The natural solution to pollution

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Abstract

Exposure to air pollutants is one of the major threats to skin health. Contaminants attack the skin on several levels: they induce oxidative stress, they stimulate inflammatory pathways, and they accelerate the ageing process of skin. As a consequence, consumers demand functional cosmetics that prevent and repair pollution-induced skin damage. In this respect, the most promising approach is using the body's endogenous detoxification machinery, which is composed of a multitude of cell-protective and detoxifying mechanisms. These powerful systems are capable of neutralising thousands of toxic molecules per second, whereas the mere application of antioxidants is much less efficient, as one antioxidant molecule is capable of neutralising only one free radical.

HerbaShield URB addresses these concerns. The COSMOS-approved multi-component active ingredient targets three mechanisms to naturally reduce pollution-induced skin damage: (1) It strengthens the skin's barrier through hydrogenated lecithin; (2) it protects from radical oxygen species through natural antioxidants; and (3) it enhances the endogenous detoxification machinery through natural activators of detoxifying enzymes.

The presented anti-pollution ingredient is a perfect fit for anti-ageing cosmetics and to be formulated in skin care applications, such as face care, body care, and cleansing products.

Undoubtedly, pollution-induced skin damage will be a fundamental focus of the cosmetics industry in the next decades. A typical constituent of air pollution is so-called particulate matter (PM) – tiny particles, classified by their size into inhalable PM (< 2.5 µm in size) and not inhalable PM (2.5 - 10 µm in size). These PM disrupt the integrity of the skin barrier and, in addition, function as carriers of pollutants that adsorb to their surface.[1] This way, PM enhances the penetration and accumulation of harmful substances in the skin.[2]

Pollutants that enter the skin by way of PM include substances from the class of polycyclic aromatic hydrocarbons (PAH), heavy metals, endotoxins, ions, or reactive gases. The PAH Benzo[a]pyrene (BaP) is considered one of the most dangerous.[3]

Pollutants, and harmful substances carried by them, affect the skin on several levels: first, they induce oxidative stress, second, they stimulate inflammatory pathways, and finally they accentuate the visible signs of ageing.[3,4] In fact, scientific studies demonstrated that air pollutants induce the formation of wrinkles and pigmentation spots.[5]

The skin's own detoxification tools

The skin, which is exposed to pollutants and toxins, is equipped with a powerful, endogenous, two-phase detoxification machinery. This system first identifies and chemically activates toxic substances by specific enzymes, and second, it enzymatically conjugates these activated toxins to water-soluble carriers, which can then be eliminated from the body via the kidneys.

Phase I: Pollutants are activated for further processing, but become toxic

The aryl hydrocarbon-receptor (AHR) is a pollutant sensor that constitutes the starting point of the detoxification mechanism.[6] In phase I, aromatic hydrocarbons (e.g. present in cigarette smoke) bind to and activate AHR. The activated receptor, then, induces defence-related genes, such as enzymes of the cytochrome P450 superfamily.[7] These enzymes oxidise the corresponding toxins for further conjugation. However, this reaction itself also generates reactive intermediates and reactive oxygen species that collaterally cause cell damage.[8] Therefore, a stimulation of phase I may be harmful to the skin.[9]

Phase II: Activated toxins are conjugated to S-glutathione and eliminated from the body

During phase II, the reactive intermediates are transformed into excretable products.[10] This final detoxification is controlled by a nuclear factor called Nrf2.[7] Nrf2 activates a broad range of antioxidative genes,[11] and initiates their transcription, including glutathione-S-transferase (GST). GST enzymatically transfers a highly soluble tripeptide (S-glutathione) to the activated toxin, which can now be excreted via the kidneys (Fig 1).

Anti-pollution strategy for cosmetics

Strengthening the skin's barrier and its endogenous detoxification machinery is a promising anti-pollution strategy for cosmetics. To this end, reinforcement of the skin's barrier would reduce the penetration and accumulation of pollutants and avoid the initiation of Phase I and its negative side effects. Once entered into Phase I, the administration of natural antioxidants (e.g. from botanical extracts) would reduce oxidative stress. To support Phase II of the detoxification process, natural activators of Nrf2 (e.g. flavonoids from botanical extracts) would enhance the activity of detoxifying enzymes, such as GST, and reinforce fast elimination of toxic substances.[7,12] In summary, cosmetic treatment directed at individual stages of the skin's endogenous detoxification machinery, would better protect our skin from environmental pollutants and from the health risks associated with them.

The natural approach to skin detoxification

The active ingredient presented here is a three-component natural ingredient based on watercress, horsetail, and nettle extract (INCI: Maltodextrin, Nasturtium Officinale Flower/Leaf/Stem Extract, Hydrogenated Lecithin, Equisetum Arvense Extract, Urtica Dioica (Nettle) Leaf Extract, Sodium Chloride). The aqueous-ethanolic extract is embedded

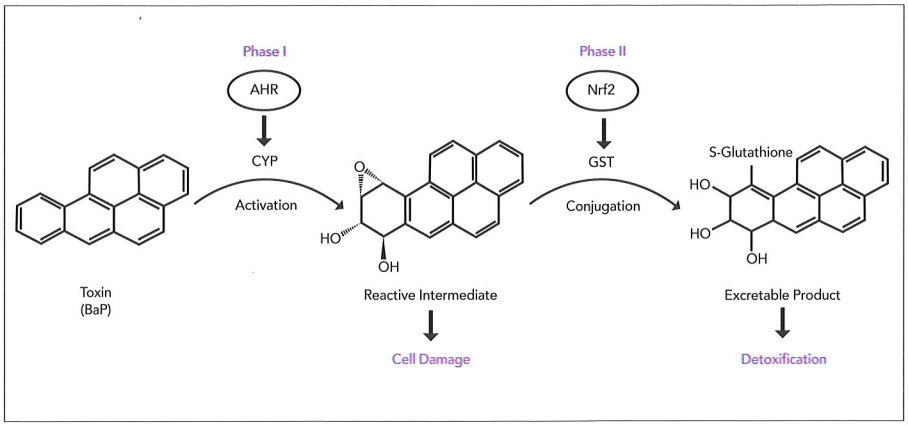

Figure 1: The joined action of AHR and Nrf2 trigger the elimination of toxins in a two-phase process. In phase I the toxin Benzo[a]pyrene (BaP) becomes activated by oxidation. Subsequently, in Phase II, S-glutathione is conjugated to the activated toxin. The now water-soluble conjugate can be excreted via the kidneys.

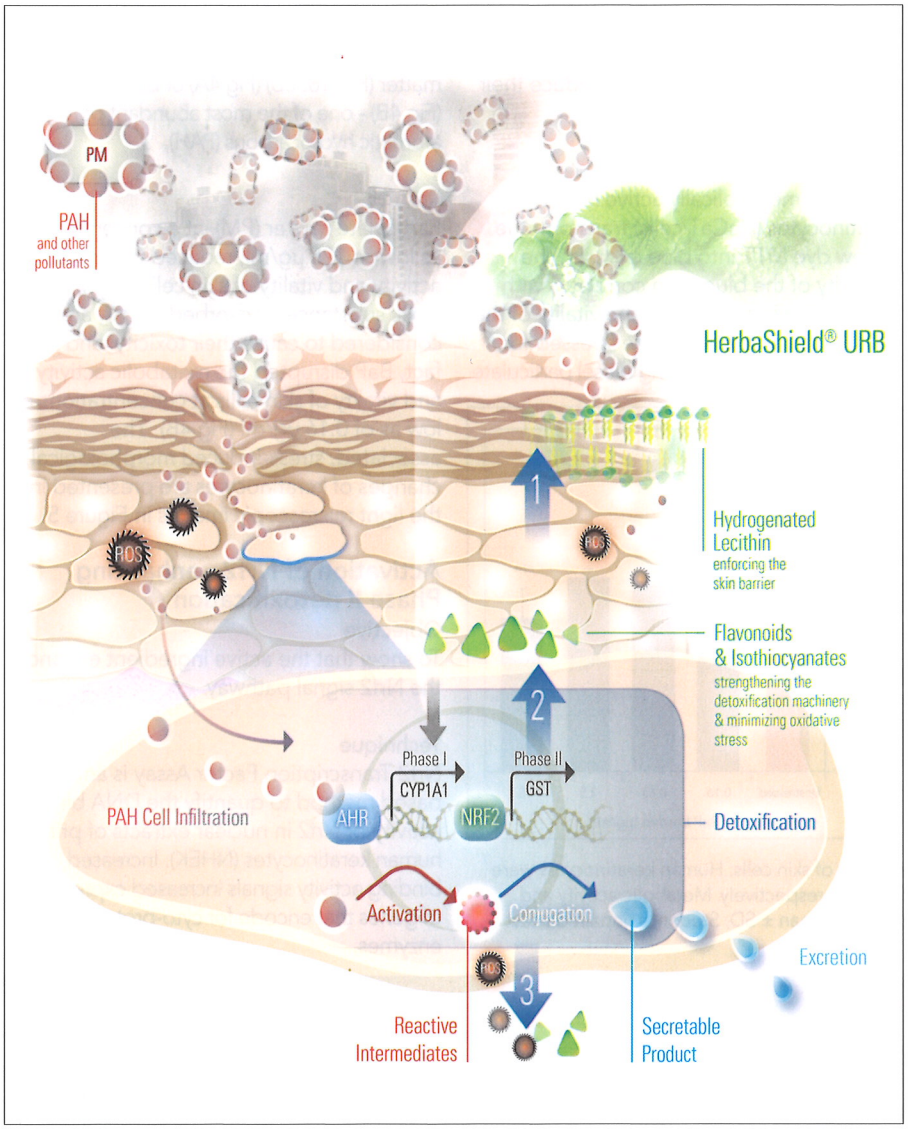

Figure 2: Mode of action - Pollutants, such as polycyclic aromatic hydrocarbons (PAH), adhere to particulate matter (PM) and may penetrate the skin. As a first line of defence, hydrogenated lecithin and silica strengthen the barrier function of the skin and impede skin contact with pollutants.[22] Pollutants, once entered the epidermis, bind to AHR which triggers the expression of enzymes of Phase I. At this stage, pollutants become activated for further processing, but harmful substances are also formed. As a second line of defence, antioxidants of nettle and horsetail extracts neutralise these reactive oxygen substances and prevent cellular damage. In Phase II of the detoxification process, active ingredients derived from watercress, nettle, and horsetail activate Nrf2 and shift the balance in favour of phase II[15,16] which ensures rapid detoxification of these reactive intermediates.

in a unique matrix of phospholipids and maltodextrin. The active ingredient targets all stages of endogenous detoxification and reduces pollution-induced skin damage:

Watercress (*Nasturtium officinale*) – detoxifying

The detoxifying effect of botanical extracts from watercress is predominantly ascribed to isothiocyanates.[13,14] Isothiocyanates activate phase I and phase II detoxifying enzymes and support the skin's endogenous detoxification machinery.[15] In fact, a current study among smokers lead to significantly less damage through toxins in cigarette smoke after administering watercress extracts.[16]

Nettle (*Urtica dioica*) – antioxidant, detoxifying

Quercetin, one of the active components of nettle,[17] enhances the activity of Nrf2 - the master regulator of the detoxification machinery - and helps to activate detoxifying and antioxidant enzymes.[7,12] Moreover, quercetin and the phenylpropanoid caffeoylquinic acid, present in nettle extract, protect from antioxidative stress generated in Phase I of the detoxification process.

Horsetail extract (*Equisetum arvense*) – protecting, antioxidant, detoxifying

A high silica content is a hallmark trait of horsetail, which already absorbs pollutants before they come into contact with the skin's surface. Furthermore, horsetail extract contains flavonoids, in particular, kaempferol, quercetin and protogenkwanin glycoside[14,18,19] that support the detoxification process in Phase I and Phase II by their antioxidant and Nrf2-enhancing activity.

Hydrogenated lecithin – protecting, barrier-forming

Hydrogenated phospholipids reinforce the skin's barrier function. They are essential constituents of cellular membranes, and facilitate skin renewal.[20,21] This way, topical formulations containing hydrogenated lecithin prevent pollutants from entering the epidermis.

In summary, the active ingredient presented here is a powerful anti-pollution cosmetic active ingredient.

Phytochemical analysis

Objective

To identify the main active constituents of the active ingredient and to evaluate their stability.

Techniques

High performance thin layer chromatography (HPTLC) was used to assess the overall flavonoid composition. Dereplication analysis, using liquid chromatography coupled with mass spectrometry as well as UV - and light scattering detectors (LC-MS/UV/ELSD)

allowed database-assisted identification of secondary metabolites. Antioxidant power, a measure of antioxidant capacity and reactivity, were analysed by electron spin resonance spectroscopy. The overall antioxidant power is expressed as antioxidant units, where one unit corresponds to the activity of a 1 ppm solution of pure ascorbic acid as a benchmark.[23]

Results

Flavonoid HPTLC fingerprint analysis of the aqueous-alcoholic pre-material of the active ingredient revealed substantial amounts of flavonoids, which correlate with the antioxidative power.

HPTLC also demonstrated excellent heat and storage stability. Heating to 80°C for 2 h or storage at 40°C for 1 month had no effect on the overall flavonoid pattern. Dereplication analysis identified flavonoids such as quercetin-, kaempferol-, and protogenkwanin-derivatives as well as phenylpropanoids of caffeoylquinic acid, or cichoric acid, as main constituents (Fig 3). In addition, the presence of isothiocyanates such as gluconasturtiin was confirmed by extracted ion chromatograms (not shown).

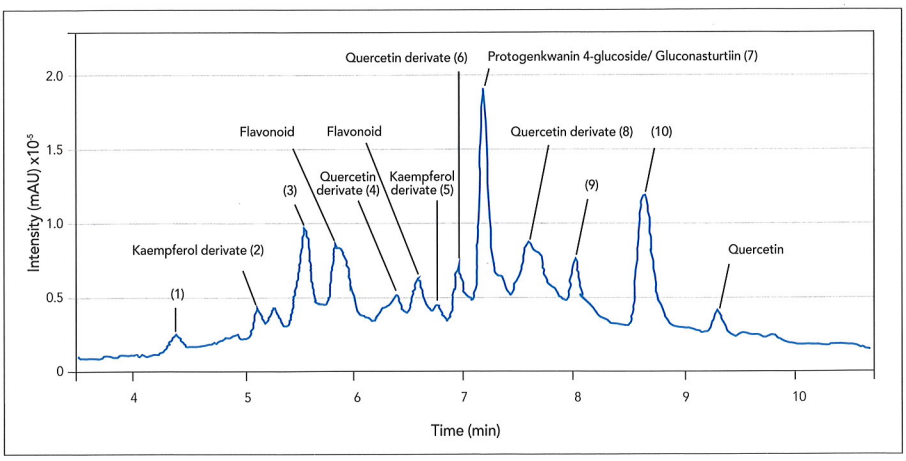

Figure 3: The active ingredient combines a wealth of powerful metabolites. Dereplication analysis of the aqueous-alcoholic pre-material identified: (1) Tryptophan; (2) Kaempferol3-glucoside7-sophoroside; (3) 5-O-caffeoylquinic acid; (4) Quercetin 3,5-digalactoside; (5) Kaempferol-3-O-sophoriside; (6) Quercetin-3-O-rutinoside; (7) Protogenkwanin 4-glucoside/ Gluconasturtiin (an isothiocyanate); (8) Quercetin-3-0-(6"-malonylglucoside); (9) 1,5-Di-O-caffeoylquinic acid; (10) Mesocichoric acid.

Particulate matter and pollutants impair skin cells

Objective

To show that particulate matter (PM) and pollutants stress skin cells and reduce their metabolic activity and vitality.

Technique - MTT Assay

Cultured, metabolically active human keratinocytes (HaCaT cells) transform the yellow dye MTT into blue crystals. The intensity of the blue stain correlates with the metabolic activity and the vitality of the cells. In the assay, cells were stressed for 48h with naturally occurring diesel particulate matter (PM 1650b) (Fig 4A) or benzo[a]pyrene (Fig 4B) - one of the most abundant polycyclic aromatic hydrocarbons (PAH).

Results

Particulate matter (PM), at a concentration as low as 125 µg/ml, reduced the metabolic activity and vitality of skin cells (Fig 4A). Toxic substances, adsorbed to PM, are considered to cause their toxicity[3] and in fact, BaP disrupted the metabolic activity and vitality of skin cells at concentrations as low as of 0.16 µg/ml (Fig 4B). The associated, stress-induced morphological changes of keratinocytes are presented in the light microscopic images in Figure 5.

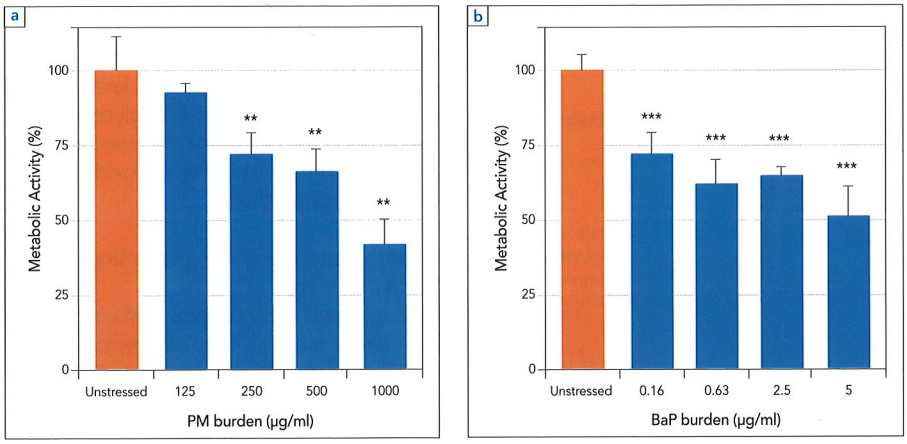

Figure 4: Particulate matter and pollutants reduce the vitality of skin cells. Human keratinocytes were incubated for 48 h with particulate matter (A; PM) and BaP (B), respectively. Metabolic activity and vitality decline with increasing PM and pollutant stress. N = 4; Mean ± SD; Student's unpaired t-test versus unstressed; ** = p <0.01; *** = p <0.001.

Activation of Nrf2 - enhancing Phase II detoxification

Objective

To show that the active ingredient enhances the Nrf2 signal pathway.

Technique

Nrf2 Transcription Factor Assay is an ELISA-based method to quantify the DNA binding activity of Nrf2 in nuclear extracts of primary human keratinocytes (NHEK). Increased binding activity signals increased expression of genes that encode for cyto-protective enzymes.

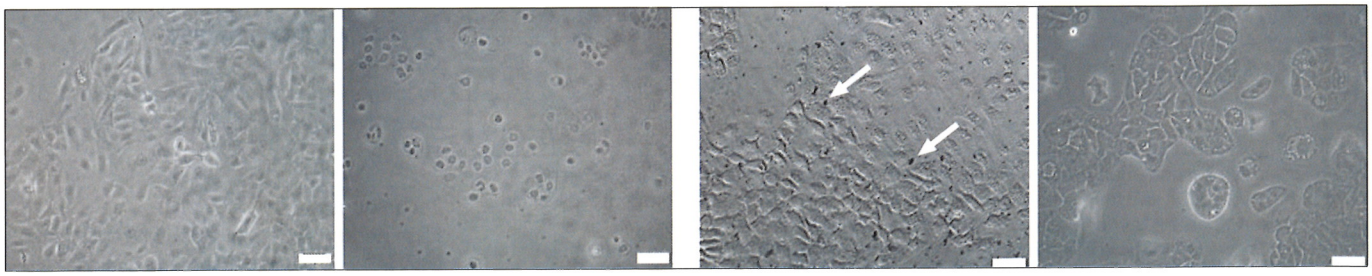

Figure 5: Pollutants damage cultured human keratinocytes. Light microscopic images following 48 h of exposure to stressors. Unstressed keratinocytes showed a characteristic structure and monolayer organisation. Positive control cells, devitalized with detergents, shrank and became nodular or detached from the bottom of the culture dishes. These stress signals were also observed with cells that were treated with 500 µg/ml particulate matter (PM; note the particles in the image) or with 5 µg/ml BaP. Scale bars = 50 µm.

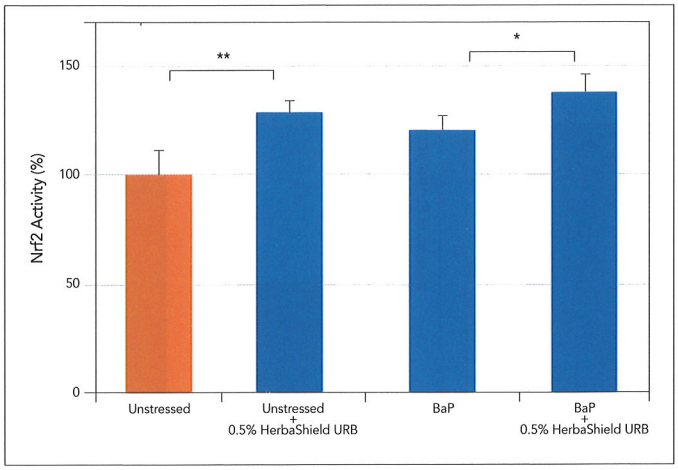

Figure 6: The active ingredient empowers the cellular detoxification machinery. Human keratinocytes were stressed for 48h with 0.05 µg/ml BaP or not (unstressed). Nrf2 activity was significantly enhanced upon treatment with the multi-component active ingredient in a concentration of 0.5%. N = 4; Mean ± SD; Student's unpaired t-test versus untreated; * = p <0.05; ** = p <0.01.

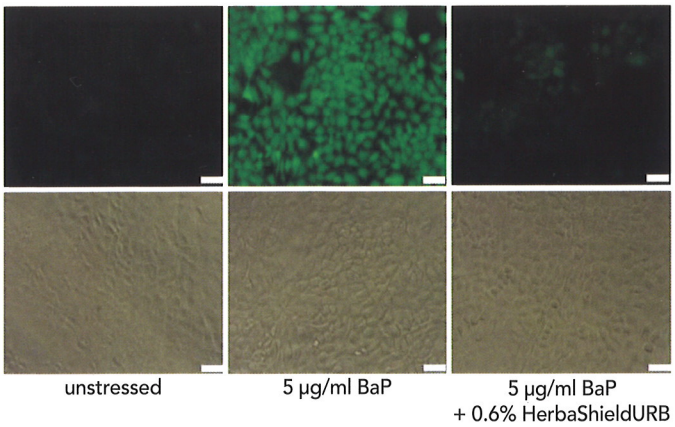

Figure 8: The active ingredient protects against pollutant-induced oxidative stress. Unstressed cells produce low amounts of free radicals as shown by a low fluorescence signal (left panel - unstressed). Cells incubated with BaP generate oxidative stress as shown by a high fluorescence signal (center panel - 5µg/ml BaP). The additional presence of the active ingredient completely relieves oxidative stress (right panel - 5µg/ml BaP + active ingredient), as shown by the absence of fluorescence. Upper images show fluorescence signals, lower images show phase contrast images. Scale bars = 50 µm.

Results

The treatment with the active ingredient stimulated Nrf2 activity, indicating that the extract can induce the skin's endogenous detoxification machinery (Fig 6). The active ingredient provided additional detoxification power. Cells stressed with BaP showed higher Nrf2 activity than unstressed cells, implying that the cellular detoxifying machinery is reinforced (Fig 6).

Reducing antioxidative stress - enhancing Phase I detoxification

Objective

To show that the active ingredient provides additional detoxification power and counteracts pollutant-induced skin stress.

Technique - DCF Assay

A non-fluorescent precursor of a fluorescent dye diffuses into keratinocyte cells, where radical oxygen species convert the precursor into the fluorescent dye. The enhanced fluorescence signal is monitored and taken as a measure of oxidative stress. Pre-incubation with the active ingredient for 24 h followed by 1 h incubation with 1 mM H_2O_2, 2.5 µg/ml BaP, or 100 µg/ml particulate matter (PM) in the presence of the active ingredient.

Results

Incubation with particulate matter (PM) or BaP led to massive oxidative stress. The formation of intra-cellular reactive oxygen species (ROS) increased threefold and was even higher as compared to incubation with 1 mM H_2O_2. The active ingredient was able to entirely inhibit the pollutant-induced formation of ROS (Fig 7). The reduced quantity of radicals was confirmed by fluorescence microscopy (Fig 8).

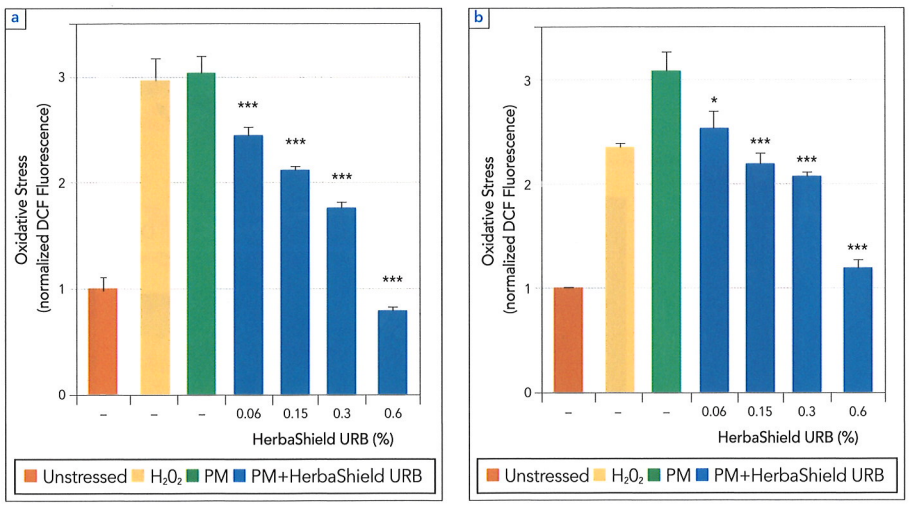

Figure 7: The active ingredient counteracts the effects of particulate matter and pollutant-induced skin stress. Human keratinocytes (HaCaT cells) were stressed for 1 h with 100 µg/ml particulate matter (A; PM), and 2.5 µg/ml BaP (B), respectively. Treatment with the extract significantly reduced the stress response. N = 4; Mean ± SD; Student's unpaired t-test versus stressed (PM or BaP) but untreated; * = p < 0.05; *** = p < 0.001.

Pollution causes skin ageing - an *in vivo* study

Objective

To study the anti-pollution effect of the active ingredient and to evaluate anti-age parameters, such as skin firmness, skin elasticity, skin roughness and wrinkles in a double-blind, placebo-controlled, randomised *in vivo* study, with 2x21 Caucasian female volunteers, between 30–65 years of age, living in an urban area, and smoking at least 5 cigarettes per day. One group applied a cream formulation containing 1% active ingredient to their face, twice a day, for 4 weeks. The other group applied the same formulation without the active ingredient (placebo).

Techniques

(1) Skin firmness and elasticity was measured by cutometry determining R0 and R2, respectively. (2) Skin roughness and wrinkles were determined evaluating the RZ (a marker for skin roughness and fine lines) and wrinkle volume by Primos 3D. In addition, an expert dermatologist scored these parameters by using the 8-degree Japanese Cosmetic Industry Association's wrinkle grading system. (3) Skin complexion was measured using VISIA-CR, a standardised imaging system, taking high-resolution photos and allowing the calculation of brownish and reddish skin irregularities. Moreover, an expert dermatologist evaluated skin tone, spots, evenness, and complexion on a 5-degree scale. (4) Skin oiliness, the so-called lipidic index, was evaluated using sebumeter tape and grease spot photometry. (5) Changes in epidermal barrier function were evaluated by measuring transepidermal water loss (TEWL). Finally, the *in vivo* test included a self-assessment using a questionnaire completed before and after the study.

Results are shown in Figures 9-12.

Conclusion

Air pollution is one of the world's biggest environmental concerns and a major threat to skin health. As a result, the development of anti-pollution strategies will be a central task of the cosmetics industry in the upcoming years.

The present study analyses the impact of pollutants to skin-health and proposes cosmetic solutions to prevent and repair pollution-induced skin damage. The *in vitro* data presented here demonstrate that pollutants impair skin cells: in cultured human keratinocytes, they induce stress, reduce metabolic activity, decrease cellular vitality, activate detoxifying enzymes, but also change the morphology of affected cells. Notably, the study shows that efficient protection from pollutants, as well as improved endogenous detoxification processes, can alleviate these effects. The multi-component active ingredient, which was used throughout the study, reduced the formation of radicals, activated detoxifying enzymes, improved the epidermal barrier, and protected against radical skin damage - all factors that potentially lead to premature and accelerated skin ageing.

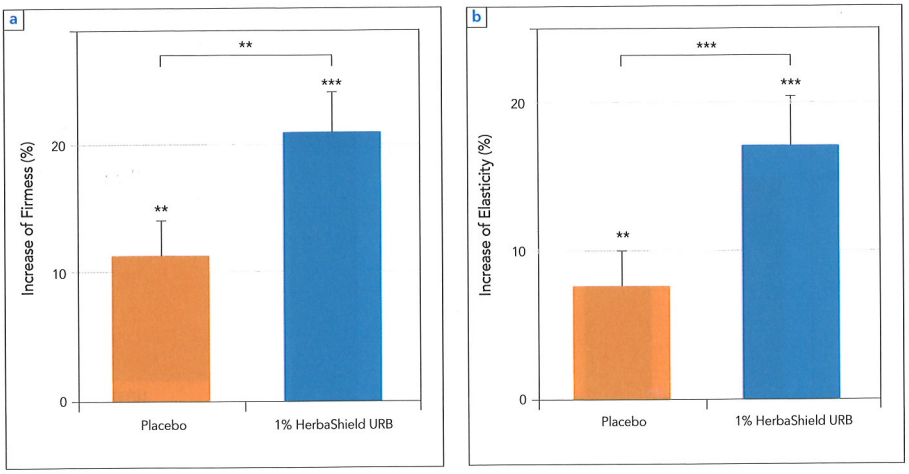

Figure 9: A cream containing 1% active ingredient improves skin firmness and elasticity. Relative skin firmness (A) and elasticity (B) increase after 4 weeks of treatment compared to placebo. N = 21; Mean ± SEM; Student's t-test versus untreated and between treatments; ** = p <0.01; *** = p <0.001.

The *in vivo* data of the present study confirm that the active ingredient reduces visible signs of skin ageing. The use of 1% of active ingredient in a cosmetic formulation, applied to pollution-exposed facial skin, resulted in visible, sensible and measurable skin benefits after 4 weeks: the skin's firmness and elasticity increased significantly (Fig 9). Additionally, the skin became markedly smoother, as the appearance of fine lines and wrinkles was reduced around the eye and nose area (Fig 10); the overall skin complexion improved, as it became more even and less sallow (Fig 11); the skin became significantly

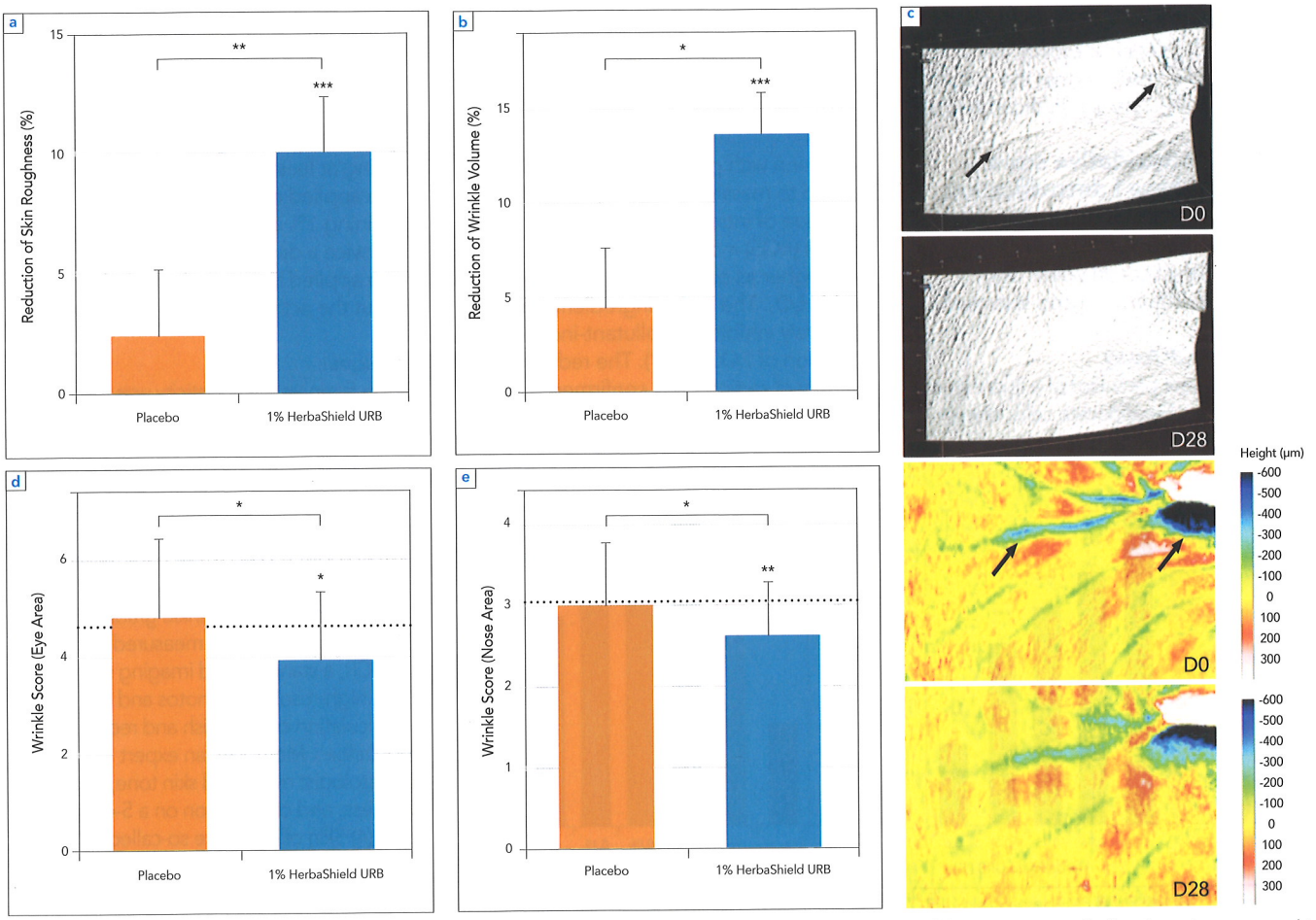

Figure 10: A cream containing 1% active ingredient smoothed fine lines and wrinkles. (A) Relative reduction of the parameter RZ after 4 weeks compared to placebo. RZ is a marker for skin roughness and fine lines. (B) Relative wrinkle volume reduction compared to placebo. (C) Representative 3D and pseudocolor images of the crow feet area of two volunteers before and after treatment. (D, E) Visual scoring of the wrinkle manifestation by a dermatologist confirmed a significant improvement of the overall appearance of wrinkles after 4 weeks treatment around the eye area (D) and nose area (E). Score 5 = deep wrinkles; Score 3 = Shallow wrinkles. The dashed lines indicate the scores before treatment. N = 21; Mean ± SEM; Student's t-test versus untreated and between treatments; * = p <0.05; ** = p <0.01; *** = p <0.001.

less oily and had improved barrier function (Fig 12). Finally, these benefits were substantiated by self-assessment via a questionnaire. Indeed, the volunteers reported significantly improved skin radiance, overall skin beauty and visible skin health. PC

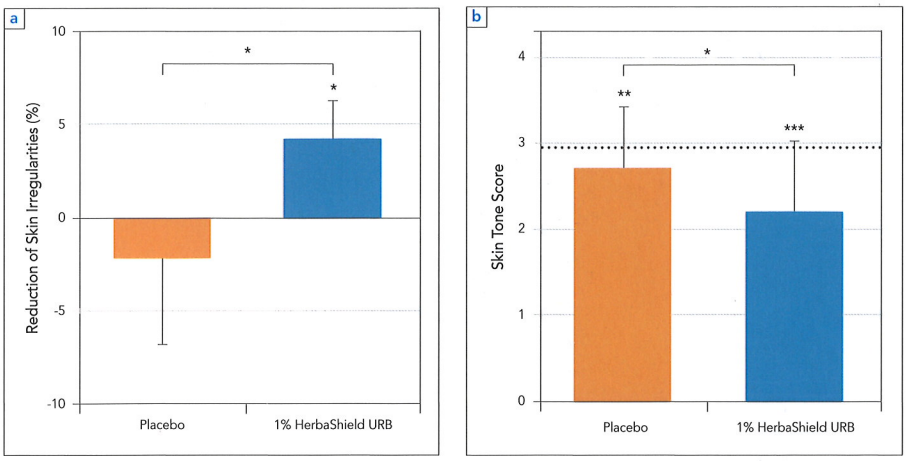

Figure 11: A cream containing 1% active ingredient reduces skin irregularities, such as spots and freckles, and improves complexion of the skin. (A) Relative reduction of brownish and reddish skin irregularities after 4 weeks compared to placebo. Visible spots were measured instrumentally. (B) Visual scoring of skin tone, evenness, appearance and spots by a dermatologist confirmed a significant improvement of the overall skin complexion after 4 weeks. Score 3 = marked dullness/ lack of skin brightness/ unhealthy appearance; Score 2 = noticeable dullness/ low skin brightness/ somewhat low vitality. The dashed line indicates the score before treatment.

References

1 Pöschl U. Atmospheric aerosols: composition, transformation, climate and health effects. *Angew Chem Int Ed Engl.* 2005;**44**(46): 7520-7540.

2 Pan TL, Wang PW, Aljuffali IA, Huang CT, Lee CW, Fang JY. The impact of urban particulate pollution on skin barrier function and the subsequent drug absorption. *J Dermatol Sci.* 2015;**78**(1):51-60.

3 Mancebo SE, Wang SQ. Recognizing the impact of ambient air pollution on skin health. *J Eur Acad Dermatol Venereol.* 2015;**29**(12):2326-2332.

4 Kim KE, Cho D, Park HJ. Air pollution and skin diseases: Adverse effects of airborne particulate matter on various skin diseases. *Life Sci.* 2016;152:126-134.

5 Vierkötter A, Schikowski T, Ranft U, et al. Airborne particle exposure and extrinsic skin aging. *J Invest Dermatol.* 2010;**130**(12): 2719-2726.

6 Esser C, Bargen I, Weighardt H, Haarmann-Stemmann T, Krutmann J. Functions of the aryl hydrocarbon receptor in the skin. *Semin Immunopathol.* 2013;**35**(6):677-691.

7 Köhle C, Bock KW. Activation of coupled Ah receptor and Nrf2 gene batteries by dietary phytochemicals in relation to chemoprevention. *Biochem Pharmacol.* 2006;**72**(7):795-805.

8 Denison MS, Nagy SR. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu Rev Pharmacol Toxicol.* 2003;43:309-334.

9 Henkler F, Brinkmann J, Luch A. The role of oxidative stress in carcinogenesis induced by metals and xenobiotics. *Cancers (Basel).* 2010;**2**(2):376-396.

10 Hines RN, McCarver DG. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. *J Pharmacol Exp Ther.* 2002;**300**(2):355-360.

11 Lee JM, Li J, Johnson DA, et al. Nrf2, a multi-organ protector? *FASEB J.* 2005;**19**(9): 1061-1066.

12 Saw CL, Guo Y, Yang AY, et al. The berry constituents quercetin, kaempferol, and pterostilbene synergistically attenuate reactive oxygen species: involvement of the Nrf2-ARE signaling pathway. *Food Chem Toxicol.* 2014;72:303-311.

13 Wichtl M, Blaschek W. Teedrogen und Phytopharmaka: ein Handbuch für die Praxis. In. 6 ed: Stuttgart: Wissenschaftliche Verlagsgesellschaft; 2016.

14 Blaschek W, Hilgenfeldt U, Holzgrabe U, Reichling J, Ruth P. In. 13 ed. Hagers Enzyklopädie der Arzneistoffe und Drogen: Berlin Heidelberg: Springer-Verlag; 2015.

15 Hecht SS. Inhibition of carcinogenesis by isothiocyanates. *Drug Metab Rev.* 2000; **32**(3-4):395-411.

16 Yuan JM, Murphy SE, Stepanov I, et al. 2-Phenethyl Isothiocyanate, Glutathione S-transferase M1 and T1 Polymorphisms, and Detoxification of Volatile Organic Carcinogens and Toxicants in Tobacco Smoke. *Cancer Prev Res (Phila).* 2016;**9**(7):598-606.

17 Marrassini C, Acevedo C, Miño J, Ferraro G, Gorzalczany S. Evaluation of antinociceptive, antinflammatory activities and phytochemical analysis of aerial parts of Urtica urens L. *Phytother Res.* 2010;**24**(12):1807-1812.

18 Asgarpanah J, E. R. Phytochemistry and pharmacological properties of Equisetum arvense L. In. Vol 6. *Journal of Medicinal Plants Research 2012*:3689 - 3693.

19 Stajner D, Popović BM, Canadanović-Brunet J, Anackov G. Exploring Equisetum arvense L., Equisetum ramosissimum L. and Equisetum telmateia L. as sources of natural antioxidants. *Phytother Res.* 2009;**23**(4):546-550.

20 Ghyczy M, Vacata V. Phosphatidylcholine and Skin Hydration. In: Leyden JJ, Rawlings AV, eds. Skin Moisturization. Marcel Dekker; 2002.

21 Van Hoogevest P, Prusseit B, Wajda R. Phospholipids: Natural Functional Ingredients and Actives for Cosmetic Products. *SOFW.* 2013;**139**(8):9 - 14.

22 Furue M, Uchi H, Mitoma C, et al. Antioxidants for Healthy Skin: The Emerging Role of Aryl Hydrocarbon Receptors and Nuclear Factor-Erythroid 2-Related Factor-2. *Nutrients* 2017;**9**(3).

23 Jung K, Richter J, Kabrodt K, Lücke IM, Schellenberg I, Herrling T. The antioxidative power AP—A new quantitative time dependent (2D) parameter for the determination of the antioxidant capacity and reactivity of different plants. *Spectrochim Acta A Mol Biomol Spectrosc.* 2006;**63**(4): 846-850.

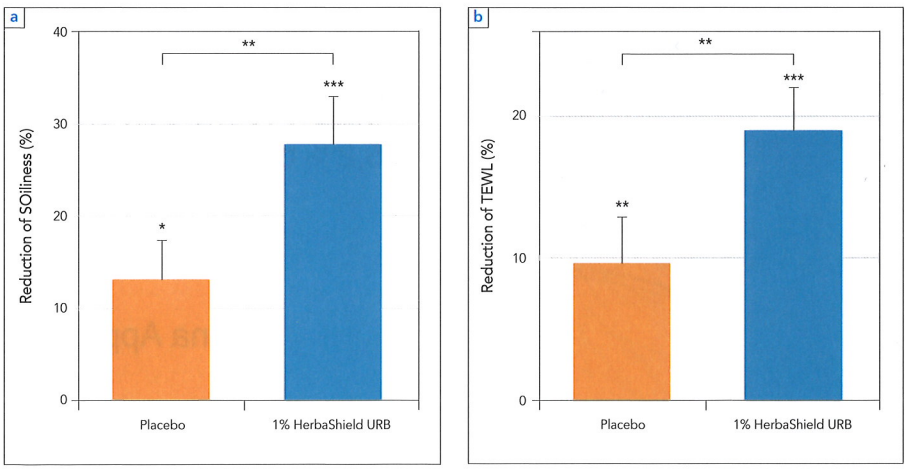

Figure 12: A cream containing 1% active ingredient reduces oiliness and strengthens the skin barrier. Relative reduction of the skin lipidic index (A) and transepidermal water loss (TEWL; B) after 4 weeks compared to untreated skin (before treatment). Pollutants are known to increase sebum production, giving skin an oily skin appearance. Pollutants also impair the skin's barrier function, eventually causing dry and sensitive skin. A reduction in TEWL is indicative for a strengthened barrier. N = 21; Mean ± SEM; Student's t-test versus untreated and between treatments; * = p <0.05; ** = p <0.01; *** = p <0.001.