IFSCC 2025 full paper (IFSCC2025-947)

"A Gentle Alternative to AHAs in Improving Desquamation"

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1. Introduction

Desquamation is the natural process of shedding dead skin cells, known as corneocytes, from the outermost layer of the skin, the stratum corneum. This renewal process helps maintain the skin's texture and barrier function. In addition, regulated desquamation reduces hyperpigmentation and fine lines, helps prevent clogged pores, improving skin appearance (1, 2).

The desquamation process is regulated by enzymes such as kallikrein-related peptidases (KLKs), which are mainly secreted from granular keratinocytes. These enzymes are serine proteases and exhibit trypsin- or chymotrypsin-like activities. KLKs are responsible for degrading protein structures linking corneocytes. Corneocytes are held together by corneodesmosomes and lipids, with proteins like desmogleins (DSG), desmocollins (DSC), and corneodesmosins (CDSN) playing key roles. The breakdown of corneodesmosomes by KLKs leads to the desquamation and exfoliation of the corneocytes. Effective regulation of KLKs is vital for maintaining skin health and preventing various skin conditions (1, 2).

Chemical peelings, such as alpha-hydroxy acids (AHAs), are widely recognized in skincare for their effective exfoliating properties (3, 4). AHAs, including glycolic acid (GA), citric acid (CA), malic acid (MA), tartaric acid (TA), and lactic acid (LA), are water-soluble acids derived from fruits and milk sugars and are considered as benchmark for skin desquamation in cosmetics. However, the application of AHAs can lead to side effects such as skin irritation, UV sensitivity, redness, and dryness. Incorrect usage or excessively high concentrations can exacerbate these side effects (4, 5).

To meet the high demand of the cosmetic industry for exfoliating agents and mitigate the side effects of chemical peelings such as AHAs, it is essential to find natural and gentle desquamation options. In our study, we aimed to investigate the impact of wild carrot (*Daucus carota subsp. carota*) on natural skin exfoliation. By optimizing KLK5 activity via our botanical extract, we anticipate improving the desquamation process. To evaluate this, we utilized an *ex vivo* desquamation model on skin explants and compared the effects of long-term wild carrot application with short-term treatment using AHAs.

2. Materials and Methods

2.1 Extract development

Wild carrot extract was prepared by extracting 2.5% dried wild carrot herb (*Daucus carota subsp. carota*) in 30 mM sodium dihydrogen phosphate, 9.5% glycerol aqueous solution for 2 h at 80°C. For the *in vitro* and *ex vivo* studies, the wild carrot extract was sterile filtered.

2.2 Gene expression analysis in NHEK

2.2.1 Cell cultivation and treatment

Normal human epidermal keratinocytes (NHEK) were cultured at 37°C and 5 % CO_2. Cells were treated or not (control) with different concentrations of wild carrot extract for 24 h. Replicates were pooled and harvested for mRNA extraction.

2.2.2 Gene expression analysis

mRNA was extracted and reverse transcribed into cDNA. qRT-PCR analysis was performed on genes selected for their importance in skin physiology and for mitochondrial function. Normalization was performed using house-keeping genes and the resulting gene expression data was compared to the untreated control.

2.3 Skin explant evaluation compared to AHAs treatment

3.1 Skin explants cultivation and treatment

Organotypic skin explants (donor: 35 years old, female, Caucasian) were cultured at 37°C and 5 % CO_2. They were treated once daily for 4 consecutive days with 0.5 % wild carrot extract. On day 5, another skin explant was treated topically for 3 min with a solution containing (25% v-v) a mix of alpha-hydroxy acids (AHAs): 15% glycolic acid, 5% lactic acid and 5% malic acid, as previously reported by Karwal et al (6). The control skin explant sample did not receive any treatment. 24 h after the last application of wild carrot extract and immediately after 3 min contact with AHAs, each explant was harvested and sampled for further evaluation. For tape strip sampling, the skin explants were gently dried, then a tape strip was applied to the surface of the explants and collected. Five strips were sampled consecutively from the same skin explants using a single application per tape strip (D-squame® D100, 22 mm diameter). The first strip was removed and strips 2 to 5 were used for further protein extraction. The strips were immediately stored at -80°C. The skin explants were divided into two parts for optimal cutting temperature (OCT) cryopreservation and snap freezing with liquid nitrogen. Skin samples were stored at -80°C until analysis.

2.3.1 Kallikrein 5 activity assay

Four tape strips (tape 2 to 5) were pooled for protein extraction using an optimized buffer. Samples were gently sonicated on ice. After quantification of protein content, the same amounts of protein were used for an enzymatic assay to determine KLK5 trypsin-like activity (Trypsin Activity Assay Kit, ab102531, Abcam). Absorbance (OD=405 nm) was recorded during the time of the assay and linear ranges of kinetics were used for analyses.

2.3.2 Immunofluorescence analysis

Skin explant sections of 5 µm thickness were cut from the OCT cryopreserved samples using a cryostat (Leica) and fixed. For immunofluorescence analysis, skin explant sections were blocked and incubated with either the primary antibody anti-desmoglein 1 (32-6000, Invitrogen) or anti-β-defensin 2 (ab63982, Abcam) or anti-aquaporin-3 (ab125219, Abcam). After washing,

the skin explant sections were incubated with the fluorophore-labeled secondary antibody (A21235, Invitrogen). Cell nuclei were labeled with diluted DAPI. Fluorescence images were captured using an epi-fluorescence microscope (EVOS M5000 Imaging System, ThermoFisher).

2.3.3 Protein carbonylation analysis

Skin explant sections of 5 µm thickness were cut from the OCT cryopreserved samples using a cryostat (Leica) and fixed. To analyze oxidative damage, carbonylated proteins in skin explant sections were labeled with a proprietary fluorescent probe (Ex = 647 nm / Em = 650 nm) functionalized to specifically bind to carbonyl moieties (7). Cell nuclei were labeled with diluted DAPI. Fluorescence images were captured using an epi-fluorescence microscope (EVOS M5000 Imaging System, ThermoFisher).

2.3.4 Analysis of ATP/ADP ratio

For ATP and ADP analysis, 10 mg of frozen tissue of each sample was crushed either for ATP or ADP analysis and prepared according to the manufacturer's instructions (ATP and ADP ELISA Kits, ATP: ab83355; Abcam, ADP: ab83539, Abcam). ATP and ADP levels were measured using the Varioskan system (ThermoFisher).

3. Results

3.1. Wild carrot extract affects desquamation, mitochondrial function and the organization of the epidermal extracellular matrix

Desquamation is the natural process of shedding corneocytes from the stratum corneum. This process is crucial for maintaining healthy skin as it promotes cell renewal and helps to remove dead skin cells, allowing new cells to surface. To examine the effects of wild carrot extract on the epidermis, gene expression analyses on keratinocytes were performed. Treatment of keratinocytes with the wild carrot extract induced the expression of kallikrein 5 (KLK5) and small leucine-rich proteoglycans (SLRPs) DCN and BGN (Figure 1). KLK5 is crucial for the desquamation process, whereas SLRPs contribute to the structural integrity and organization of the epidermal extracellular matrix. In addition, the wild carrot extract increased the expression of the mitochondrial genes UCP2, MIEF2 and NQO1 that are involved in regulating mitochondrial function, energy metabolism and oxidative stress response (Figure 1).

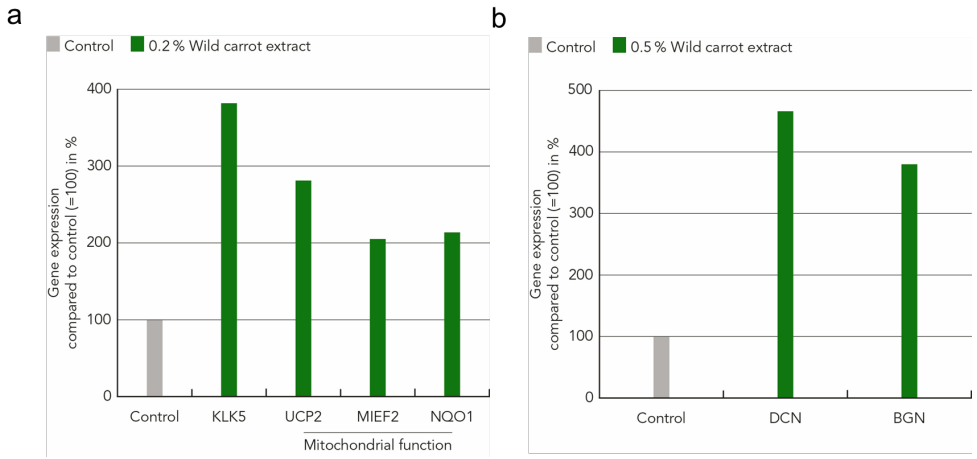

Figure 1. Wild carrot extract increases genes involved in desquamation, mitochondrial function and in the organization of the epidermal extracellular matrix. (a) Gene expression analysis of KLK5, UCP2, MIEF2, and NQO1 in keratinocytes treated with 0.2 % wild carrot extract. (b) Gene expression analysis of DCN and BGN in keratinocytes treated with 0.5 % wild carrot extract. Gene expression was normalized to control cells (100%).

3.2. Wild carrot extract induces the desquamation process

Since wild carrot extract has shown to upregulate the expression of KLK5, we aimed to investigate its effects in a desquamation model. Wild carrot extract was applied topically to skin explants for four days. In parallel, another skin explant was treated with benchmark AHAs for 3 minutes. After harvesting the samples, KLK5 activity and desmoglein-1 levels were analyzed. Application of 0.5 % wild carrot extract to the skin explant significantly increased KLK5 activity in the stratum corneum by 156 % compared to the untreated control (Figure 2). Treatment with 25 % AHAs had a similar effect, significantly increasing KLK5 activity by 115 % compared to the untreated control (Figure 2). Both treatments significantly increased the activity of KLK5, a serine protease involved in the final step of the natural skin exfoliation process.

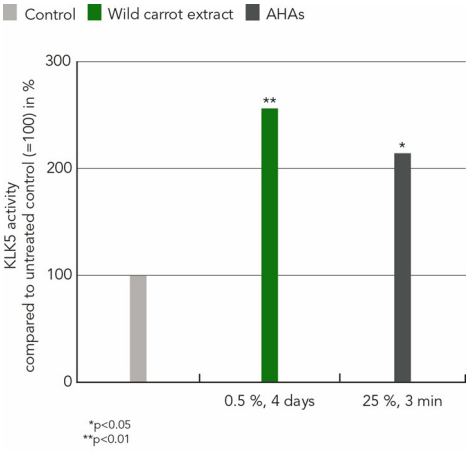

Figure 2. Wild carrot extract increases the activity of kallikrein 5 (KLK5). (a) KLK5 activity analysis of tape strips from skin explants treated with 0.5 % wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting.

In addition to KLK5 activity, desmoglein-1 levels were analyzed in skin explant sections by immunofluorescence. Desmoglein-1 is involved in cell-cell junctions in the upper layers of the epidermis and serves as a substrate for KLK5. It is associated with natural skin peeling as desmoglein-1 levels are reduced when KLK5 activity is increased. Application of wild carrot

extract significantly reduced desmoglein-1 by 26 % compared to the untreated control (Figure 3a). Treatment with 25 % AHAs significantly reduced desmoglein-1 by 46 %. These results are also shown in the representative images (Figure 3b). The reduction of desmoglein-1 in both treatments confirmed the activity of KLK5 and its exfoliating effect on the skin.

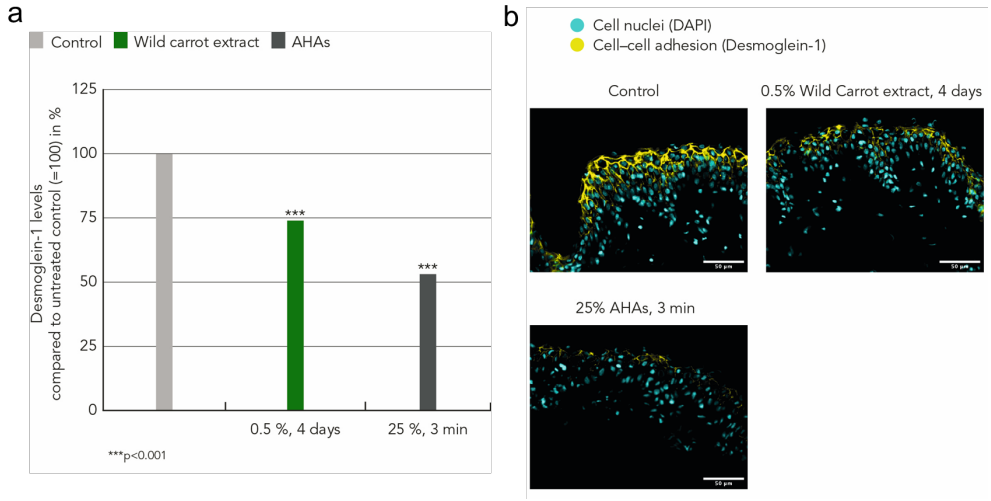

Figure 3. Wild carrot extract reduces desmoglein-1 levels. (a) Quantitative representation of desmoglein-1 levels from immunofluorescence analysis of skin explants treated with 0.5 % wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting. (b) Immunofluorescence images of desmoglein-1 levels from skin explants treated with 0.5 % wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting.

3.3. Compared to AHAs, wild carrot extract does not induce stress to the skin

Both the long-term application of wild carrot extract and the short-term treatment with AHAs have an exfoliating effect in the desquamation model. Therefore, we aimed to investigate the stress response of both applications to the skin. To this purpose, we analyzed oxidatively damaged (carbonylated) proteins and β-defensin 2 levels by immunofluorescence in the skin explant sections. Carbonylation levels are indicators of oxidative stress and skin irritation. Treatment with 25 % AHAs significantly increased carbonylation levels in whole skin by 203 % (Figure 4a). Spatial analysis of carbonylation in the skin explant showed increases of 108 % in the stratum corneum, 219 % in the epidermis and 162 % in the dermis. The wild carrot treatment showed a significantly reduced effect on carbonylation levels in the whole skin compared to the AHAs treatment, especially in the epidermis and dermis. Application of wild carrot extract increased whole skin carbonylation levels by 10 % (Figure 4a). Spatial analysis showed that carbonylation levels were reduced by 13 % in the stratum corneum and by 9 % in the dermis. Carbonylation levels in the epidermis were significantly increased by 62 %. These results are also shown in the representative images (Figure 4b). In comparison to AHAs, wild carrot extract had only a minor effect on protein damage in the skin. This mild increase may reflect a physiological adaptation without inducing overall oxidative damage.

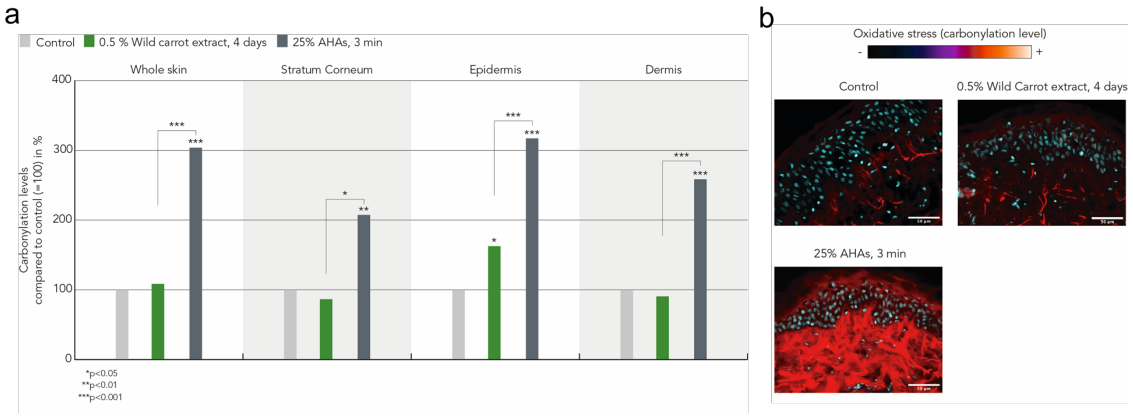

Figure 4. Compared to AHAs, wild carrot extract causes only a minor increase in protein damage. (a) Quantitative representation of carbonylation levels from immunofluorescence analysis of skin explants treated with 0.5 % wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting. (b) Immunofluorescence images of carbonylation levels from skin explants treated with 0.5 % wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting.

In addition to carbonylation, β-defensin 2 is a stress marker of skin inflammation and barrier disruption. Treatment with 25 % AHAs significantly increased β-defensin 2 by 23 % compared to the untreated control (Figure 5a). Application of wild carrot extract had no effect on β-defensin 2 levels in the skin (Figure 5a). These findings are also shown in the representative images (Figure 5b). These results confirm that compared to AHAs, wild carrot extract did not have an overall harmful effect on the skin.

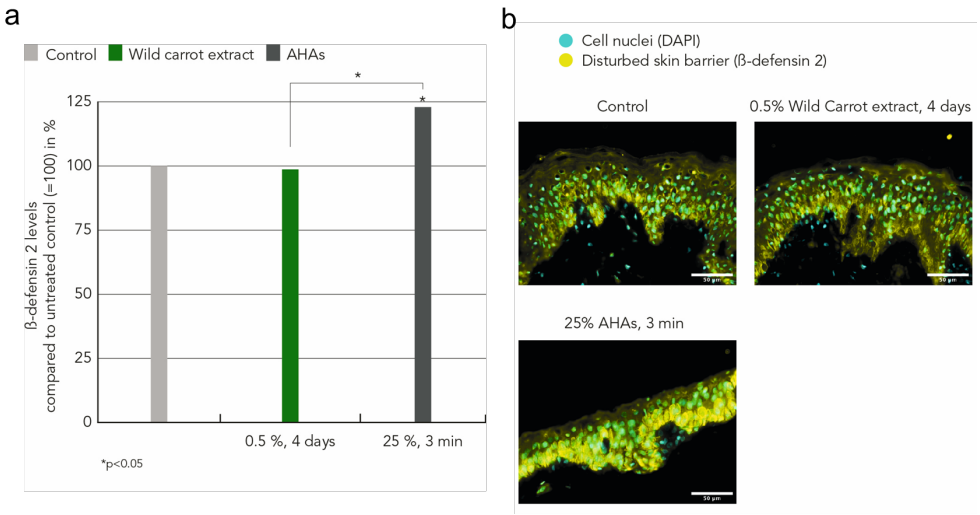

Figure 5. Compared to AHAs, wild carrot extract has no increase in β-defensin 2 levels. (a) Quantitative representation of β-defensin 2 levels from immunofluorescence analysis of skin explants treated with 0.5% wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting. (b) Immunofluorescence images of β-defensin 2 levels from skin explants treated with 0.5 % wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting.

3.4 Wild carrot extract improves energy metabolism and epidermal hydration

Since wild carrot extract was suggested to induce mitochondrial function via gene expression, the ATP/ADP ratio was analyzed as a parameter of cellular energy metabolism. An increased ATP/ADP ratio indicates high energy potential. Treatment with 0.5 % wild carrot extract resulted in a significant increase in the ATP/ADP ratio, indicating an improvement in energy

availability (Figure 6). On the other hand, the application of 25 % AHAs did not result in any significant change in the ATP/ADP ratio.

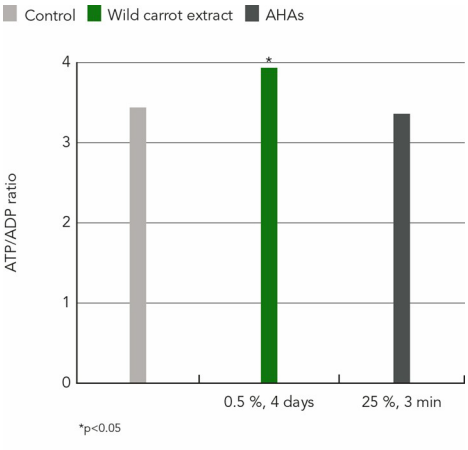

Figure 6. Wild carrot extract increases energy metabolism. ATP/ADP ratio analysis of skin explants treated with 0.5 % wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting.

The effect of both treatments on epidermal hydration was investigated. Aquaporin-3, which is involved in epidermal hydration, was analyzed. Treatment with 25 % AHAs significantly increased aquaporin-3 by 24 % compared to the untreated control (Figure 7a). Similarly, the application of wild carrot extract significantly increased aquaporin-3 by 38 %. These findings are also shown in the representative images (Figure 7b). These results suggest that the improved desquamation process leads to improved epidermal hydration.

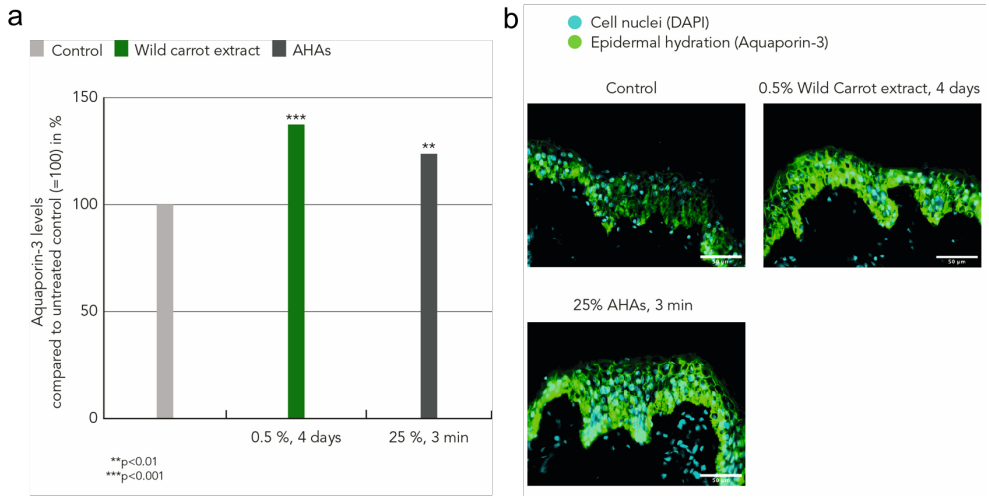

Figure 7. Wild carrot extract increases epidermal hydration. (a) Quantitative representation of aquaporin-3 levels from immunofluorescence analysis of skin explants treated with 0.5% wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting. (b) Immunofluorescence images of aquaporin-3 levels from skin explants treated with 0.5 % wild carrot extract for four consecutive days or with 25 % AHAs for 3 min prior to harvesting.

4. Discussion

Desquamation and exfoliation involve the removal of dead skin cells, promoting regeneration and achieving a smoother texture. These procedures are primarily used to reduce fine lines, treat acne scars, improve skin texture, and address hyperpigmentation. The skincare peeling market has experienced significant growth and is projected to continue evolving rapidly, driven by dynamic trends. Rising consumer interest in non-invasive aesthetic procedures and increasing awareness of skincare are fueling the demand for desquamation methods, such as chemical peelings. However, chemical peelings, such as AHAs, have limitations, including potential side effects, which can discourage amateur users. Potential opportunities in the market include the development of innovative products, such as gentler peeling active ingredients. The integration of organic and natural ingredients can cater to the growing trend towards clean and natural beauty.

In our research, we evaluated the effect of wild carrot (*Daucus carota subsp. carota*) extract on keratinocytes and found that it increased the expression of KLK5, which is believed to be the main activator of the skin desquamation process. KLK5 can cleave all three proteins from the corneodesmosome, desmoglein-1 (DSG1), desmocollin-1 (DSC1), and corneodesmosin (CDSN), thereby removing dead corneocytes. We then utilized an *ex vivo* desquamation model to investigate the effects of long-term wild carrot application compared to short-term treatment with AHAs. The wild carrot application increased the enzymatic activity of KLK5 and reduced the protein levels of desmoglein-1, confirming its exfoliation effect on the skin. The short-term AHAs treatment had a similar effect on desquamation. To compare the skin irritation potential, we additionally investigated the carbonylation and β-defensin 2 levels in the same desquamation model. Carbonylation is a marker for damage, whereas β-defensin 2 indicates skin inflammation and irritation. Interestingly, compared to AHAs, the long-term wild carrot application had only minor skin-damaging effects and did not increase β-defensin 2 levels, whereas AHAs had a strong irritating effect, as demonstrated by increased carbonylation and β-defensin 2 levels. Previous research and gene expression analysis demonstrated that the wild carrot extract has a beneficial effect on mitochondrial function. We further investigated energy metabolism by analyzing ATP/ADP ratios and found that only the wild carrot application improved energy metabolism. This finding suggests that the wild carrot extract enhances epidermal renewal and regeneration by boosting mitochondrial function, leading to improved metabolism. Finally, our *ex vivo* desquamation study also demonstrated that both AHAs and wild carrot extract increased epidermal hydration by elevating aquaporin-3 levels.

In the future, we aim to perform a placebo-controlled randomized clinical study to investigate the effect of wild carrot extract on skin renewal and appearance *in vivo*.

5. Conclusion

Our research indicates that wild carrot extract is a promising natural alternative to chemical peelings like AHAs for promoting skin desquamation. The extract enhances KLK5 activity, leading to effective exfoliation while having a minor effect on skin irritation and inflammation compared to AHAs. Additionally, wild carrot extract improves mitochondrial function and energy metabolism, contributing to better epidermal renewal and regeneration. Both AHAs and wild carrot extract increase epidermal hydration, but the latter offers a gentler approach for skin peeling with fewer side effects. To our knowledge, this study is the first to demonstrate the potential of *Daucus carota subsp. carota* extract as a natural exfoliating agent. Therefore, wild carrot extract represents a promising active ingredient for desquamation, providing a gentle solution for clean and natural beauty.

6. Acknowledgements

The authors would like to thank the development team at Mibelle Biochemistry for the optimization of the wild carrot extract.

7. References

1. Nauroy P, Nyström A. Kallikreins: Essential epidermal messengers for regulation of the skin microenvironment during homeostasis, repair and disease. Matrix Biol Plus. 2019 Nov 21;6-7:100019. doi: 10.1016/j.mbplus.2019.100019. PMID: 33543017; PMCID: PMC7852331.
2. Zani MB, Sant'Ana AM, Tognato RC, Chagas JR, Puzer L. Human Tissue Kallikreins-Related Peptidases Are Targets for the Treatment of Skin Desquamation Diseases. Front Med (Lausanne). 2022 Mar 4;8:777619. doi: 10.3389/fmed.2021.777619. PMID: 35356049; PMCID: PMC8959125.
3. Landau M, Bageorgeou F. Update on Chemical Peels. Dermatol Clin. 2024 Jan;42(1):13-20. doi: 10.1016/j.det.2023.06.005. Epub 2023 Jul 23. PMID: 37977680.
4. Almeman AA. Evaluating the Efficacy and Safety of Alpha-Hydroxy Acids in Dermatological Practice: A Comprehensive Clinical and Legal Review. Clin Cosmet Investig Dermatol. 2024 Jul 16;17:1661-1685. doi: 10.2147/CCID.S453243. Erratum in: Clin Cosmet Investig Dermatol. 2024 Aug 30;17:1973-1974. doi: 10.2147/CCID.S493491. PMID: 39050562; PMCID: PMC11268769.
5. Tang SC, Yang JH. Dual Effects of Alpha-Hydroxy Acids on the Skin. Molecules. 2018 Apr 10;23(4):863. doi: 10.3390/molecules23040863. PMID: 29642579; PMCID: PMC6017965.
6. Karwal K, Mukovozov I. Topical AHA in Dermatology: Formulations, Mechanisms of Action, Efficacy, and Future Perspectives. Cosmetics. 2023; 10(5):131. https://doi.org/10.3390/cosmetics10050131
7. Baraibar MA, Ladouce R, Friguet B. Proteomics quantification & identification of carbonylated proteins upon oxidative stress and during cellular aging. J Proteomics. 2013 Oct 30;92:63-70. doi: 10.1016/j.jprot.2013.05.008.