A MULTI-FUNCTIONAL AND ECOFRIENDLY INGREDIENT TO FIGHT AGAINST PHOTO-AGING

ID #448

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INTRODUCTION

Sunrays may cause photo-aging which consists in biological, and clinical skin alterations [1]. Ultra-violet (UV) traditional filters and chemicals are widely used in cosmetic products as a preventive protection. However, they tend to have side effects on health and their impact on environment is now condemned.

In order to further protect the skin from photoaging, and for answering the market's need for natural, eco-friendly cosmetic products, Exsymol designed an original plant extract with an accurately defined content in photoprotective metabolites and antioxidants.

Entada phaseoloides is a widespread plant found in tropical regions (Asia, Southern Africa, Australia, …). It has been used for long in folk medicine, and multiple medicinal properties have been reported, including anti-inflammation, analgesic activity, anti-pyretic, anti-arthritis, anti-diabetic, … [2]. It produces huge seed pods holding large disk-shaped seeds (Figure 1A) that are especially rich in bioactive constituents such as entadamide A, phaseoloidin and saponins.

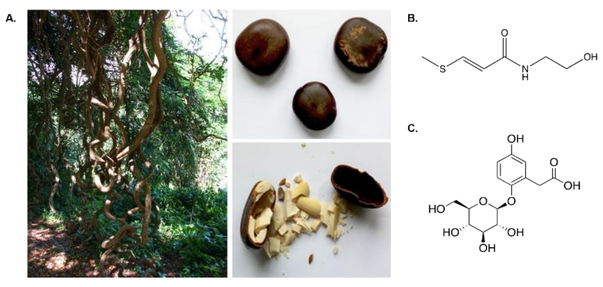

Figure 1 – ESPE is an optimized extract rich in bioactive molecules. *(A) Pictures of Entada phaseoloides and its seeds. Molecular structure of entadamide A (B) and phaseoloidin (C).*

Entadamide A (Figure 1B) is a methylthiopropenoic acid conjugates (MTPC) that readily absorbs UVB and releases the absorbed photons energy by a "non-sacrificial" absorption mechanism of *trans* to/from *cis* isomerisation (Figure 5A). This accounts for entadamide A high UVB-absorbing efficacy (ε(290nm) = 9900 L/mol/cm), and also avoids photo-degradation and formation of potentially harmful by-products.

The seeds contain several antioxidant phenolic compounds [3], and especially phaseoloidin (Figure 1C), a stable phenolic glycoside that is by far the most abundant (>10% of the seeds' dry mass).

Several constituents of the seeds, including saponins, have proven anti-inflammatory activities [4].

All these constituents may provide benefits that are key for fighting photoaging.

A patented extraction process based on endogen hydrolases (glycosidases) was developed in order to maximize the concentration and the stability of the aforementioned molecules of interest in our *Entada phaseoloides* seeds extract or EPSE (publication No WO2019197548) [5].

As a result, EPSE contains more than 0.25% of entadamide A, 3% of phaseoloidin and 1.5-2.0% of saponins.

Here, we present the efficacy of an original, and patented extract obtained from the seeds of *Entada phaseoloides* (EPSE) to protect the skin from the noxious effects of sunrays and artificial irradiations. To that end, we evaluated the impact of UV and blue light exposure in the presence or in the absence of EPSE on several key features, namely genotoxicity, pro-inflammation, immunosuppression and melanogenesis.

RESULTS

EPSE prevents UVB-induced DNA mutations and cell death

UV cause direct and ROS-mediated indirect DNA damages.

In order to assess the ability of EPSE to prevent DNA alteration and the resulting cell death, reconstructed human epidermis (RHE) were exposed to UVB in the presence or in the absence of EPSE (applied during irradiation for assessing the direct DNA alterations, and for an additional 5 hours for assessing the indirect DNA alterations).

The number of resulting cyclobutane pyrimidine dimers (CPD) was measured and the presence of sunburn cells (SBC) with pyknotic nuclei and the morphological aspect of the RHE was assessed by histological analysis.

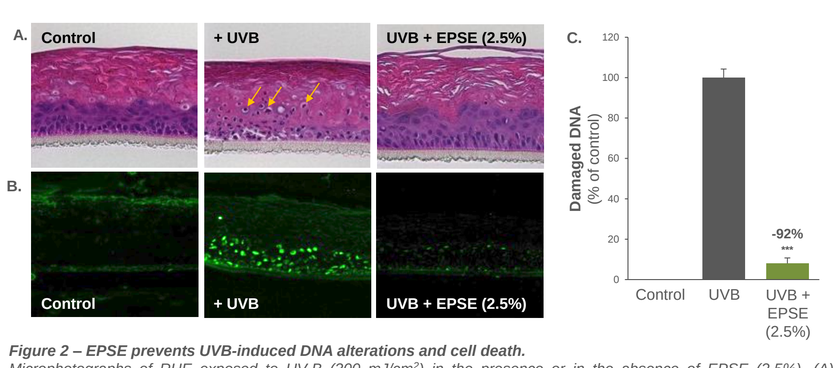

Figure 2 – EPSE prevents UVB-induced DNA alterations and cell death. *Microphotographs of RHE exposed to UV-B (300 mJ/cm²) in the presence or in the absence of EPSE (2.5%). (A) Morphological analysis. Sunburn cells are highlighted by the yellow arrows. (B) CPD were observed (in green) and (C) quantified.*

After UVB exposure, RHE demonstrated a damaged morphology compared to control condition. Keratinocytes at the basal layers showed an impaired cohesion with the acetate cellulose filters. In the upper cell layers the keratinocytes presented vacuoles and SBC (Figure 2A).

The treatment with EPSE (2.5%) applied only during irradiation led to a 92% reduction of the number of UVB-induced DNA damages (Figure 2B, 2C).

Taken together, these results confirm that the active ingredient reduces cell death by preventing DNA damage in UVB-irradiated RHE.

EPSE protects the skin from the "dark sun"

Both UVA and UVB produce reactive oxygen and nitrogen species (ROS and RNS respectively), which together create a higher-energy form of melanin (chemoexcitation process). This new form of melanin contains the energy of a UV photon that is later transfered to DNA (chemiluminescence process) and may cause a delayed mutation, a "dark CPD" [6].

Since EPSE have antioxidant and chromophore abilities, we assessed its ability to protect skin cells from the "dark sun" that may cause melanin-induced delayed DNA alterations, the "dark CPD". Melanocytes were thus exposed to solar irradiation (1.8 J/cm²) and EPSE was later applied for 2 h. The relative number of "dark CPD" was then quantified.

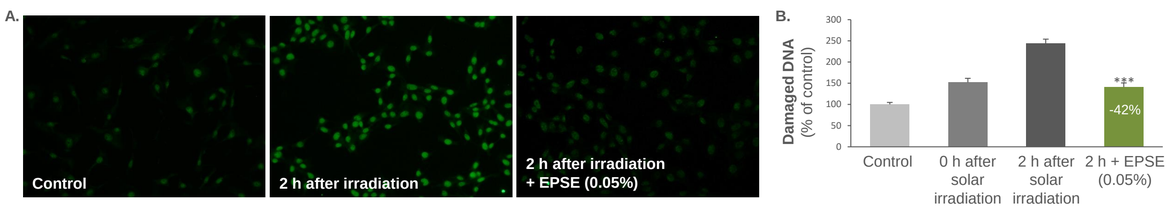

Figure 3 - EPSE prevents the apparition of Dark CPD.
Microphotographs of melanocytes exposed to sun light and then treated with ESPE for 2 h. Dark CPD were observed (A, in green) and quantified (B).

2 h after sun light exposure, a dramatic increase in the number of CPD was observed. The treatment with EPSE (0.05%) led to a 42% decrease in the number of CPD after 2 h of treatment (Figure 3A, 3B).

EPSE protects skin cells from the dark sun as it reduces melanin-induced delayed DNA alterations.

EPSE has anti-inflammatory properties

Exposure to sunlight and especially UVB may cause an inflammatory response. Also known as erythema or sunburn, this reaction is characterized by redness, skin dehydration, painful oversensitivity, desquamation… [7].

In order to assess EPSE ability to reduce UV-induced inflammation, RHE were exposed to UVB (300 mJ/cm²) in the presence or in the absence of EPSE (1.5%). The secretion of several proinflammatory cytokines (IL-6, IL-8 and TNF-α) was measured using ELISA assays.

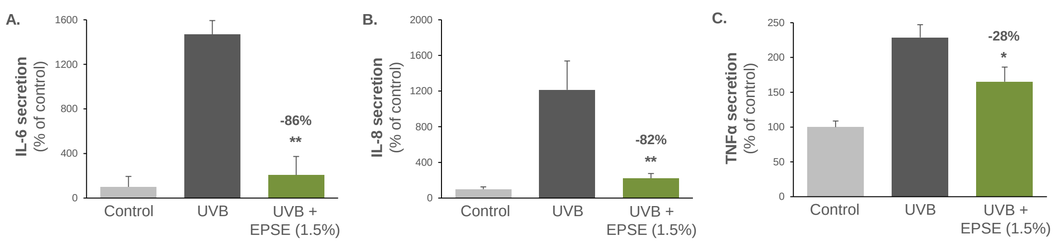

Figure 4 – EPSE has anti inflammatory properties.
Quantification of IL-6 (A), IL-8 (B) and TNFα (C) secreted by RHE exposed to UVB (300 mJ/cm²) in the presence or in the absence of ESPE (1.5%).

The treatment with EPSE (1.5%) decreases UVB-induced secretion of IL-6 by 86% hence completely negating the noxious effect of the UVB exposure (Figure 4A).

The treatment of RHE with EPSE (1.5%) led to a 82% and 28% decrease in the UV-induced secretion of IL-8 and TNF-α respectively (Figure 4B, 4C).

Taken together, these results show that a topical treatment with EPSE led to a strong anti-inflammatory response after an UV exposure.

EPSE prevents UV-induced immunosuppression

Several UV-induced mechanisms such as DNA damages, the release of pro-inflammatory cytokines and the isomerization of *trans*-urocanic acid (UCA) into *cis*-UCA (Figure 5A), have all been reported to cause an immunosuppression in skin [8-10].

As a result, we investigated EPSE's anti-immunosuppressive capacities.

EPSE is rich in entadamide A that is capable of absorbing UV in the same wavelength as trans-UCA (284 nm, Figure 5B) thus suggesting that EPSE could reduce the UV-induced isomerization of UCA. So, in order to assess this feature, *trans*-UCA was exposed to UVB in the presence or in the absence of EPSE. The formation of the immunosuppressive *cis*-UCA was monitored by HPLC.

EPSE limits UV-induced *cis*-UCA formation, indicating that UVB absorption by entadamide A partially avoids *trans*-UCA photo-isomerization (Figure 5C). Thus, EPSE can limit UVB-induced immunosuppression.

Galectin-7 is involved in UV-induced immunosuppression [9]. So, in order to assess the ability of EPSE to prevent the UV-induced galectin-7 overexpression, RHE were exposed to UVB (300 mJ/cm²) in the presence or in the absence of EPSE and galectin-7 secretion was measured using an ELISA assay.

The topical treatment with EPSE (2.5%) led to a strong decrease (-90%) of galectin-7 expression (Figure 5D).

This observation is consistent with the previous result and, taken together, they show that EPSE is capable of preventing UV-induced immunosuppression.

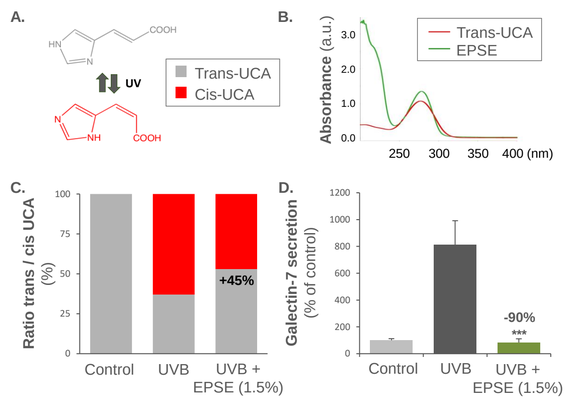

Figure 5 – EPSE has an anti-immunosuppressive activity

(A) UV-induced isomerization of trans-UCA into the immunosuppressive cis-UCA. (B) Ultraviolet absorption spectra of EPSE and trans-UCA. (C) Ratio of trans / cis UCA after an exposure to UVB (300 mJ/cm²) in the presence or in the absence of EPSE (1.5%). (D) Quantification of galectin-7 secretion by RHE exposed to UVB in the presence or in the absence of EPSE (1.5%).

EPSE decreases melanin production

While UCA and melanin are the main defensive mechanisms of the skin against UV, a sustained exposure to sunrays, chronic inflammation (inflamm'aging), oxidative stress, and/or hormonal changes may still trigger local melanin overproduction, resulting in non aesthetic hyperpigmentary disorders, such as melasma, lentigines, dark circles or post-inflammatory hyperpigmentation [10].

The most widely used melanogenesis inhibitors (kojic acid, ascorbic acid and arbutin…) are inhibitors of tyrosinase, an enzyme that catalyzes the conversion of tyrosine to L-DOPA and further oxidizes it to dopaquinone, which is used for the ultimate formation of melanin. It was recently reported that entadamides, and especially entadamide A, show inhibitory activity for melanin production at the same level as arbutin [11].

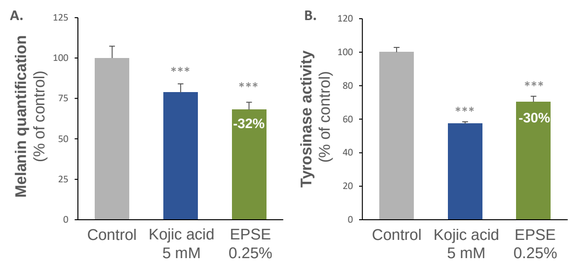

Figure 6 – EPSE reduces melanin production by inhibiting tyrosinase activity. *Quantification of melanin production (A) and tyrosinase activity (B) in melanocytes treated with EPSE.*

So, in order to assess EPSE ability to inhibit melanogenesis, melanocytes received a 48 h treatment with EPSE (0.25%). Melanin production (Figure 6A) and tyrosinase activity (Figure 6B) were measured by spectrophotometry at 405 nm and 475 nm respectively.

The treatment with EPSE at 0.25% led to a drop in both the amount of melanin synthesized by melanocytes and tyrosinase activity with a 32% and 30% reduction respectively.

Taken together, these results suggest that EPSE is capable of reducing melanogenesis by inhibiting tyrosinase activity. Both observations support the use of EPSE to reduce skin colour (claims, skin brightener, skin lightener, skin tone unifier or skin whitening) or pigmentary disorders related to photo-ageing such as lentigines or pigmentary dark circles.

EPSE protects the skin from blue light

The visible spectrum of the light is also involved in the photoaging process. This is especially true for the blue light (that has the shortest wavelength of the visible spectrum and is therefore the most energetic) and which was described to promote matrix degradation by stimulating MMP-1 production [12]. Furthermore, the question of domestic artificial light participation to the aging process was raised.

In order to assess the ability of EPSE to protect the skin against artificial light, NHDF were exposed to blue light (30 J/cm²) and incubated for 24 h in the presence or in the absence of EPSE (0.05%). MMP-1 expression was then quantified using ELISA assay.

Blue light exposure led to a 2 folds increase in MMP-1 expression. The treatment with EPSE led to a 79% decrease in MMP-1 expression thus completely negating the effect of the blue light exposure (Figure 7).

This result suggests that EPSE is also capable of protecting the skin from an exposure to blue light, and more broadly from visible light.

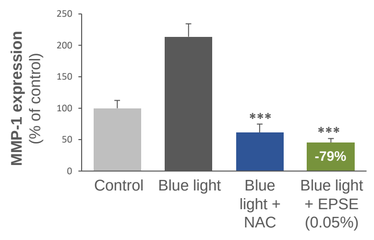

Figure 7 – EPSE protects skin from blue light. *Quantification of MMP-1 secreted by fibroblasts exposed to blue light in the presence or in the absence of EPSE (0.05%).*

DISCUSSION & CONCLUSION

Our *in vitro* and *ex vivo* experiments have shown that EPSE, an extract obtained from *Entada phaseoloides* seeds, is effective against a large range of wavelengths (UVB, UVA, visible light). EPSE very efficiently limits UV-induced DNA damages, prevents the overexpression of key inflammatory cytokines (IL-6, IL-8…) and the setup of immunosuppressive mechanisms. Our mechanistic studies indicate that entadamide A, a specific UVB chromophore present in EPSE, is an important contributor to the photoprotective properties of the extract. Entadamide A is especially effective against photo-immunosuppression as it inhibits the isomerization of *trans*-UCA to the immunosuppressive *cis*-UCA. However, other constituents of EPSE significantly participate to its photoprotective effect. Antioxidant polyphenols such as phaseoloidin efficiently scavenge ROS generated by UV and visible light, and anti-inflammatory constituents such as saponins contribute to reducing the formation of downstream inflammatory mediators. The combination of three mechanisms: antioxidation and anti-inflammation, enables a broad-spectrum photoprotection and supports the usefulness of EPSE for sun care or photoaging prevention. Furthermore, EPSE is also capable of inhibiting melanogenesis and may therefore prevent the apparition of melanin spots responsible for an uneven skin tone.

Taken together, the data presented here suggest that EPSE is an interesting active ingredient for any sun care products, by itself or in combination with sun filters, as it provides the skin with a broad-spectrum protection and prevent the apparition of melanin spots for a protected and even skin.

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