# STUDY: SLOWING DOWN INFLAMMATORY AGEING

**Research results** | Various factors and receptors influence the speed and intensity of the ageing process in the skin. In a study, Stefan Fellner examined what effects an extract from alpine healing moor can have on ageing and oxidative stress as well as on the skin microbiome and presents his results.

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With the ageing and oxidative stress study the role of an extract form alpine healing moor (AHE) was determined in preventing ageing and age-related diseases and suppressing oxidative stress by determining gene expression of AGE receptors; RAGE, AGE-R1, AGER2 and RANC1 and markers of oxidative damage; ROS, nitric oxide, isoprostane, and hydrogen peroxide in Normal Human Fibroblasts (NHDF) and RAW cells incubated with this extract from healing moor.

The expression of RAGE, AGER1 and RCAN1 was determined by RT-qPCR. Total RNA was extracted using RNA isolation kit (Roboklon), retrotranscribed using the iScriptTM cDNA Synthesis Kit (Bio-Rad; Hercules, CA, USA), and the cDNA analysed using the SYBR Green Supermix (ThermoFischer) performed with a RT-qPCR Detection System (Analytik Jena). NO production was determined using the Griess method (Sigma-Aldrich, Deissenhofen, Germany) by measuring nitrite concentration based on sodium nitrite standard curve. 8-Isoprostanes and PGE2 were measures in RAW cells incubated with healing moor extract using 8-Isoprostane/PGE2 ELISA Kits (Cayman/Biomol, Germany). ROS in NHDF were determined by treating cells with 2, 7-dichlorodihydrofluorescein (DCFH-DA), various concentrations of AHE, and $H_2O_2$ where vitamin C and Trolox served as ROS inhibitors in positive controls. Fluorescent DCF formed by ROS and DCFH was mea-sured with FluoStar spectrofluorometer (BMG LabTechnologies Instruments Offenburg, Germany). AHE was solved for these studies in 100mg/mL PBS.

The skin microbiome test was performed in presence and in absence of the extract of healing moor product in accordance with DIN EN ISO 17516, DIN EN ISO 21149 and DIN EN ISO 16212. The growth behaviour of the selected microorganisms in direct and indirect contact in the presence of the test product was investigated in this part of the test. Microbiome biodiversity maintenance and skin protection in presence of the extract of healing moor were also investigated.

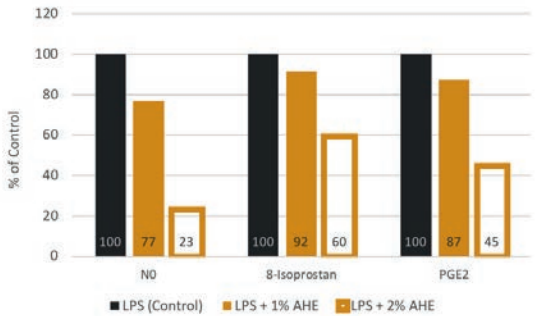


figure 1: Effect of 1% and 2% extract of alpine healing moor on NO, Isoprostane and PGE2 production in RAW cells. Data were normalised as percentage of negative control with LPS (100ng/ml). Results are expressed as means ± SEM from N=2

### Oxidative stress markers

Age-related functional losses and diseases are associated with accumulation of **reactive oxygen (ROS)** and nitrogen species[1], such as **nitrogen monoxide (NO)**. Most isoprostanes are produced by ROS that catalyse lipid peroxidation. Measurement of



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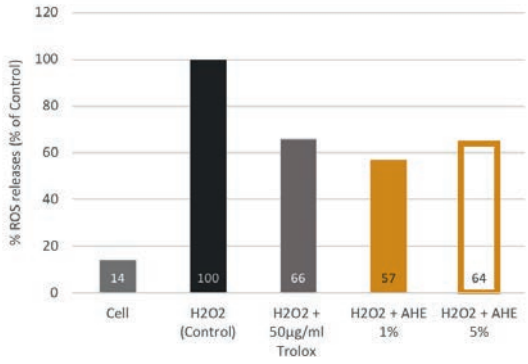


figure 2: Effect of 1% and 5% extract of healing moor on ROS production in NHDF cells measured by DCFH-DA. Values after 3h exposure to $H_2O_2$ were normalised to the value of the control with 100 µM of $H_2O_2$.

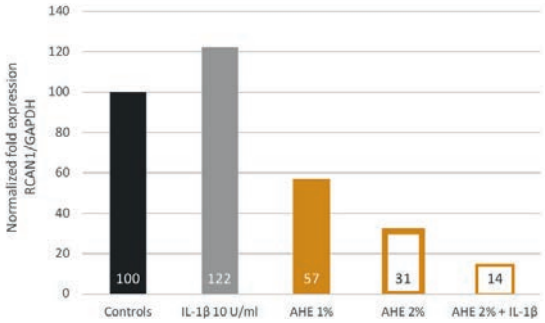


figure 3: Effect of 1% and 2% extract of healing moor on RANC1 expression. GAPDH was used as an internal control for normalisation.

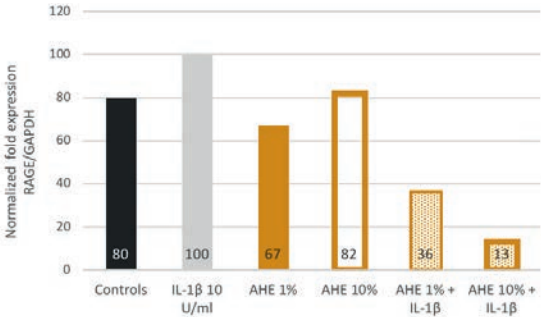


figure 4: Effect of healing moor extraction on gene expression of advanced glycation end products receptors (RAGE) in IL-1β stimulated NHDF cells. GAPDH was used as an internal control for normalisation and data were quantified by using the comparative cycle threshold Ct method. Results are expressed as means ± SEM

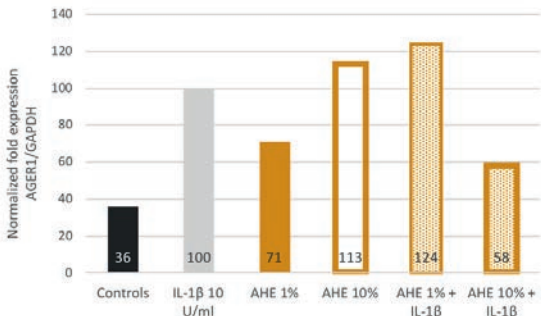


figure 5: Effect of healing moor extraction on gene expression of advanced glycation end products receptors (AGE-R1) in IL-1β stimulated NHDF cells. GAPDH was used as an internal control for normalisation and data were quantified by using the comparative cycle threshold Ct method. Results are expressed as means ± SEM

isoprostanes is an accurate way to assess oxidative stress in-vivo and can be correlated with numerous diseases[2]. **Prostaglandin E2 (PGE2)**-induced inhibition of collagen expression and promotion of **Matrix-Metalloprotease-1 (MMP1)**, respectively, are two further mechanisms related to ageing[3].

In this study, the effect of the extract of healing moor on **lipopolysaccaride (LPS)**-induced formation of NO, 8-Isoprostane and PGE2 in RAW cells (figure 1) was investigated. Treatment with the extract elicited a marked decrease in the formation of NO and 8-Isoprostane as compared to the LPS-treated controls. Additionally, the expression of PGE2 was reduced by half upon treatment with 2% extract. This data indicates that the extract exerts an anti-ageing effect due to the reduction of oxidative stress markers, such as NO and 8-Isoprostane, and PGE2.

## Slowdown

The reduction of oxidative stress by reactive oxygen species (ROS) scavengers followed by the delay of the age-associated decline in physiological processes and marked prolongation in the mean lifespan can be considered as a confirmation of the oxidative stress theory of ageing. Mitochondria are primary sites for ROS accumulation. **Regulator of calcineurin-1 (RCAN1)** regulates mitochondrial functions and increases the susceptibility to oxidative stress[4]. Here, it was determined whether the extract of healing moor produces a reduction in ROS as a marker of oxidative stress by using NHDF cells and $H_2O_2$ as a model of oxidative stress. We also investigated the effect of the extract on RCAN1 expression. Our data showed that the treatment with the extract has a significantly reductive effect on the formation of ROS compared to Trolox positive controls. Trolox is an antioxidant and a structural analogue of vitamin E (figure 2). Furthermore, a significant decrease in expression of RCAN1 could be observed (figure 3).

This data indicates that the extract is a potent inhibitor of ROS formation and can be used as an anti-ageing agent through the oxidative stress pathway.

## Counteracting the effects of AGEs

**Advanced glycation end products (AGEs)** originate from non-enzymatic reactions of proteins with reducing sugars. AGEs also seem to highly accumulate in extrinsically aged skin[5]. Previous studies reported that AGEs could accumulated in dermal elastin and collagens and interact non-specifically with the cell membrane of dermal fibroblasts[6]. Therefore, AGEs may play a role in skin ageing, since the binding of AGEs with their multiligand receptors, RAGEs, is known to induce oxidative stress and inflammatory responses. However, other AGE-specific cell surface receptors, such as AGE-R1, AGE-R2 and AGE-R3, counteract RAGE function. These receptors are involved in AGE-homeostasis[7], thus reducing AGE levels, and thereby suppressing oxidative stress and inflammation. Therefore, the effect of the extract on gene expression patterns of the different classes of AGE receptors was investigated. A significant decrease in expression of RAGE genes was observed when cells were incubated with the extract followed by interleukin 1β (IL-1β) (figure 4). Furthermore, a stable up-regulation of AGE-R1 (Figure 04b) upon incubation with the extract was also observed. These results suggest that the extract reduces AGE-induced oxidative stress and inflammatory response by influencing the expression of antagonistic AGE-receptors, RAGE (downregulation) and AGE-R1 (up-regulation).

## Preserving skin microbiome

First of all, the primary goal is that cosmetic products must not have a damaging effect on the skin's microbiome, meaning that the microorganisms must remain vital and the natural biodiversity and the protective function, which is built up and maintained by skin's microorganisms, remains unimpaired by the application of a product. Skin care products

figures: Premium Organic

containing extract of healing moor exhibited all of these properties in the test procedure.

Furthermore, a growth test showed that **both Staphylococcus and Corynebacterium species, which are central components of the skin microbiome system, grew faster** in the presence of the extract than in its absence. This can lead to a faster restoration of normal skin microbiome in dysbiosis or in neurodermatitis, where the skin flora is usually disrupted (figure 6). Considering these positive results, it would be conceivable to use the extract in the field of microbiome balancing.

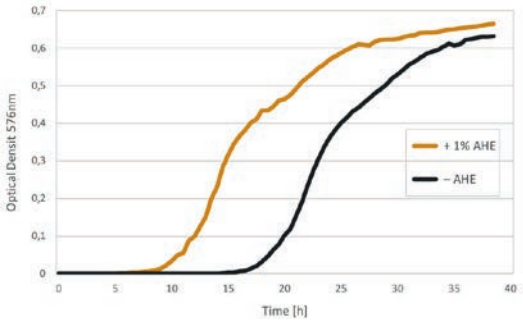


figure 6: Growth Curve of Styphylococcus epidermis with and without the extract of healing moor.

## Conclusion

We found the extract of healing moor to be involved in suppression of oxidative stress and preventing ageing factors. In addition, it shows to balance the skin microbiome and maintains skin microbiome diversity and vitality. □

References

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