SCLAREOLIDE, THE NATURAL ACTIVE COMBATING HYPERPIGMENTATION AND SPOTS

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SUMMARY

Skin lightening has come a long way from the basic 'whitening' approach, aimed at ethnic consumers with darker skin to new targets like illuminating skin and spot-targeting to reduce the appearance of brown / age spots.

During our continuous screening for new skin lighteners, we discovered sclareolide, a sesquiterpene lactone obtainable from clary sage (*Salvia sclarea* L.), as highly potent active. Furthermore, due to inhibition of cytokine gene expression and biosynthesis sclareolide is capable to protect against postinflammatory hyperpigmentation. These properties make sclareolide an attractive material for deo, depilation, and acne applications but also the day cream to protect against harming influences all day long.

Clary sage and other Salvia species contain sclareol in stems, leaves and flowering parts. Biotransformation of sclareol results in sclareolide. By subsequent extraction and concentration processes sclareolide is obtained in high purity. As sclareolide is used since decades for fragrance production and in fragrance mixtures the safety profile is well-known and the material is a China registered cosmetic ingredient.

INTRODUCTION

Skin lightening has come a long way from the basic 'whitening' approach, aimed at ethnic consumers with darker skin to new targets like illuminating skin and spot-targeting to reduce the appearance of brown / age spots. For many consumers uneven skin tone can be a source of shame. 58% of consumers feel bad with an uneven skin tone [1]. The demand for skin perfection is rising in women of all ages.

For these purposes it is important to understand the pigmentation mechanisms in the epidermis. It is well accepted that tyrosinase inhibitors in cosmetics contribute to the reduction of the pigmentation of the skin. But to protect against the formation of freckles and melasma the cross-talk of keratinocytes and melanocytes has to be taken into account. The stimuli resulting in this undesirable inhomogeneous pigmentation are divers: UV irradiation is a well-known pigmentation enhancer; acne scars and depilation or shaving lesions are increasingly demanding effective products [2]; but also air pollution was identified recently as a major inducer of lentigines [3,4]. All stimuli unify the underlying mechanism of keratinocytes releasing a bunch of cytokines triggering melanocytes to proliferate, differentiate, produce melanin and transfer melanosomes to keratinocytes [5], with the most prevalent mechanism known as postinflammatory hyperpigmentation (PIH). As pigment spots may only resolve over a protracted period

of time if left untreated [6] a protection against PIH combined with a depigmenting effect is the most promising cosmetic treatment.

MATERIALS AND METHODS

Skin pigmentation *in vitro*: B16V melanoma cells were seeded into a 96-well microtitre plate. After adherence, various concentrations of the test substances and 10 nM α-MSH (α-melanocyte stimulating hormone) were added. After 96h incubation, alkaline extraction of the cellular melanin was performed, the absorption was measured at 400 nm.

Skin pigmentation *ex vivo*: Full thickness human skin explants (7 x 3 mm; Ø x thickness) obtained from abdominal plastic surgery were transferred into culture plates and placed on a cotton pad, soaked with culture medium. 4-6 skin explants were used per test concentration and placebo. Sclareolide formulated into a hydrodispersion gel was topically applied once per day. On day 6, histological sections were prepared and melanin was quantified by image analysis after Fontana-Masson staining. Kojic acid served as positive control and was applied at a concentration of 0.1% in DMSO.

Interleukin-1α *in vitro*: HaCaT keratinocytes were seeded into a 96-well microtitre plate. After adherence, various concentrations of the test substances were added and incubated for 1h. Interleukin-1 (IL-1) α biosynthesis was stimulated by addition of 0.4 µM A23187 Calcium Ionophore. After 6h cells were lysed and intracellular IL-1α was analysed by ELISA. Dexamethasone served as positive control.

PIH gene array: HaCaT keratinocytes were seeded into 12well plates. After adherence, 50 µM Sclareolide were added and incubated for 2h. For the irradiation with 20 mJ/cm² UVB (UVITEC Cambridge, absorption maximum at 312 nm) medium was exchanged by phosphate buffered saline. Subsequently medium containing sclareolide was added. After 24h incubation RNA was prepared and endothelin-1 (EDN1), chemokine (C-X-C motif) ligand 1 (CXCL1), interleukins (ILs) IL-6 and IL-8 genes were quantified by qRT-PCR.

RESULTS

Sclareolide showed a potent lightening effect when tested on B16V melanoma cells *in vitro* with an IC_{50} of 10.2 µM (Fig. 1A). In the next step, sclareolide was tested on *ex vivo* human skin (photo-type intermediate) dosed at 0.1% in a hydrodispersion gel formulation. A significant lightening effect was observed with a reduction of 41% versus placebo (Fig. 1B). In comparison the positive control kojic acid applied at 0.1% in DMSO reduced melanin content by 30%.

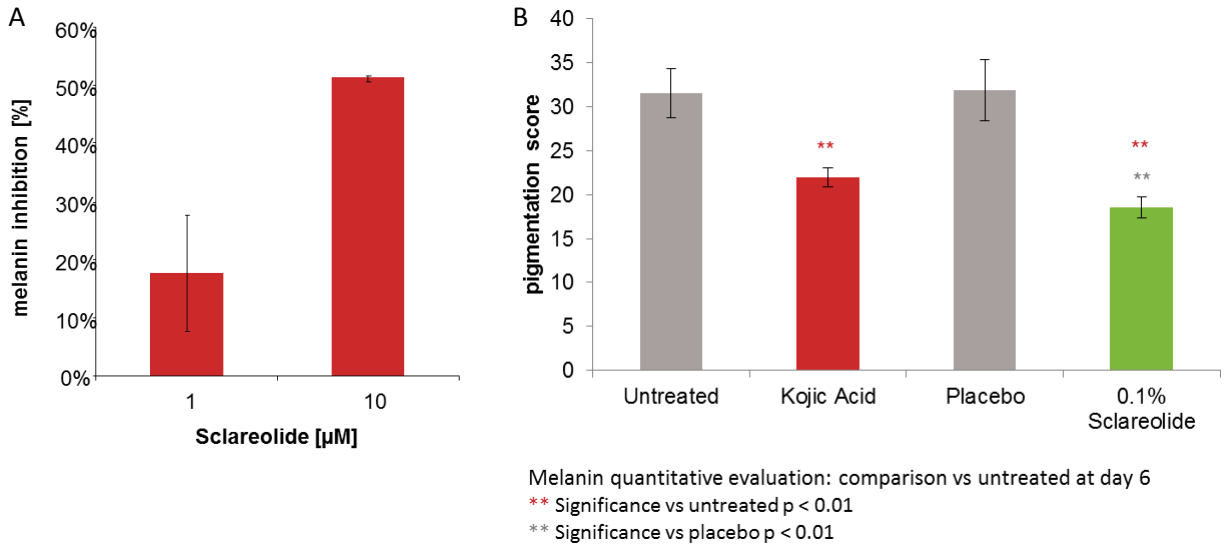

Fig. 1: Inhibition of melanogenesis: Sclareolide inhibited melanin production in B16V cells *in vitro* (A). Melanin in human *ex vivo* skin explants was reduced by 0.1% Sclareolide applied in a gel formulation after 6 days; positive control kojic acid was applied 0.1% in DMSO (B).

Additionally sclareolide showed a potent dose-dependent IL-1α inhibitory activity with an IC_{50} of 56.5 µM on HaCaT keratinocytes (Fig. 2).

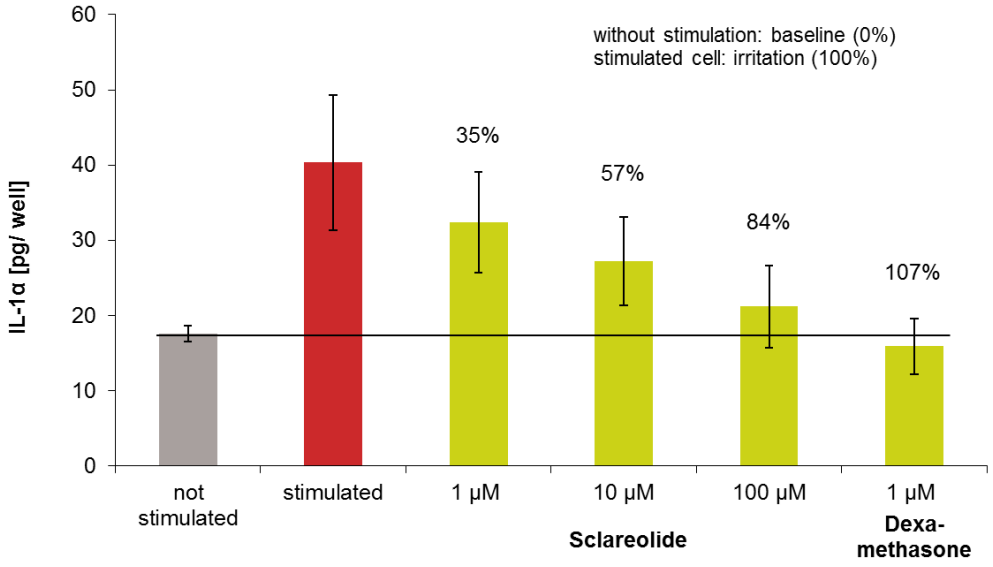

Fig. 2: Inhibition of IL-1α biosynthesis in HaCaT keratinocytes by sclareolide 6 h after stimulation with A23187 calcium ionophor.

After irradiation of HaCaT keratinocytes with UVB gene expression of EDN1, CXCL1, IL6 and IL8 was increased. Sclareolide applied 2 h before irradiation with UVB inhibited these upregulations (Fig. 3).

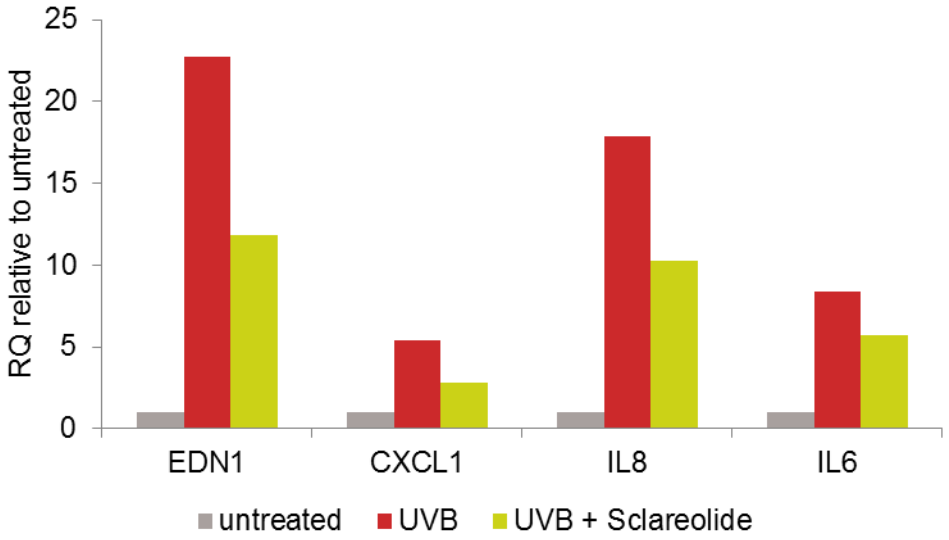

Fig. 3: Upregulation of gene expression in HaCaT keratinocytes 24 h after irradiation with 20 mJ/cm² UVB; inhibition by 50 µM sclareolide applied 2 h before irradiation.

By inhibition of IL-1α biosynthesis and inhibition of upregulation of genes mediating PIH sclareolide is capable to protect against postinflammatory hyperpigmentation. Thus, the active influences skin pigmentation via two mechanisms: the direct depigmenting effect on melanocytes and the inflammatory protecting effect on keratinocytes influencing melanocytes via a paracrine mechanism.

DISCUSSION AND CONCLUSION

Our continuous screening for new skin lightening agents led to the discovery of sclareolide, a new, natural, highly potent active. Furthermore, due to inhibition of cytokine gene expression and biosynthesis sclareolide is capable to protect against postinflammatory hyperpigmentation. These properties make sclareolide an attractive material for deo, depilation, and acne applications but also the day cream to protect against harming influences all day long.

Sclareolide can be produced in high purity from clary sage by biotransformation of sclareol making it a natural, sustainable product.

A clinical study is underway to show the protection against pigmentation induced by UV in suberythemal dose.

REFERENCE

1. CMI Data Source: Symrise Cosmetic Ingredients Consumer data base 2012-2013 (36 000 consumers in 10 markets)
2. Ortonne and Bissett (2008) J Investig Dermatol Symp Proc 13(1):10-4
3. Vierkötter et al (2010) J Invest Dermatol 130(12): 2719-2726
4. Nakamura et al (2015) Exp Dermatol 24(6): 407-11
5. Yamaguchi and Hearing (2009) Biofactors 35(2): 193-9
6. Davis and Callender (2010) J Clin Aesthet Dermatol 3(7):20-31