

A multiparametric, stepwise *in vitro* approach to identify anti-dark circle and anti-puffiness ingredients

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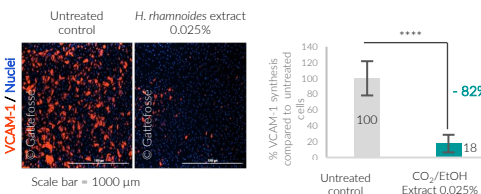
Introduction:

- Dark circles are a cosmetic concern worldwide, often associated with tiredness or aging. Owing to its thinness the highly vascularized eye contour area easily shows blood and lymphatic circulation disorders [1]. Environmental stress alters skin microcirculation, endothelial barrier function and increases oxidation in the subocular area [2]. The multifactorial nature of dark circles and puffiness represents a real challenge for *in vitro* efficacy testing of active ingredients.
- Therefore, to select an active ingredient with both anti-puffiness and anti-dark circle potentials, we have implemented a screening strategy that combined different biological models addressing targets relevant to skin microcirculation and endothelial barrier function. Using this screening approach on 22 plant extracts, we identified that ground leaves of seabuckthorn (*Hippophae rhamnoides*) extracted using supercritical CO₂ added with ethanol as co-solvent (herein after referred to as CO₂/EtOH extract) displayed high potency.

Results & Discussion:

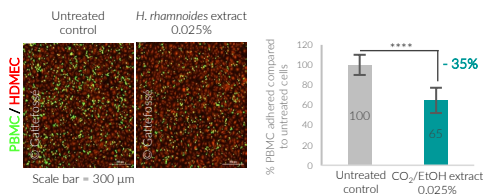
A CO₂/EtOH extract of *H. rhamnoides* leaves decreased vascular permeability and improved endothelial barrier function

The CO₂/EtOH extract decreased VCAM-1 protein synthesis in Human Dermal Microvascular Endothelial Cell (HDMEC) monolayers in inflammatory conditions



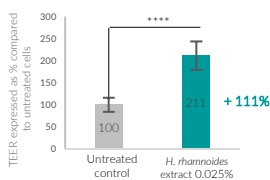
Immunofluorescence imaging and image analysis of VCAM-1. Analysis of 6 images per experiment, n=3 independent experiments, Mann-Whitney non-parametric statistical test, ****p<0.0001. The extraction solvent alone did not reduce VCAM-1 synthesis (data not shown).

The CO₂/EtOH extract decreased adhesion of peripheral blood mononuclear cells (PBMC) to HDMEC monolayers in inflammatory conditions



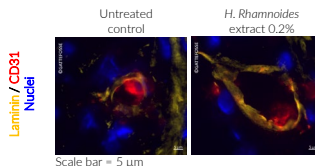
Immunofluorescence imaging and image analysis of PBMC adhesion to HDMEC membranes. Analysis of 6 images per experiment, n=3 independent experiments, Mann-Whitney non-parametric statistical test, ****p<0.0001. The extraction solvent alone did not decrease PBMCs adhesion (data not shown).

CO₂/EtOH extract increased Trans-Endothelial Electrical Resistance (TEER) in HDMEC monolayers in inflammatory conditions



n=3 independent experiments. Statistical analysis was conducted using non-parametric Mann-Whitney test, ****p<0.0001. The extraction solvent alone did not induce an increase in TEER (data not shown).

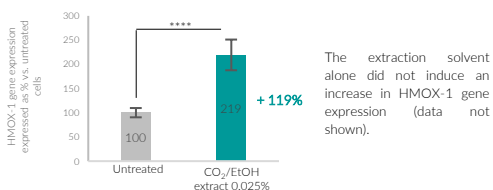
CO₂/EtOH extract stimulated laminin expression in a vascularized 3D dermis model in inflammatory conditions



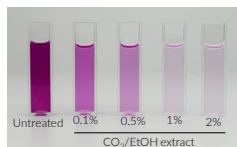
Treatment with CO₂/EtOH extract at 0.2% in inflammatory conditions (TNF-α) during dermal maturation (from day 14 to day 28). Laminin expression was visibly increased in dermal equivalents treated with CO₂/EtOH extract.

A CO₂/EtOH extract of *H. rhamnoides* leaves favors degradation of hemoglobin, whose accumulation is one of the primary causes of hyperpigmentation

CO₂/EtOH extract increase HMOX-1 gene expression in Normal Human Dermal Fibroblast (NHDF) cultures



CO₂/EtOH extract dose-dependently chelated ferrous ions



CO₂/EtOH extract dose-dependently chelated ferrous ions using *in tubo* chelation assay whereas the extraction solvent alone did (data not shown).

CO₂/EtOH extract could contribute to reduce hyperpigmentation, characteristic of dark circles by chelating ferrous ions, which accumulate in the extracellular space due to hemoglobin degradation.

Conclusions:

- The stepwise selection model we used allowed us to identify a unique plant extract with promising anti-puffiness and anti-dark circle potential, based on combined proteomic, genomic and biochemical methods using acellular assays as well as 2D and 3D cell models.
- Dedicated clinical study should be used in the near future to demonstrate the *in vivo* benefits of the ingredient.

References:

[1] Rho S-S, Ando K and Fukuhara S 2017 J Nippon Med Sch **84** 148-59. [2] Swift A, Liew S, Weinkle S, Garcia J K and Silberberg M B 2021 Aesthetic surgery journal **41** 1107-19