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abstract

Although some skin-lightening cosmetic raw materials already exist, their effectiveness particularly on human tyrosinase has not been directly measured in every case or has not shown convincing performance in-vitro. Some active ingredients are even irritating to the skin and are restricted accordingly by some cosmetics legislations. The search for new, skin-friendlier skin-lightening raw materials has led to the development of promising candidates in recent years. Here we present a barley germ extract containing a new class of active ingredients - hordatines - with superior tyrosinase inhibition properties. The great potential of these molecules was revealed with the help of artificial intelligence by molecular docking to the tyrosinase structure.

Introduction

Skin pigment variations and colour are among the most important determinants of our skin's appearance. Even if they are interpreted differently by different ethnic groups, an even skin tone is generally regarded as a sign of health, well-being and beauty.

Skin colour is based on genetic differences that have developed depending on people's geographical location and access to vitamin D synthesis due to UV-B radiation [1]. At the equator with high (UV) solar radiation, skin colour is very dark, whereas in the far north it is very light. The current theory of evolution states that a balance must be established between UV-B-dependent vitamin D synthesis and the protection of DNA from UV radiation [2].

Skin colour was judged very differently depending on the time and culture. In Europe, at times it was fashionable to have a very pale skin colour, at other times it was fashionable to be tanned. It always reflected the ability of the upper classes not to be forced to work hard physically and made their status in society apparent through skin colour. In some parts of Asia, having the lightest possible skin colour has long been an expression of beauty. However, as today's digital generation grows up, a certain change is taking place. Instead of following a general social trend, the new generation is celebrating difference, individuality and diversity.

Nevertheless, there is a desire to optimise the skin tone, but away from "simple brightening to the limits" and towards the aspects of healthy skin such as radiance, luminosity, brightness and reduction of redness. All these attributes are an expression of a wellbeing lifestyle that you can see on the skin of the person.

Skin and hair colour depend on two types of melanin. Melanocytes are able to synthesise a black pigment called eumelanin and a light brown/reddish pigment called pheomelanin. The individual combination of the two pigments accounts for the marvellous variety from dark skin to light skin and from black hair to blond or red hair. The central step is the formation of L-DOPAquinone from L-tyrosine **(Figure 1)**. This is done by the

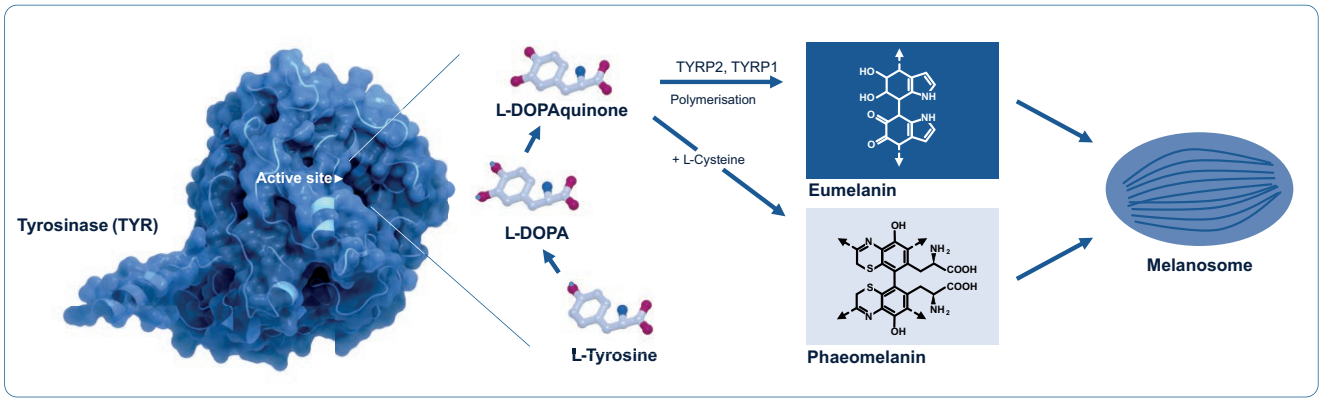


Fig. 1 Tyrosinase is the key enzyme that determines the pigmentation of the skin and the intensity of pigment spots.

enzyme tyrosinase. L-DOPAquinone is finally converted to eumelanin by the enzymes TYRP1 and TYRP2 or to pheomelanin with the help of L-cysteine. Both pigments are transferred to the keratinocytes via melanosomes.

The inhibition of tyrosinase is the central mechanism of action for lightening the skin. The active site of the enzyme is blocked by an inhibitor that binds to the same location as the substrate would do. The substrate (here: L-tyrosine) then no longer has access to the enzyme and cannot be converted. Known inhibitors in cosmetics include hydroquinone or arbutin, kojic acid, azelaic acid, glabridin and others (see **Table 1**). However, some of these substances are banned or restricted in some markets and can irritate the skin.

The extract from barley sprouts (ILLUMISCIN®-GLOW; Hordeum Vulgare Extract) adds a new, previously unknown but highly effective class of compounds: hordatines. These act as competitive inhibitors of tyrosinase, which are very gentle on the skin. Hordatines **(Figure 7)** are formed by the dimerisation of hydroxycinnamic acid agmantins, e.g. p-cumarylagmantin and feruloylagmantin in various combinations [3]. They have an L-tyrosine or L-DOPA-like head group that fits perfectly into the active site of the human tyrosinase. It is a family of substances that differs only in variations of methyl groups and hydroxyl groups at certain positions of the backbone [4]. Molecular modelling and tyrosinase inhibition assays suggest that hordatines are a very effective new class of tyrosinase inhibitors (see results section).

Materials and methods

To evaluate the efficacy of Hordeum Vulgare Extract *in-vivo*, an emulsion containing 1% Hordeum Vulgare Extract or no active ingredient (placebo) was applied to the face twice daily for 56 days. Skin pigmentation was assessed using mexametry, colourimetry and VISIA-CR. In addition, skin colour was assessed objectively and subjectively using questionnaires. The area of pigmentation was measured using VISIA-CR. Skin redness and ITA° values were measured by colourimetry and VISIA-CR. The test panel consisted of 64 Chinese men and women aged 30 - 60 years. The study was conducted in spring in East-central China.

Inhibition of tyrosinase: melanoma cells were stimulated with 400 µM IBMX (3-isobutyl-1-methylxanthine) and incubated for 72 hours with Hordeum Vulgare Extract at various concentrations. IBMX is a proven and effective stimulator of melanin synthesis. It increases the amount of tyrosinase in the melanocytes. In parallel, a non-stimulated control was performed. The cells were washed in PBS and the tyrosinase was extracted in PBS-Triton X-100. The enzymatic activity was measured at 540 nm using L-DOPA as substrate and adding different concentrations of Hordeum Vulgare Extract. This assay is comparable to a cell-free enzymatic assay.

Melanin synthesis: B16 melanoma cells were stimulated with 0.1 µM NDP-MSH (afamelanotide) and incubated for 72 hours with Hordeum Vulgare Extract at various concentrations. NDP-MSH is a stronger variant of the natural hormone α-MSH (melanocyte-stimulating hormone), which increases tyrosinase expression. A non-stimulated control was performed in parallel. The absorbance at 405 nm was measured and compared with a melanin standard curve.

Molecular modelling: For this approach, the Selnergy® platform (Greenpharma) was used with an AI-optimised 3D model of human tyrosinase. Known inhibitors and hordatines were modelled into the active site and the binding energies were calculated based on the inhibitor-protein interactions.

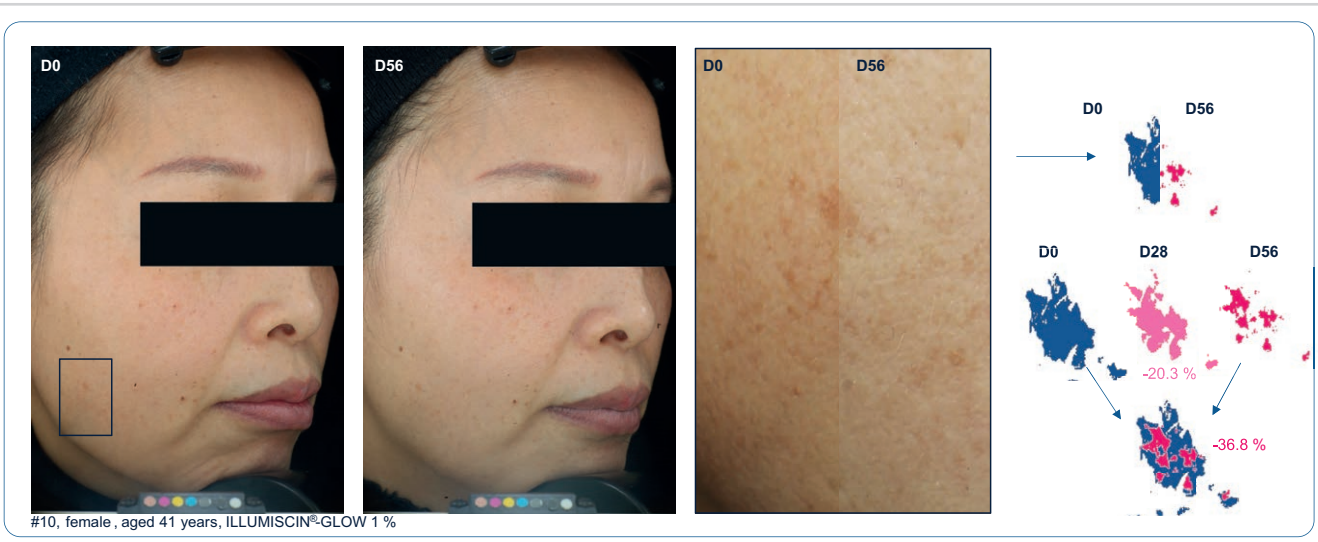


Fig. 2 Hordeum Vulgare Extract lightens pigmentation spots and the surrounding skin. Right panels: The marked section in the image on the far left has been divided into two halves: Left before treatment, right after treatment. The lightening effect after 56 days is clearly visible, enhanced in the panel on the far right using pixel analysis on a pigment spot.

Results

For the studies, an aqueous Hordeum Vulgare Extract was used, which is produced from the root seedlings of malting barley. These root seedlings are separated for the brewing process of beer and usually discarded. The resulting product from the extraction, ILLUMISCIN®-GLOW, is therefore an upcycling product that repurposes otherwise neglected plant material to provide valuable ressources for the cosmetic industry.

***In-vivo* efficacy:** 1% Active ingredient and placebo were applied to the face for 56 days in an emulsion. Measurements were taken after 28 and 56 days. 1% Hordeum Vulgare Extract significantly reduced the total area of pigmented spots by 36% measured with VISIA-CR **(Figure 2)**. The visibility of pigmented spots was significantly reduced and the brightness of the skin was significantly improved. The overall reduction in the pigmentation area in the entire panel was almost 15%. The active ingredient was four times more effective than placebo after 28 days and twice as effective after 56 days **(Figure 3)**.

The skin-lightening effect was confirmed in an objective visual examination and underpinned by various measurement methods (colourimeter, mexameter, VISIA-CR). A 20% reduction in skin pigmentation was visibly observed, significantly more than baseline and placebo (not shown). The lightening effect of 1% active ingredient was not only limited to pigment spots, but was also visible to the surrounding skin. **Figure 4** shows the summary of the colour parameters in the Lab* colour space. The changes in the L* and b* values resulted in an improvement in the ITA° value of over 3 units for pigment spots and surrounding skin. Placebo had no effect or even in some cases the opposite effect. This is probably due to the increasing sun exposure during the study period and the resulting increase in natural tanning (January to March). A reduction in reddening of the skin is also evident, which suggests that the active ingredient is extremely skin-friendly. This was also confirmed in a separate dermatological test on 30 people with sensitive skin.

In addition to the pure skin-lightening effect of Hordeum Vulgare Extract, there was also an increase in skin luminosity, radiance and an improvement in the feeling of the skin. In addition to the instrumental measurements, this was impressively demonstrated in the subjective survey of the study participants. In almost all parameters relating to skin feel, radiance and brightening

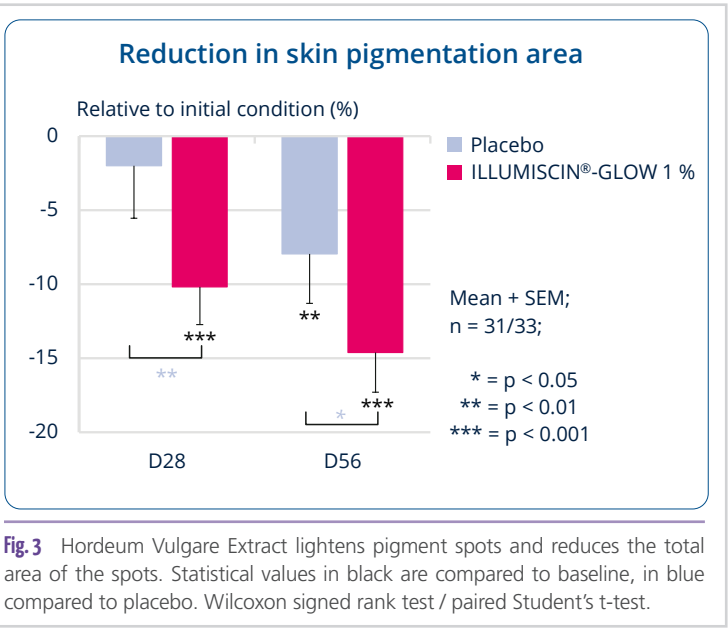
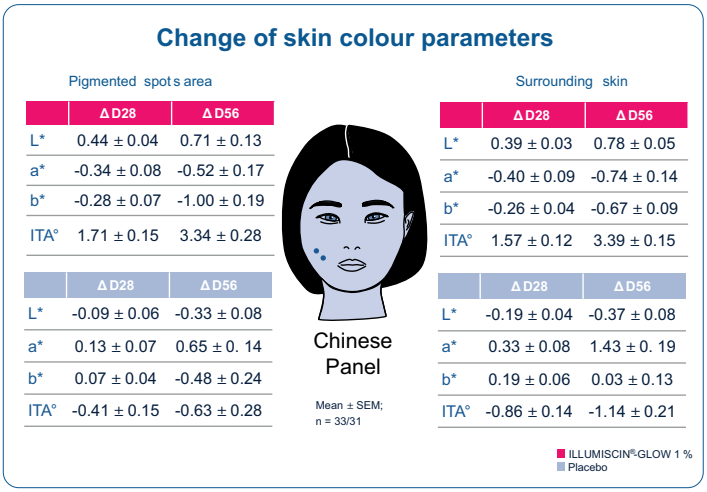


Fig. 3 Hordeum Vulgare Extract lightens pigment spots and reduces the total area of the spots. Statistical values in black are compared to baseline, in blue compared to placebo. Wilcoxon signed rank test / paired Student's t-test.



Pigmented spot s area	Δ D28	Δ D56
L*	0.44 ± 0.04	0.71 ± 0.13
a*	-0.34 ± 0.08	-0.52 ± 0.17
b*	-0.28 ± 0.07	-1.00 ± 0.19
ITA°	1.71 ± 0.15	3.34 ± 0.28

	Δ D28	Δ D56
L*	-0.09 ± 0.06	-0.33 ± 0.08
a*	0.13 ± 0.07	0.65 ± 0. 14
b*	0.07 ± 0.04	-0.48 ± 0.24
ITA°	-0.41 ± 0.15	-0.63 ± 0.28

Surrounding skin	Δ D28	Δ D56
L*	0.39 ± 0.03	0.78 ± 0.05
a*	-0.40 ± 0.09	-0.74 ± 0.14
b*	-0.26 ± 0.04	-0.67 ± 0.09
ITA°	1.57 ± 0.12	3.39 ± 0.15

	Δ D28	Δ D56
L*	-0.19 ± 0.04	-0.37 ± 0.08
a*	0.33 ± 0.08	1.43 ± 0. 19
b*	0.19 ± 0.06	0.03 ± 0.13
ITA°	-0.86 ± 0.14	-1.14 ± 0.21

Fig. 4 Hordeum Vulgare Extract lightens the skin and reduces yellow and red tones. Placebo has the opposite effect. Active ingredient was significantly superior tobaseline and placebo in all cases ($p < 0.01$). Wilcoxon signed rank test / paired Student's t-test.

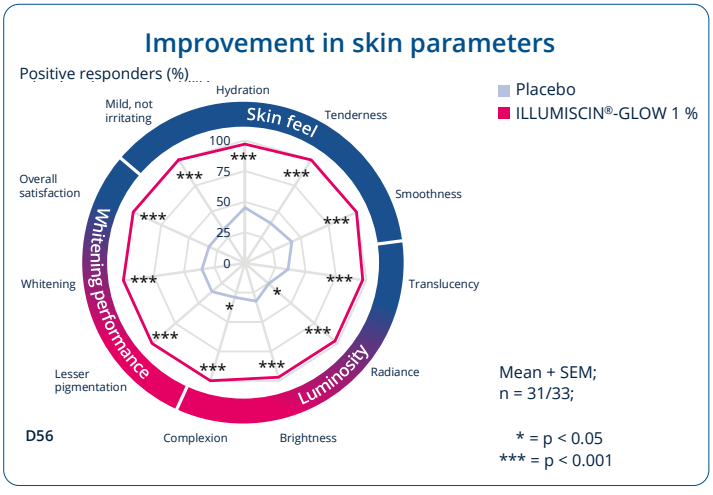


Fig. 5 Hordeum Vulgare Extract has an impressive efficacy performance (subjective survey). It improves the skin's appearance with a 3-fold effect in terms of skin feel, luminosity and brightening. Significance compared to initial value and placebo. Paired Student's T-test.

power, 100% of the participants were convinced of the effect, compared with only 25 – 40% for placebo **(Figure 5)**.

***In-vitro* efficacy:** Hordeum Vulgare Extract showed a clear concentration-dependent effect on the inhibition of tyrosinase and melanin production **(Figure 6)**. The extract inhibited melanin production with an IC_{50} of 0.22%. When using 2.5% extract, the tyrosinase inhibition was 65%. At 1%, the inhibition was comparable to 3 mM kojic acid used as a positive control. The amount (dry mass) of 3 mM kojic acid corresponds to the dry mass of 1% Hordeum Vulgare Extract.

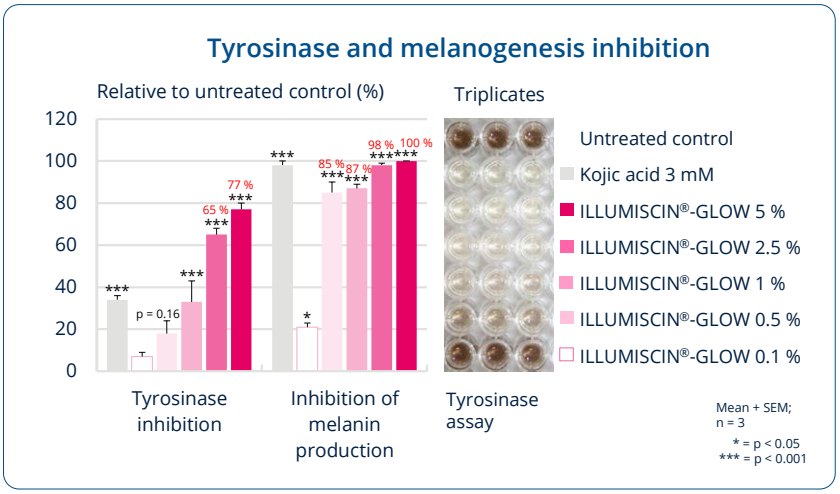


Fig. 6 Hordeum Vulgare Extract inhibits tyrosinase and melanin synthesis. Right: The brown colour of the melanin in the tyrosinase assay decreases with increasing concentration of the active ingredient. Unpaired Student's T-test.

***In-silico* study:** There are significant differences between the commonly used mushroom tyrosinase to determine inhibition and the human analog [5]. Therefore, it is important to determine the binding properties of potential tyrosinase inhibitors explicitly using human tyrosinase. Since an X-ray crystal structure of human tyrosinase is not currently available, it was modelled based on a bacterial tyrosinase structure using the AI model of human tyrosinase created by Alpha Fold [6,7]. The binding values of hordatines from Hordeum Vulgare Extract and known tyrosinase inhibitors were modelled (AI tool Selnergy® from Greenpharma) and compared with literature values **(Table 1)**. Hordatines turned out to be promising candidates that fit perfectly into the active site of human tyrosinase. As shown in **Figure 7**, hordatine C2 forms multiple interactions with amino acids in the active site region and binds and occupies both copper ions, rendering the tyrosinase inactive. Hordatines are classic competitive inhibitors, as are other known tyrosinase inhibitors such as kojic acid or Thiamidol® (Beiersdorf). Based on the binding values, hordatines can be assumed to have a similar inhibitory effect on human tyrosinase as Thiamidol®.

Inhibitor	IC_{50} on mushroom tyrosinase	IC_{50} on human tyrosinase	Binding value
Arbutin	40 µM[8]	> 4000 µM[5]	6.10
L-ascorbic acid	> 20 µM[9]	> 100 µM[9]	6.60
Azelaic acid	~1000 µM[10]	n.d.	7.45
Glabridin	11.4 µM[11]	n.d.	5.69
Kojic acid	6 µM[12]	500 µM[5]	6.70
Thiamidol®	108 µM[5]	1.1 µM[5]	6.44
Tranexamic acid	n.d.	n.d.	7.05
Hordatin A	n.d.	n.d.	8.95
Hordatin C2	n.d.	n.d.	9.52
Hordatin C	n.d.	n.d.	10.28

n.d.: no data found in literature

Table. 1 Inhibition values for various tyrosinase inhibitors. The binding value indicates the calculated binding strength based on in-silico modelling.

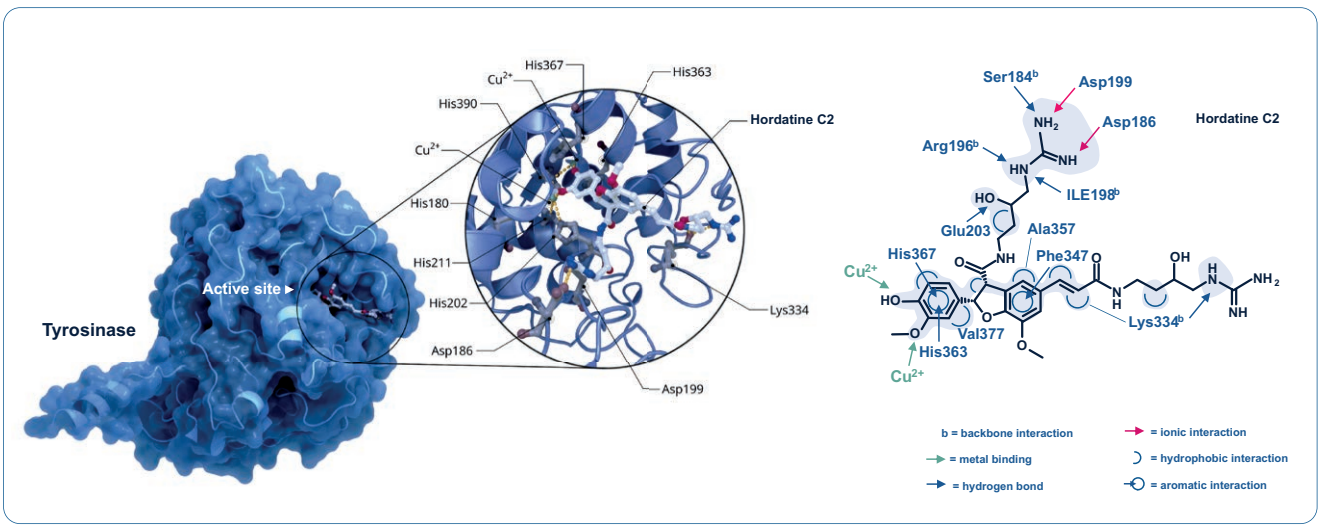


Fig. 7 Tyrosinase is the key enzyme that determines the pigmentation of the skin and the intensity of pigment spots.

Discussion

Hordeum Vulgare Extract (ILLUMISCIN®-GLOW, RAHN-Cosmetic Actives) provides a new class of tyrosinase inhibitors for cosmetics that goes beyond pure "whitening" and gives the skin new radiance and a good skin feel. Its outstanding effectiveness was confirmed in further studies in a multi-ethnic study panel with Asian, African, Indian and European test subjects. ILLUMISCIN®-GLOW is therefore effective for all skin types, ages, genders and ethnicities. A multi-functional product for a multi-ethnic and diverse world.

RAHN-Cosmetic Actives - unveiling the secrets of beauty

References:

[1] *Del Bino S, Duval C, Bernerd F.* Clinical and Biological Characterization of Skin Pigmentation Diversity and Its Consequences on UV Impact. Int J Mol Sci 2018, 19.

[2] *Jablonski NG, Chaplin G.* Colloquium paper: human skin pigmentation as an adaptation to UV radiation. Proc Natl Acad Sci U S A 2010, 107 Suppl 2: 8962-8968.

[3] *Pihlava J-M, Kurtelius T, Hurme T.* Total hordatine content in different types of beers. Journal of the Institute of Brewing 2016, 122: 212-217.

[4] *Becker D, Permann S, Bakuradze T, Stegmüller S, Richling E.* Isolation and Characterisation of Hordatine-Rich Fractions from Brewer’s Spent Grain and Their Biological Activity on α-Glucosidase and Glycogen Phosphorylase &alpha. Sustainability 2022, 14: 8421.

[5] *Mann T, Gerwat W, Batzer J, Eggers K, Scherner C, Wenck H, et al.* Inhibition of Human Tyrosinase Requires Molecular Motifs Distinctively Different from Mushroom Tyrosinase. Journal of Investigative Dermatology 2018, 138: 1601-1608.

[6] *Jumper J, Evans R, Pritzel A, Green T, Figurnov M, Ronneberger O, et al.* Highly accurate protein structure prediction with AlphaFold. Nature 2021, 596: 583-589.

[7] *Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, et al.* AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucleic Acids Research 2021, 50: D439-D444.

[8] *Jin YH, Lee SJ, Chung MH, Park JH, Park YI, Cho TH, et al.* Aloesin and arbutin inhibit tyrosinase activity in a synergistic manner via a different action mechanism. Archives of pharmacal research 1999, 22: 232-236.

[9] *Nokinsee D, Shank L, Lee VS, Nimmanpipug P.* Estimation of Inhibitory Effect against Tyrosinase Activity through Homology Modeling and Molecular Docking. Enzyme Res 2015, 2015: 262364.

[10] *Wang Z, Xiang H, Dong P, Zhang T, Lu C, Jin T, et al.* Pegylated azelaic acid: Synthesis, tyrosinase inhibitory activity, antibacterial activity and cytotoxic studies. Journal of Molecular Structure 2021, 1224: 129234.

[11] *Jirawattanapong W, Saifah E, Patarapanich C.* Synthesis of glabridin derivatives as tyrosinase inhibitors. Arch Pharm Res 2009, 32: 647-654.

[12] *Curto EV, Kwong C, Hermersdörfer H, Glatt H, Santis C, Virador V, et al.* Inhibitors of mammalian melanocyte tyrosinase: in vitro comparisons of alkyl esters of gentisic acid with other putative inhibitors. Biochemical pharmacology 1999, 57: 663-672.

authors

Stefan Hettwer, Emina Besic Gyenge, Loya Schoeffel, Brigit Suter, Barbara Obermayer

Corresponding author: Stefan Hettwer | RAHN AG

Dörflistrasse 120 | Switzerland

phone +41443254200

stefan.hettwer@rahn-group.com