyl radicals destroy the fluorescence of the fluorescent probe. This assay continues until completion, which means the antioxidant's inhibition time equals the inhibition



percentage of free radical damage. The sample antioxidant capacity correlates with the fluorescence decay curve, which is usually represented as the area under the curve (AUC). The AUC is used to quantify the total peroxyl radical antioxidant activity in a sample and is compared to an antioxidant standard curve of synthetic molecules:

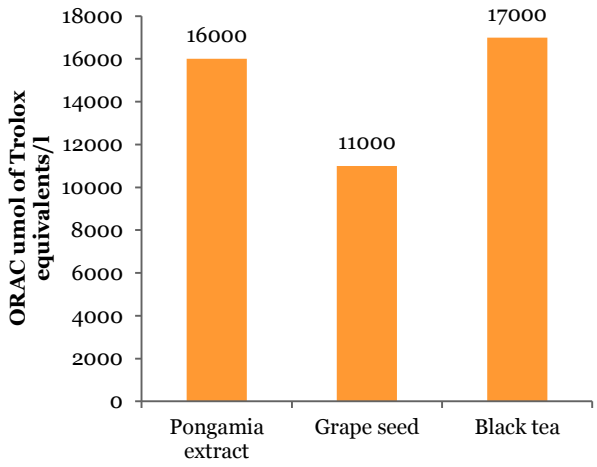
The AUC can be calculated from the equation below:

$$AUC = 1 + RFU1/RFU0 + RFU2/RFU0 + ...+ RFU60/RFU0$$

RFU0 = relative fluorescence value of time point zero
RFUx = relative fluorescence value of time points (eg. RFU5 is relative fluorescence value at minute five)

Figure 1: Measurement of the oxidative degradation of a fluorescent after being mixed with free radical generators.

Results



Pongamia extract is a powerful natural antioxidant.

Anti-oxidant activity after UV
Ex vivo test

The aim of this study is to evaluate the anti-oxidant and anti-free radical activities of a Pongamia Extract after UV irradiation of human living skin explants.

Explants coming from a 47-year-old Caucasian woman were prepared.
Pongamia extract was dissolved as a final concentration of 1% or 2% in nut oil excipient and topically applied at the surface of the explant at D0, D2, D3, D6 and D7.
On day 7, explants were irradiated using 18 J/cm2 of UVA for 4 hours. Non irradiated ones were kept in the dark during the irradiation.
4 groups of explants have been established:

Group	Number of explants	UV
Control D0	3	-
Control D7	4	-
Nut oil	4	-
PE 1%	4	-
PE 2%	4	-
Control D7	4	+
Nut oil	4	+
PE 1%	4	+
PE 2%	4	+

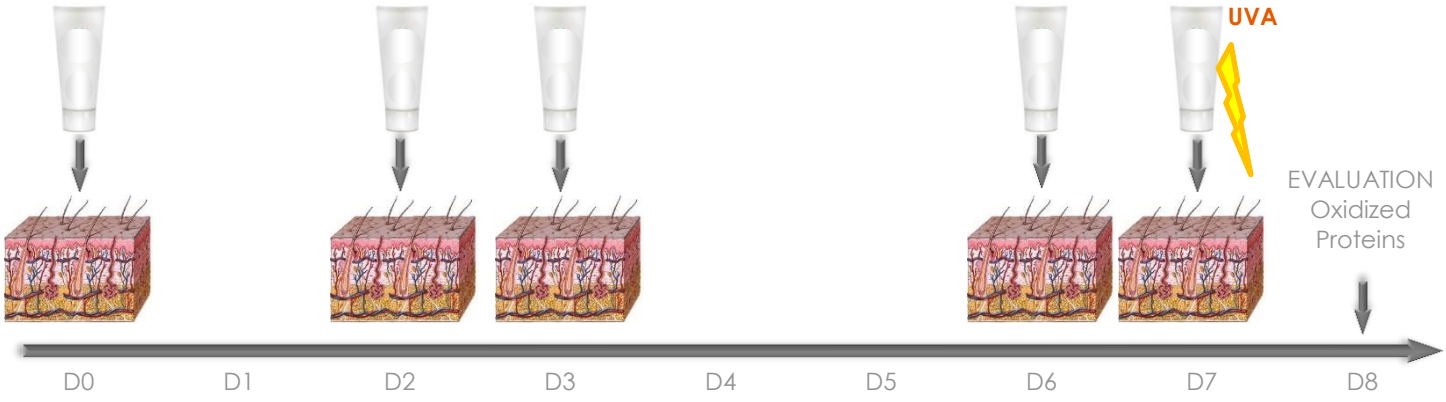
Four parameters have been analyzed:

General morphology
The observation of the general morphology was performed after staining of paraffinized sections according to Masson's trichrome, Goldner variant.

Immunostaining of oxidized proteins
The immunostaining of oxidized proteins was made using the OxyBlotTM protein oxidation kit allowing the immunoblot detection of carbonyl groups introduced into proteins by oxidative reactions with ozone or oxides of nitrogen or by metal catalyzed oxidation. The immunostaining was assessed by an image analysis.

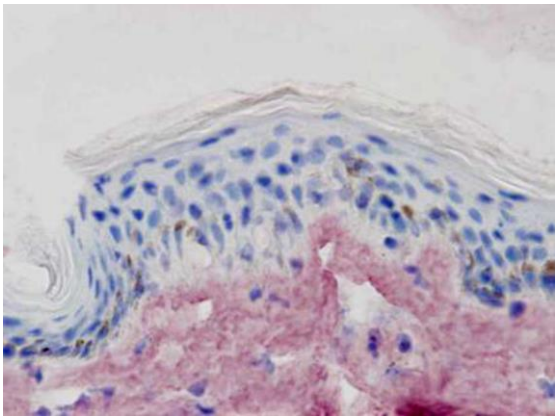
Immunostaining of OXSR1 (Oxidative stress responsive-1)
OXSR1 is a protein of 527 amino acids. It is classified as serin/threonine kinase, and it is overexpressed following oxidative environmental stress including osmotic shock or UV exposure. OXSR-1 activate by phosphorylation of Na+/K+/2Cl-, which in turn are implicated in the maintenance of cellular homeostasis.

The immunostaining of OXSR1 was performed on paraffinized sections with anti-OXSR1 antibody; the staining was enhanced with a streptavidin/biotin system and revealed using peroxydase substrat. The immunostaining was assessed by microscopical observation.

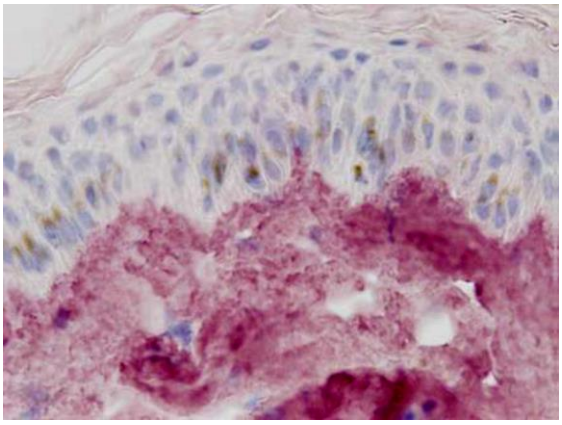


Results

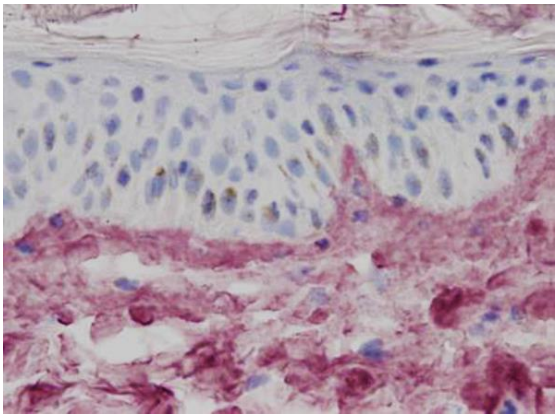
General morphology



Control on D0



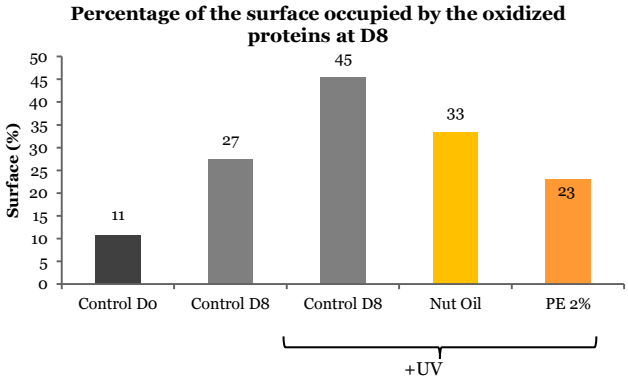
UV-irradiated untreated batch on D8



UV-irradiated treated explant with Pongamia Extract at 2% on D8

2% of Pongamia Extract induces a slight protective activity of the general morphology with a slight decrease of the spongiosis.

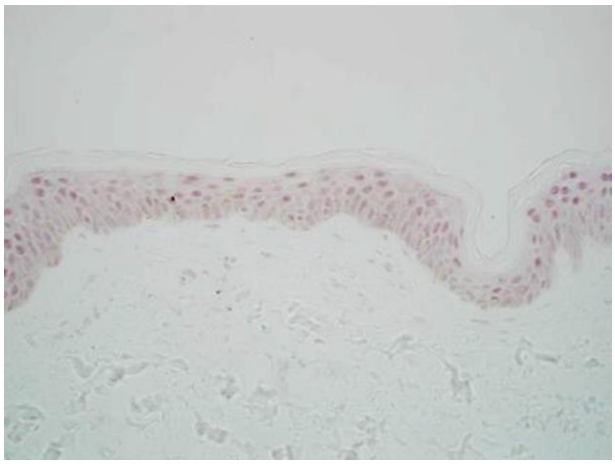
Immunostaining of oxidized proteins



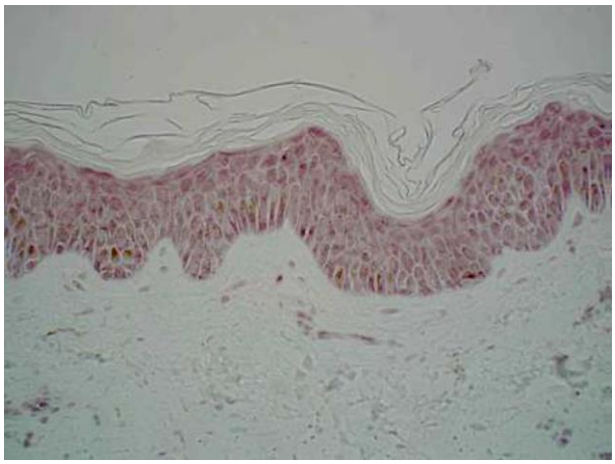
On D8, the UV irradiation induces a significant increase of 66%** of the oxidized proteins in the papillary dermis compared to the untreated control D8.

2% of Pongamia Extract induces a significant decrease of 31%* of the oxidized proteins.

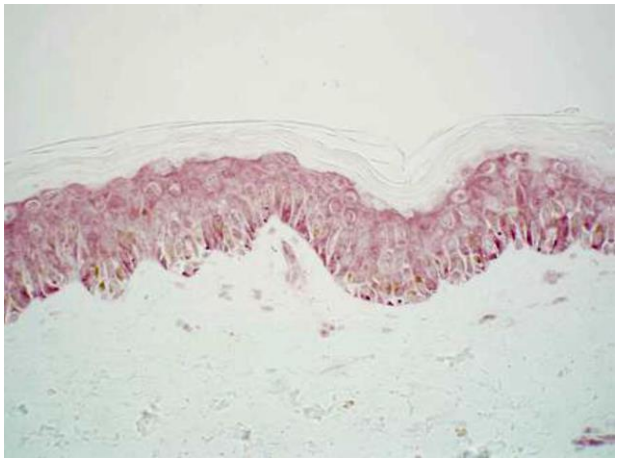
Immunostaining of OXSR1



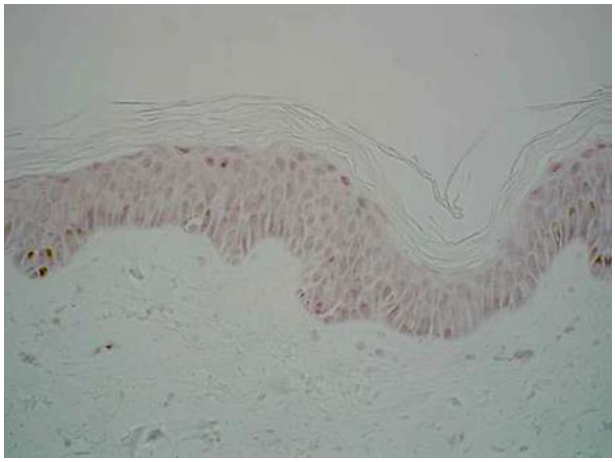
Control D0



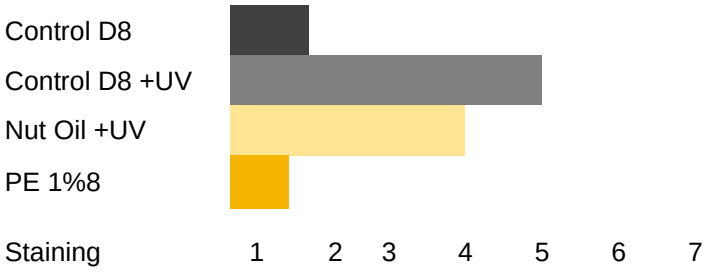
Nut Oil after UV-irradiation on D8



Control after UV irradiation on D8



Pongamia Extract 1% after UV-irradiation on D8

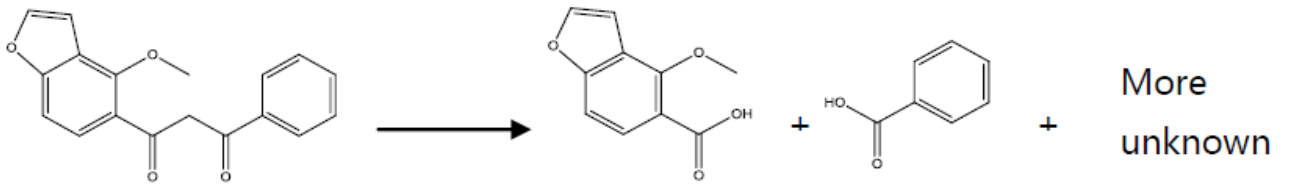


The UV irradiation induces a strong increase of OXSR-1 expression in the epidermis.

The Pongamia extract CQ U/A at 1% showed a very good activity, by inducing a clear decrease of OXSR1 expression following UV exposure.

Photo stability of Pongamol under UVA exposure
In tubo test

An alcoholic solution of Pongamia Extract QC (95% Pongamol) was exposed during 72h to UVA at 0.420 mW/cm². The percentage of degradation of Pongamol was measured by LC-UV and the degradation products were analyzed by LC-MS/MS.



Pongamol

4-methoxy-benzofuran-5-carboxylic acid *(tentative structure assigment)*

Benzoic acid *(confirmed by reference sample)*

After 72h of UV exposure, a degradation of only 22.9% was measured and the following degradation products were postulated.

Characterists

Pongamia extract INCI name is Pongamia Glabra Seed Oil

The recommended dosage is 1 to 2%

Cosmetic uses

Pongamia extract could be used in every sun care products to limit cell damage.

Conclusion

Pongamia Extract offers a total anti-ageing protection for the skin.
It contains an unique molecule; Pongamol, purified at more than 90% from Karanja Oil. Besides its well-known natural UVA absorbing effect, Pongamia Extract is a powerful natural antioxidant pushing down regulation of stress proteins when skin is submitted to environmental stress. Pongamia Extract works as a shield ingredient protecting skin matrix proteins from UV damages and all external aggressions.