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Seaberry (*Hippophae rhamnoides L.*) and Water Lily (*Nymphaeaceae*) Extracts Protect Human Skin against Blue Light, Environmental Pollutants and UV-A Irradiations in an *Ex Vivo* Model System

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Abstract

Background: The skin is the outer shell of the mammalian body and it is continuously exposed to a large spectrum of external stimuli including sun irradiation and atmospheric pollutants. These factors present deleterious effects on the cutaneous compartment by altering the skin barrier functions and accelerating the aging of the skin. **Objectives:** The goal of this study was to investigate the activity of Seaberry and Water Lily extracts, here called Dermina complex, against different external stresses. **Methods:** Human skin explants were exposed to different stimuli including delipidation by organic solvents, blue light, atmospheric pollutants and UV-A. The activity of the Seaberry and Water Lily extracts was assessed by immunohistochemistry and by biochemical assays. **Results**: We showed that Dermina complex prevents the delipidation-induced filaggrin decrease, suggesting that these plant extracts exhibited barrier function protecting properties. Also, we observed that Dermina complex showed an antioxidant and DNA protection activity by decreasing the activated form of Nrf2, the oxidized proteins and the formation of γ-H2AX induced upon stress conditions. The Dermina complex also decreased the pro-inflammatory cytokine IL-1 alpha released in the culture medium following atmospheric pollutants and UV-A exposure confirming its anti-inflammatory activity. Moreover, Dermina complex reduced the blue light-induced overexpression of opsin 3, indicating that its skin protection activities may be due, in part, to filter property

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against environmental stresses. **Conclusions:** Dermina complex shows a protective activity of the skin against different environmental stresses and these extracts may be able to slow down the aging process of the cutaneous compartment.

Keywords

Exposome, Environmental Pollution, Blue Light, Oxidative Stress, Plant Extracts, Skin Explants

1. Introduction

The human exposome is defined as the totality of stresses to which our body is exposed during its entire lifetime [1].

Since the skin is the outermost organ of human body and interacts daily with the environment, the skin exposome affects significantly human well-being [2].

If the effects of UV irradiations on the skin have been thoroughly analyzed so far the effects of air pollution on the skin have become a central subject of investigation of the worldwide scientific community more recently [3].

Environmental pollution is nowadays considered as one of the most important factors that impact skin physiology and integrity, and the chronic exposure of skin to atmospheric pollutants can impair cutaneous homeostasis leading to skin barrier dysfunctions. In fact, as described by a work realized by Rubio and collaborators, it has been demonstrated that atmospheric pollutants promote the phosphorylation and the subsequent activation of aryl hydrocarbon receptor (AhR) and nuclear factor erythroid-2-related factor 2 (Nrf2) specific pathways in human skin explants [4]. In parallel, in a work realized by Soeur and collaborators, it has been demonstrated that UV-A irradiations increase the cytotoxicity of PAHs and particulate matter in human keratinocytes and reconstructed epidermis [5]. Interestingly, the chronic exposure to urban particulate matter can decrease filaggrin expression through an increase of cyclooxygenase-2 (COX-2) expression and an increase and prostaglandin E2 (PGE2) production, leading to impairment of skin barrier function and increasing the penetration of other exogenous substances [6]. Indeed, urban particulate matter can penetrate easily through hair follicles *in vivo* and increase IL-8 and MMP-1 expression, causing inflammation and accelerated skin aging [7]. The impairment of skin barrier integrity could also be provoked by the pollutants-induced reactive oxygen species (ROS). ROS generation provokes the peroxidation of the highly unsaturated fatty acids and lipids of the *stratum corneum* thus modifying the permeability of the skin barrier [8].

The chronic action of the ROS, generated by the exposure to pollutants, may also cause the depletion of antioxidant enzymes in the epidermis, including catalase and superoxide dismutase. The content of molecules with antioxidant ac-

tivity such as ascorbic acid and *α*-tocopherol [9] can be also negatively affected by ROS.

Very recently, the detrimental effects of air pollutants on the dermis compartment have been also investigated [10]. Park and collaborators have shown that human dermal fibroblasts exposed for 24 hours to 10 μm diameter-particulate matter (PM_{10}) exhibit impaired collagen synthesis and increased levels of inflammatory markers [10].

As indicated previously, the effects of UV on the skin have been extensively investigated and very recently different studies analyzed the effects of visible light (390 - 700 nm), in particularly those related to the blue light (380 - 490 nm), on skin physiology.

The screens of computers, electronic notebooks and smartphone emit an important portion of blue light and if we consider that nowadays people spent several hours staring at computer screens, blue light irradiation may impact profoundly on skin biology and physiology. It has been shown that blue light is able to induce oxidative stress in the skin by inducing the formation of ROS [11]. Moreover, blue light can induce hyperpigmentation of the skin through opsin 3 activation in melanocytes suggesting that blue light could participate in the development of pigmentary disorders [12].

In this context the development and the commercialization of dermocosmetics able to protect human skin against the damaging effects of the exposome, including environmental pollution and blue light irradiation, have increased dramatically during recent years [8] [13].

Seaberry (*Hippophae rhamnoides L.*) plant is a fruiting shrub largely widespread in China, Russia, Mongolia, Canada and northern Europe.

It has been shown that seaberry extract presents a strong anti-oxidant activity, mainly due to the high content of flavonoids and vitamin C [14].

Several potential applications of seaberry extracts have been documented, including cancer therapy [15] [16] and treatment of cardiac disorders [17] [18].

In the dermo-cosmetic field, it has been shown that seaberry extracts may be used to treat some inflammatory skin diseases, including atopic dermatitis [19] or to promote the regeneration of the skin tissue [20].

Water Lily (*Nymphaeaceae*) is a flowering aquatic plant growing in tropical climates all over the world. The extracts of these plants are rich several in phytochemical-like saponins, alkaloids, polyphenolics and carbohydrates. Several works have demonstrated the anti-oxidant [21] [22], immunomodulatory [23] [24], anti-inflammatory [25] [26] and skin anti-aging properties [27] [28] of water lily extracts *in vitro* and *in vivo* models.

The aim of this project was to investigate for the first time the skin barrier protection, anti-pollution and anti-blue light activity of a cosmetic compound, composed of Seaberry (*Hippophae rhamnoides L.*) and Water Lily (*Nymphaeaceae*) extracts, in an *ex vivo* skin explant model.

2. Material and Methods

2.1. Preparation of "Dermina Seaberry and Water Lily Extract Complex"

The "Dermina Seaberry and Water lily extract complex", also called "Dermina complex" was prepared by Inderma laboratory.

Briefly, Seaberry (*Hippophae rhamnoides L.*) extract was prepared from dried leafy branches in order to obtain a 20% (wt/v) solution of Seaberry extract in 35% malic acid (Sigma Aldrich, Saint Quentin Fallavier, France), 60% propanediol (Merck KGaA, Darmstadt, Germany) and 5% distilled water. The solution was successively filtered through a sieve with a 150-micron mesh (VWR International, Fontenay-sous-Bois, France) and referenced as "seaberry NaDES AZE solution".

Water lily extracts were prepared from dried flowers and roots as following:

1) Dried flowers (extract A) were incubated for 24 hours in hot water (40˚C to 60˚C) in order to obtain a 20% (wt/v) solution. After filtration through a 150-micron mesh, the solution was concentrated in an evaporator and dissolved in 80% glycerin (Sigma, Sigma Aldrich, Saint Quentin Fallavier, France).

2) 20% (wt/v) of dried roots (extract B) were incubated for 24 hours at 50˚C - 60˚C in a solution containing 58% glycerin (Sigma, Sigma Aldrich, Saint Quentin Fallavier, France), 36% betain (Sigma, Sigma Aldrich, Saint Quentin Fallavier, France) and 6% distilled water. The solution was successively filtered through a sieve with a 150-micron mesh (VWR International, Fontenay-sous-Bois, France).

The extracts a and b were mixed in order to obtain a 1:1 (v/v) solution of Water lily extracts referenced as "Water lily roots NaDES BGE and flower GL 80 solution".

The Seaberry and Water lily extract complex was prepared by mixing 33.3% of the Seaberry NaDES AZE solution and 66.6% of the Water lily roots NaDES BGE and flower GL 80 solution.

Polyphenols were extracted from both Seaberry and Water Lily extracts using a solution composed by phosphoric acid and sodium tungstate and quantified by spectrophotometry by reading the absorbance at 710 nm and using a catechin-based standard curve.

In parallel, polyphenols were extracted by acidic hydrolyse and quantified by spectrophotometry by reading the absorbance at 540 nm and using a D-glucose-based standard curve.

The chemical composition of Saeberry and Water lily extracts is shown in Table 1.

2.2. Preparation of *Ex Vivo* Human Skin Explants

Full-thickness human skin biopsies were obtained from the abdominal area of three healthy female donors (35 - 48 years) who had undergone plastic surgery. Skin samples were collected from patients and processed as described previously

Table 1. Chemical composition of Seaberry (*Hippophae rhamnoides L.*) and Water Lily (*Nymphaeaceae*) extracts.

	Polyphenols (catechin) content (g/L)	Polysaccharides content (g/L)
Seaberry (*Hippophae rhamnoides L*)	13.8	3.1
Water Lily (*Nymphaeaceae*)	3.3	3.1

by Percoco *et al.* [29].

Briefly, circular explants of 1 cm in diameter were prepared using a biopsy punch, placed in 2 ml of BIO-EC's Explant Medium (BEM) and cultured under classical conditions (37˚C in 5% CO_2). The different treatments are shown in Supplementary data S1.

The day of the skin explants preparation has been defined as day 0 for each experiment.

2.3. Delipidation of Human Skin Samples

On day 0, the plasty from the abdomen of a 39-year-old female donor has been delipidated twice on a delimitated area using a solution of ether (Sigma Aldrich, Saint Quentin Fallavier, France)/acetone (VWR International, Fontenay-sous-Bois, France) 1:1 (v/v). The delipidation was performed by applying directly the lipid extracting solution on skin surface. After the delipidation, human skin explants have been prepared as previously described and treated with 2 mg per cm^2 of the product Dermina complex diluted at 3% in distillated water.

24 hours after the application of the product, the skin explants have been collected and processed for histological analysis (Supplementary data S1).

2.4. Exposure of Human Skin Explants to Blue Light

Skin explants from an abdoplasty of a 35-year-old female donor have been exposed on day 4 to blue light irradiation (λ_{max} = 455 nm) for 3 hours with a dose of 63.7 J/cm^2 using the SolarBox® device (BIO-EC laboratory, Longjumeau, France). In order to assess the protective activity against blue light irradiation, human skin explants have been treated topically with 2 mg per cm^2 of the product Dermina complex diluted at 3% in distillated water on day 0, day 1, day 3 and on day 4 just before the blue light exposure.

On day 4, 2 hours after the blue light irradiation, skin samples have been collected and processed for histological analysis (Supplementary data S1).

2.5. Exposure of Human Skin Explants to Environmental Pollutants and UV-A Irradiation

On day 4 using the PolluBox® exposure chamber, skin explants from an abdoplasty of a 49-year-old female donor have been exposed to a nebulized solution containing a mix of toluene (Fisher Scientific, Illkirch Cedex, France), benzene (Fisher Scientific, Illkirch Cedex, France), xylene (Fisher Scientific, Illkirch

Cedex, France), heavy metals (Merck KGaA, Darmstadt, Germany), and diesel particulate matter 1650b (Gaithersburg, MD, USA) (**Supplementary data S2**). Successively, skin explants have been exposed to 13.5 J/cm^2 of UV-A using a UV-simulator Vibert Lourmat RMX 3W (Thermofisher Scientific, Montigny Le Bretonne, France).

In order to assess the protective activity against environmental pollutants and UV-A irradiation, human skin explants have been treated topically with 2 mg per cm^2 of the product Dermina Complex diluted at 3% in distillated water on day 0, day 2, day 3 and on day 4 just before the pollutant and UV-A exposures.

24 hours after the pollutant and UV-A irradiation, skin explants and culture media have been collected for histological analysis and IL-1α biochemical assay, respectively (**Supplementary data S1**).

2.6. Microscopy

For histological analysis, one half of the skin explants were frozen at −80°C and the other half was fixed for 24 h in buffered formalin solution (VWR International, Fontenay-sous-Bois, France) and successively processed as described by Percoco *et al.* [17].

The general morphology has been assessed on formalin-fixed paraffin-embedded (FFPE) sections stained with Masson-Goldner's trichrome.

Both immunohistochemistry and immunofluorescence stainings were performed on either 5 µm-FFPE skin sections or 7 µm-frozen sections. Each primary antibody was diluted in phosphate-buffered saline (PBS) (Sigma Aldrich, Saint Quentin Fallavier, France) containing 0.3% bovine serum albumin (BSA) (Sigma Aldrich, Saint Quentin Fallavier, France), supplemented with or without 0.05% Tween® 20 (Sigma Aldrich, Saint Quentin Fallavier, France) and diluted as following: mouse monoclonal anti-filaggrin antibody at 1:2000 (sc-66192, Santa Cruz Biotechnology, Santa Cruz, CA, USA); rabbit monoclonal anti-phospho Serine-40-Nrf2 (Serine-40) antibody at 1:200 (ab6672, Abcam, Cambridge, UK); mouse monoclonal anti-γ-H2AX antibody at 1:800 (ab26350; Abcam), and anti-oxidized protein antibody at 1:250 (S7150, Merck KGaA, Darmstadt, Germany).

For immunohistochemistry, a ready-to-use biotinylated universal secondary antibody has been used (Vector Laboratories, Burlingame, California, USA), combined with a streptavidine-peroxidase amplifying system and revealed by the VIP chromogen (Vector Laboratories, Burlingame, California, USA).

Filaggrin immunostaining was revealed by immunofluorescence using an Alexa Fluor® 488-conjugated secondary antibody diluted in 0.3% BSA-PBS at 1:1000 (A11001, Thermofisher Scientific, Montigny Le Bretonne, France). Nuclei was counterstained with propidium iodide at 0.02% in PBS (Sigma Aldrich, Saint Quentin Fallavier, France).

Stained skin sections were observed with an Olympus BX43 microscope (Olympus, Rungis, France) equipped with a DP72 digital camera (Olympus, France), stored and analyzed using Cell^D software (Olympus, Rungis, France).

2.7. Image Analysis

The image analyses were performed using Cell^D software (Olympus, Rungis, France). The percentage of stained surface of the region of interest of stressed explants (delipidated, exposed to the blue light or to environmental pollutants + UV-A) was compared to the control explants. A similar comparison was realized between the stressed batches with or without the "Dermina Seaberry and Water lily extract complex" treatment in order to evaluate the protective effects of these plant extracts.

The analysis of the epidermal thickness was measured on the pictures of the general morphology. Three measurements per picture were realized (n = 27).

2.8. IL-1α Dosage

Interleukin-1α (IL-1α) titer was measured in BEM media (BIO-EC, Lonjumeau, France) on day 5, 24 hours following pollutants and UV-A exposure using the human IL-1α Elisa kit (Cayman Chemicals, Ann Arbor, Michigan, USA) according to the manufacturer's instructions.

The absorbance was measured at 412 nm using a microtiterplate reader M200Pro (Tecan Lifescience, Zurich, Switzerland) using the Magellan 7 software (Tecan Lifescience, Zurich, Switzerland).

The concentration of IL-1α released into BEM culture media was expressed as pg per ml ± standard deviation (SD).

2.9. Statistical Analysis

For the histological analysis of each procedure (delipidation, blue light irradiation and UV-A/pollutants exposure), 3 explants have been analyzed (n = 3) for each condition condition (3 explants for the "untreated control", 3 explants for the "stressed condition" and 3 explants for the condition "stress + Dermina complex").

For the image analysis nine images (3 per explants) have been processed for each condition.

For IL-1 α assay, the BEM culture media of four explants per condition have been analyzed (n = 4). Untreated skin explants served as control.

The data from each experiment were analyzed statistically by the Student's t-test. Data are expressed as mean ± SD. $p \leq 0.05$ was considered statistically significant.

* for $p < 0.05$ and ** for $p < 0.01$.

3. Results

3.1. Dermina Seaberry and Water Lily Extract Complex Restores Proper Barrier Functions after *Stratum corneum* Delipidation

In order to assess the capacity of the Dermina complex to reestablish proper barrier functions, the surface of human skin explants was treated with a mix of ether/acetone. No modifications of tissue viability under the different conditions

were observed (Figure 1).

24 hours after the delipidation the *stratum corneum* of skin explants became highly keratinized, less laminated and more compact with a significant decrease of 26% in thickness ($p < 0.01$) compared to the untreated explants (Figure 1). A strong reduction of 47% of filaggrin expression ($p < 0.01$) at the bottom of the horny layer was also observed compared to the control, suggesting an impairment of skin barrier functions (Figure 1).

When skin explants were treated topically with Dermina complex immediately after the delipidation, the *stratum corneum* appeared less keratinized, more laminated and thicker with a significant increase of 15% ($p < 0.01$) indicating a better hydration of the horny layer, similar to the one observed on the control explants (Figure 1). Furthermore, the Dermina Seaberry and Water Lily extracts complex prevented the delipidation-induced decrease of filaggrin expression. Indeed, the extracts increased significantly the expression of filaggrin by 76% compared to the delipidated explants ($p < 0.05$), almost restoring the filaggrin expression found in the control explants (Figure 1).

3.2. Dermina Seaberry and Water Lily Extract Complex Protects the Skin against Blue Light

Human skin explants were exposed to blue light for 3 hours in order to investigate the protective activity of Dermina complex against this kind of irradiation. No modifications of tissue viability under the different conditions were observed (Supplement data S3).

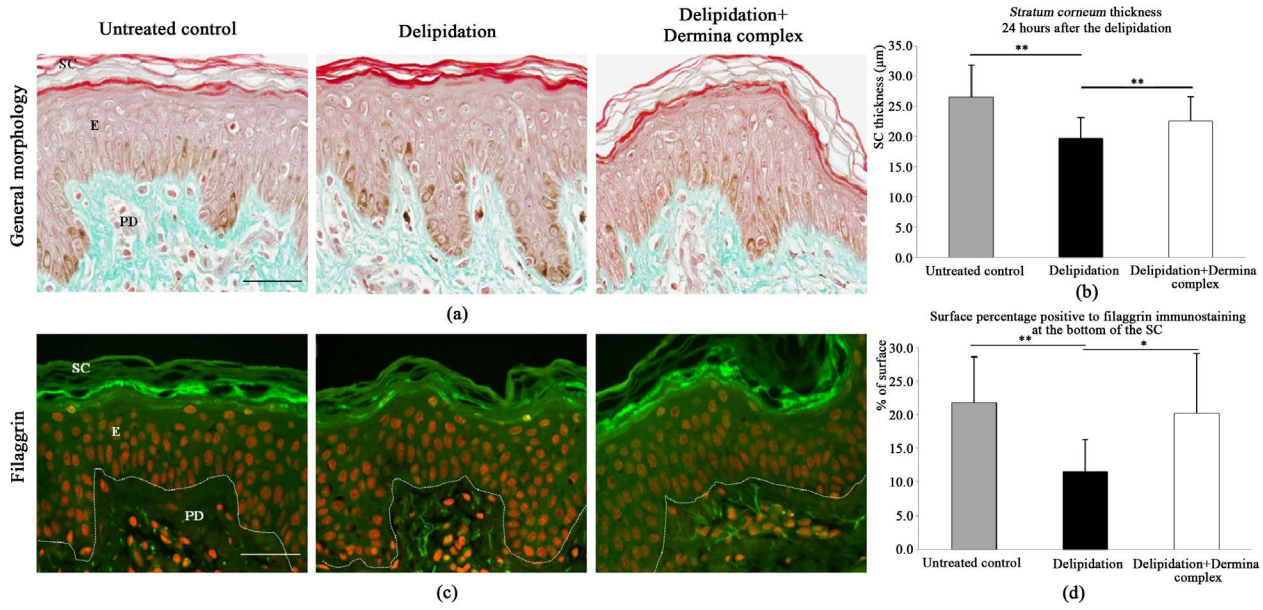

Figure 1. Dermina complex improves the aspect of the *stratum corneum* and increases filaggrin (FLG) expression following skin delipidation. In (a), analysis of the general morphology and *stratum corneum* aspect after Masson's trichrome staining of FFPE skin sections. In (b), analysis of *stratum corneum* thickness under different experimental conditions. In (c), immunolocalization of FLG in FFPE skin sections. In (d), analysis of FLG immunostained area (in %) under different conditions. Scale bar: 50 µm. Abbreviations: E, epidermis; FFPE: formalin-fixed paraffin-embedded; FLG: filaggrin; PD: papillary dermis; SC, *stratum corneum*.

2 hours after the end of blue light irradiation, a significant increase of 52% of the phosphorylated activated form on Nrf2 ($p < 0.01$) and a significant increase by 28% of opsin 3 ($p < 0.05$) expression was observed in the living epidermis compared to the control explants (Figure 2).

The preventive application of the product Dermina complex significantly reduced by 14% the activated form of Nrf2 ($p < 0.01$) and by 17% ($p < 0.05$) opsin 3 expression in the epidermis after blue light irradiation, suggesting that the product exhibited a partial protective effect against the blue light-induced oxidative stress (Figure 2).

3.3. Dermina Seaberry and Water Lily Extract Complex Defends the Skin from the Molecular Alterations Induced by Atmospheric Pollutants and UV-A Irradiation

To characterize the anti-pollution and anti-UV-A activity of Dermina complex, human skin samples were exposed to a nebulized mix of environmental pollutants through the PolluBox® exposure chamber and successively irradiated with UV-A. No modifications of tissue viability under the different conditions were observed (Supplement data S3).

We observed that the combined exposure of skin explants to the mix of environmental pollutants followed by UV-A irradiation was able to increase significantly the level of the phosphorylated and activated form of Nrf2 by 24% ($p < 0.01$) and the level of the DNA double-strand break marker γ-H2AX by 25% ($p < 0.05$) in the living epidermis, compared to the control explants. In the papillary dermis, the combined stresses could increase significantly the level of γ-H2AX

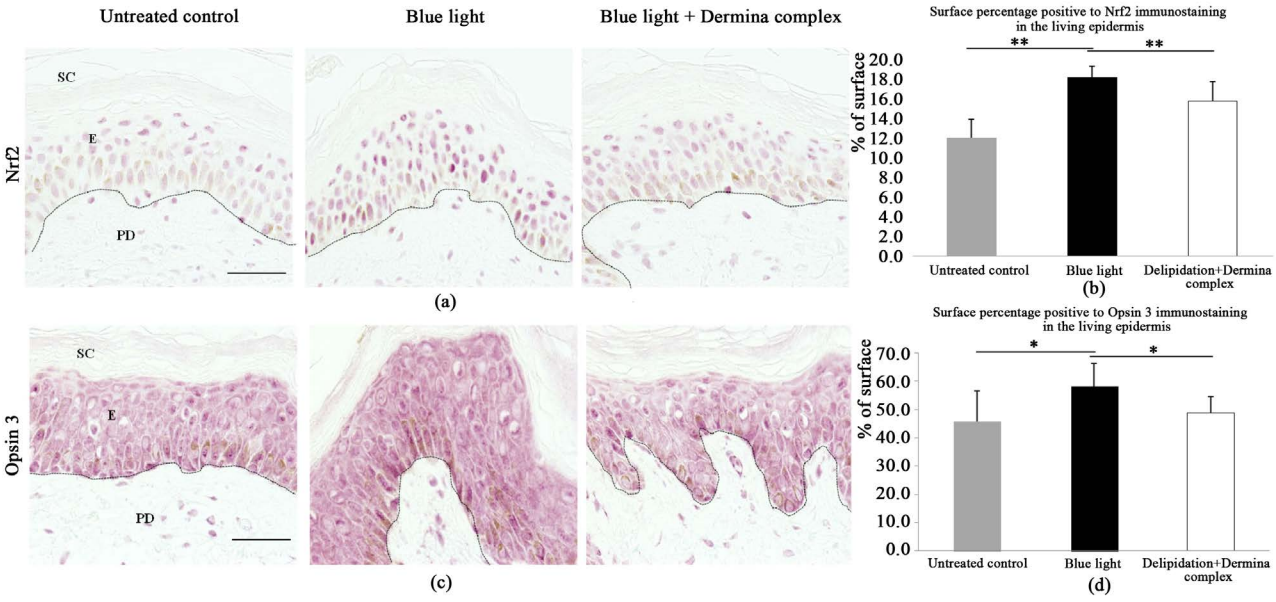

Figure 2. Dermina complex protects human skin against blue light. In (a), immunodetection of the activated form of Nrf2 in FFPE skin sections. In b), analysis of Nrf2 immunostained surface (in %) under different conditions. In (c), immunolocalization of opsin-3 on FFPE skin sections and in (d), analysis of opsin-3 immunostained area (in %) under different conditions. Scale bar: 50 µm. Abbreviations: E, epidermis; FFPE: formalin-fixed paraffin-embedded; PD: papillary dermis; SC, *stratum corneum*.

formation by 62% ($p < 0.01$) and the formation of oxidized proteins by 75% ($p < 0.01$), compared to the control explants (Figure 3).

Interestingly, the preventive application of Dermina Complex significantly reduced the active form of Nrf2 by 22% ($p < 0.01$) and the formation of γ-H2AX by 37% in the epidermis ($p < 0.01$) as well as the formation of γ-H2AX and the oxidized proteins in the papillary dermis, by 49% ($p < 0.01$) and 24% ($p < 0.01$), respectively, in comparison to the exposed explants (Figure 3).

Furthermore, we observed that the quantity of inflammatory cytokine IL-1α released into the culture medium following pollutants and UV-A exposure was also significantly increased by 68% ($p < 0.05$) compared to the control explants (Figure 4).

The preventive application of Dermina complex significantly reduced the concentration of IL-1α by 51% ($p < 0.05$) in comparison to the stressed explants (Figure 4).

Collectively these data indicate that the Dermina Complex considerably prevented the pollutant and UV-A induced stresses in the epidermis and in the dermis.

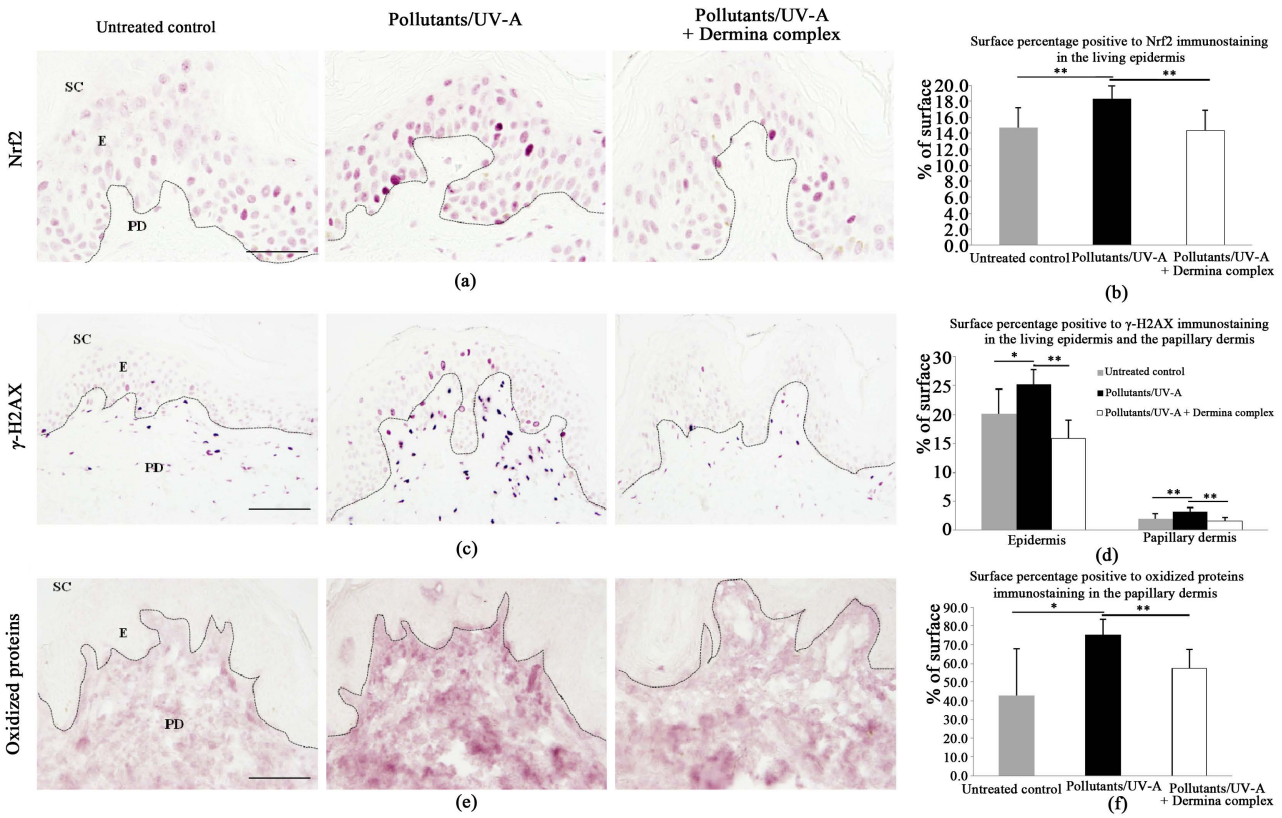

Figure 3. Dermina complex shows an anti-pollution and anti-UV-A activity. In (a), immunodetection of the activated form of Nrf2 in FFPE skin sections. In (b), analysis of Nrf2 immunostained area (in %) under different conditions. In (c), immunolocalization of γ-H2AX in FFPE skin sections and in (d), analysis of the area (in %) of γ-H2AX immunostaining by image analysis. In (e) immunodetection of oxidized proteins in frozen skin sections and in (f) analysis of oxidized proteins area (in %) by image analysis. Scale bar: (a) 50 µm; (c) and (e) 100 µm. Abbreviations: E, epidermis; FFPE: formalin-fixed paraffin-embedded; PD: papillary dermis; SC, *stratum corneum*.

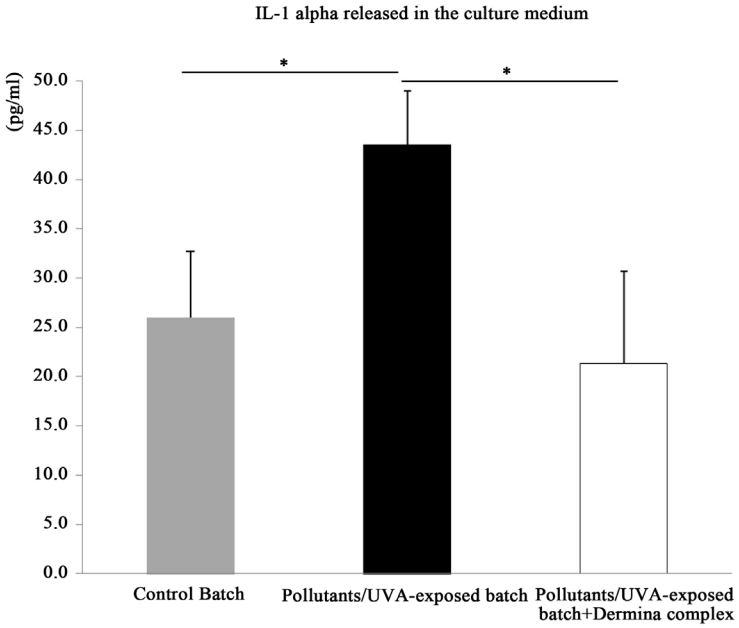

Figure 4. Dermina complex decreases the level of IL-1α induced upon environmental pollutants and UV-A exposure. Concentration of IL-1α released in the BEM culture medium following pollutants and UV-A exposure.

4. Discussion

The skin is continuously exposed to a vast range of environmental insults weakening the defense system of the epidermis and accelerating dramatically the aging of the cutaneous compartment.

The *stratum corneum* plays a major role in protecting the underlying epidermis and dermis from the assaults of the external environment acting simultaneously as physical and biological barrier. Nevertheless, as previously demonstrated the barrier function of the *stratum corneum* is reduced significantly upon the constant assaults of the environment [30].

In this context, filaggrin is an indispensable molecule for an optimal integrity of the *stratum corneum* [31].

Filaggrin plays an important role in the formation and maintenance of the epithelial barrier. It is a complex protein which is synthesized initially as high molecular-weight precursor and cleaved by different proteases into monomeric filaggrin. Successively filaggrin is cleaved into free amino acids and other molecules including urocanic acid and pyrrolidone carboxylic acid which form the natural moisturizing factor (NMF).

NMF plays a key role in skin hydration and UV protection [32].

The decrease in filaggrin expression compromises deeply the barrier function of the *stratum corneum*, causing abnormal allergen penetration and altered immune reactivity, with dramatic repercussion for the whole skin tissue [33].

In the present study we have shown that the curative application of Dermina complex prevents the decrease in filaggrin expression upon delipidation, suggesting that these plant extracts are able to protect and reinforce the barrier

functions of the skin.

As shown previously by Portugal-Cohen and collaborators, polysaccharide-rich cosmetic products could form a protective film on the surface of the *stratum corneum*, avoiding the penetration of external molecules [13]. At the same time, it has been shown that polysaccharides, including sacran, can directly stimulate the expression of pro-filaggrin [34].

In this sense, Seaberry and Water Lily extracts show a polysaccharide content of 3.3 (g/L) and 3.1 (g/L), respectively. Consequently, the skin barrier reinforcing activity of Dermina complex could be due to the polysaccharides that are naturally present in these plant extracts and that could be able to form a skin-protecting film.

In parallel, we have observed that the preventive application of Dermina complex is able to decrease significantly the opsin-3 overexpression following human skin explants exposure to blue light. These data confirm the previous hypothesis that Dermina complex could form a protective film on skin surface preventing the penetration of both chemicals and blue light. Opsin-3 has been described very recently as a possible link between visible light and skin hyperpigmentation, by activating the melanocyte inducing transcription factor (MITF) in a calcium-dependent manner, which in turn induces an increase of TYR/P protein complexes leading to skin hyperpigmentation [12]. Consequently, by avoiding blue light penetration Dermina complex may prevent the skin hyperpigmentation induced by this kind of exposure. Alternatively, Dermina complex could act as an antagonist of the G-coupled receptor Opsin-3 and decreases its blue-ligth induced overexpression.

We have also demonstrated that Seaberry and Water Lily extracts reduce significantly the formation of γ-H2AX in both epidermis and dermis after the exposure of human skin explants to environmental pollutants and UV-A. γ-H2AX is a protein involved in DNA repair which is phosphorylated upon radiation resulting in the formation of γ-H2AX. Once phosphorylated, γ-H2AX cooperates with other proteins including p53, BRCA1 and MDC1 assuring the repair of DNA double strand breaks caused by UV exposure [35].

Therefore, a diminution of gamma-H2AX is a reliable bio-marker for reduced DNA damages, indicating that Dermina complex presents an important DNA protection activity.

Very interestingly, Dermina complex is also able to reduce the phosphorylated form of Nrf-2 which is increased following both blue light and environmental/UV-A exposure. Nrf-2 is a transcription factor that under unstressed conditions binds to its inhibitor Keap-1 in the cytoplasm. Upon an oxidative stress, Keap-1 undergoes structural changes that allow the dissociation of Nrf-2. Successively, Nrf-2 is phosphorylated on serine 40 and translocates into the nucleus where it binds to a specific sequence of DNA, the Antioxidant Response Elements (ARE) and enhances the transcription of genes encoding proteins with antioxidant activity [36].

By reducing significantly the activated form of Nrf2, Dermina complex participates in a healthy maintenance of the anti-oxidative response balance upon blue light and UV-A exposure.

On the one hand, this antioxidant activity could be explained, as mentioned above, by the ability of Dermina complex to form a film on the skin surface to avoid the penetration of exogenous substances. On the other hand, the ability of Dermina complex to reduce the activated form of Nrf-2 can be explained by the fact that Seaberry and Water Lily extracts present a polyphenol content of 1.38% and 0.31% respectively. Polyphenols are natural compounds of plant origin, presenting an antioxidant activity. Consequently, the presence of these molecules in the plant extracts could confer a direct antioxidant activity to Dermina complex avoiding a premature depletion of antioxidant compounds naturally present in the skin such as retinoids [37]. Furthermore, the antioxidant activity of Seaberry and Water Lily extracts was confirmed by the ability of plant extracts to decrease the formation of oxidized proteins in the papillary dermis upon pollutants and UV-A exposure.

We have also shown that Seaberry and Water Lily extract complex reduces significantly the content of IL-1 alpha released into the culture medium of skin explants exposed to atmospheric pollutants and UV-A. IL-1 alpha is a potent inflammatory cytokine that triggers the inflammatory process and its altered regulation can cause chronic inflammation of the skin [38]. Furthermore, IL-1 alpha activation induces the over-expression of IL-6 which in turn regulates positively the expression of MMP-1 contributing thus to the premature aging of the skin (Wlasheck *et al*., 1994) [39]. The ability to reduce the IL-1 alpha overexpression under stress conditions confers to Dermina complex important anti-inflammatory properties.

Furthermore, the relative low content of polysaccharides in the plant extracts can only partially explain the anti-pollution activity of Dermina complex, and the identification of other molecules that might contribute to the observed properties is needed. In perspective, a more detailed molecular characterization and fractionation of Dermina complex could allow to determine precisely the molecules that participate principally to the observed activities. The blue light absorption proprieties or the antagonist effect on opsin-3 receptor of Seaberry and Water Lily extracts should be also investigated.

5. Conclusions

In the work described herein, we have shown that Seaberry and Water Lily extract complex exhibits a powerful skin barrier reinforcement activity, by reducing the delipidation-induced decrease of filaggrin. Moreover, the anti-oxidant, the anti-pollution and anti-inflammatory properties against multiple environmental stresses including irradiations such as blue light and UV-A, and atmospheric pollutants including benzene and heavy metals have been also demonstrated.

In order to better elucidate the molecular mechanisms involved in the anti-oxidant activity of Dermina complex, it could be interesting to explore its ROS scavenging effect or its impact on different enzymes presenting anti-oxidative damage functions, such as catalase or superoxide dismutase-1.

In regard to the anti-pollution activity demonstrated in the present work, the heavy metals chelating properties of both extracts could also be explored.

Taken together, our results show that Dermina complex is a good candidate for the development of cosmetic end-products with global anti-pollution effects.

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Conflicts of Interest

Softa Mohamed and Pauline Bony work in the company which commercialize the plant extracts tested in the present work。

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Supplement Data S1

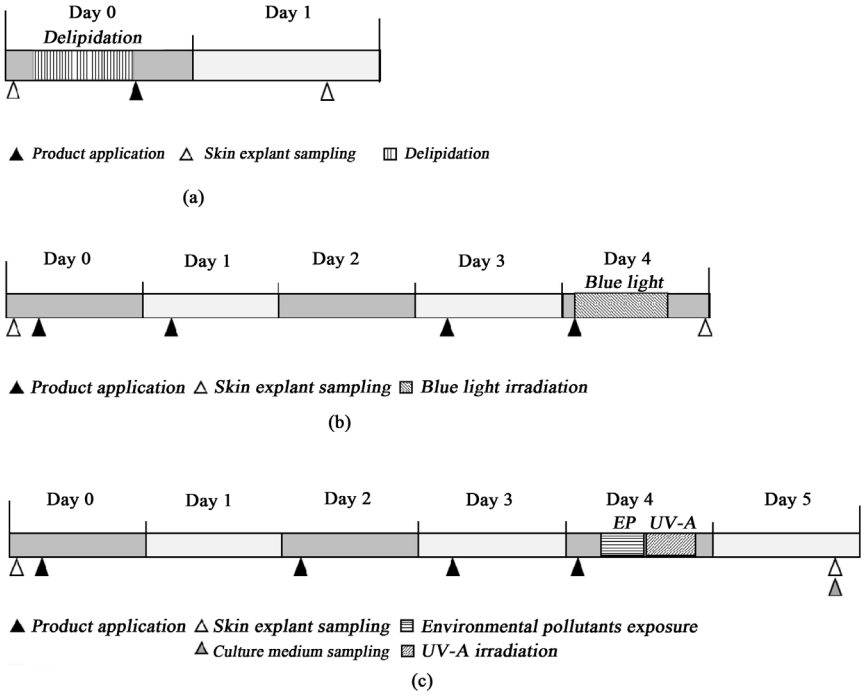

Figure S1. Schematic representations of the different experimental protocols used for (a) the delipidation of the skin explants *ex vivo*; (b) the exposure of human skin explants *ex vivo* to blue light; (c) the exposure of human skin explants *ex vivo* to environmental pollutants and UV-A irradiations.

Supplement Data S2

Table S2. Composition and concentrations of the different pollutants used to treat human skin explants.

HEAVY METALS	
Pollutant	**Concentration**
AIuminium	0.01 mg/mL
Arsenic	0.01 mg/mL
Boron	0.001 mg/mL
Barium	0.001 mg/mL
Beryllium	0.0005 mg/mL
Calcium	0.005 mg/mL
Cadmium	0.001 mg/mL
Chromium	0.001 mg/mL
Copper	0.001 mg/mL
Iron	0.001 mg/mL
Mercury	0.0025 mg/mL
Potassium	0.0495 mg/mL
Lithium	0.001 mg/mL

Continued

Magnesium	0.0005 mg/mL
Manganese	0.0005 mg/mL
Sodium	0.01 mg/mL
Nickel	0.0025 mg/mL
Phosphorus	0 .005 mg/mL
Lead	0 .005 mg/mL
Scandium	0.0005 mg/mL
Selenium	0.01 mg/mL
Strontium	0.0005 mg/mL
Tellurium	0.01 mg/mL
Titanium	0.001 mg/mL
Yttrium	0.0005 mg/mL
Zinc	0.001 mg/mL
HYDROCARBONS	
Pollutant	**Concentration**
Benzene	1 µL/mL
Xylene	1 µL/mL
Toluene	1 µL/mL
PARTICULATE MATTER	
Pollutant	**Concentration**
Diesel particulate matter	0.1 mg/mL

Supplement Data S3

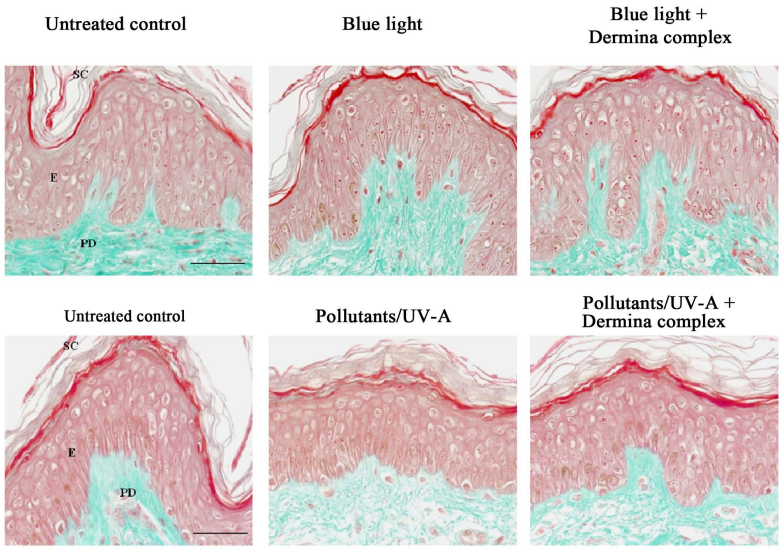

Figure S3. The tissue viability is not affected under the different experimental conditions. In (a), analysis of the general morphology of skin tissue upon blue light exposure or pollutants/UV-A exposure in (b). Scale bar: 50 µm. Abbreviations: E, epidermis PD: papillary dermis; SC, *stratum corneum*.