Ecocert Certified Alpine Rose Active Combats Aging by Protecting Skin Proteins

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Abstract

Mibelle Biochemistry investigated the symbolic Alpine Rose (Rhododendron Ferrugineum), a plant adapted to extreme conditions with a specific morphological and phytochemical set up. The leaves form as example specific proteins called dehydrins to survive the freezing and dehydrating conditions of the alpine environment. The Alpine Rose showed interesting in vivo activities to reduce the formation of carbonylated proteins, a marker of skin aging. The extract inhibits the adhesion of the herpes virus to cells in vitro which combines well with the in vivo activity. UVB leads to carbonylated proteins, skin aging and is also suppressing the immune-system, a condition which favours the outbreak of viral infections. The extract counteracts both problems synergistically.
Mibelle Biochemistry decided to develop an Ecocert certified cosmetic active and to start the agronomic development and cultivation of Rhododendron Ferrugineum in the Swiss Alps with a specialized institute. The supply of the raw material is not dependant on wild harvesting but respects the sustainability of this precious slow-growing alpine plant.

Introduction

Oxidative stress damages skin proteins and accelerates the formation of carbonylated proteins. Environmental stress factors such as UV light, infrared radiation, tobacco smoke and pollution generate reactive oxygen species (ROS). ROS oxidize proteins and lipids, the main components of cell membranes. The oxidation of proteins leads first to carbonylation, an irreversible and unrepairable modification of protein structure (Fig. 1). Normally, the resulting carbonylated proteins are recycled by the proteasome, the cell's own cleaning system which degrades damaged and oxidized proteins. But age and an increase in oxidative stress factors impair the proteasome activity, leading to an accumulation of carbonylated proteins, a marker of aging. Because they are highly reactive, carbonylated proteins aggregate and form high-molecular-weight super-structures which resist degradation. These cytotoxic structures accumulate with time and accelerate skin aging and have been associated with a large number of age-related disorders including Parkinson, Alzheimer's disease and cancer. Therefore, the content of carbonylated proteins is the major indicator of oxidative damage and a marker of aging.
Herpes is a viral disease caused mainly by herpes simplex virus type 1 (HSV-1). Most individuals are carrier of the herpes simplex virus, staying in a dormant status. Distress, caused by an unhealthy lifestyle, is immune-suppressing as is UVB, able to cause an outbreak of a herpes simplex infection on the skin or around the lips.

The Alpine Rose (Rhododendron Ferrugineum) is one of the most typical and prominent Swiss alpine plants. It grows at high altitudes in acidic and nutrient-poor soils and has developed impressive strategies to protect itself against dehydration and the attack of radicals and pathogens. The Alpine Rose is very difficult to cultivate, a reason that this plant is not used in cosmetics so far.
Mibelle Biochemistry investigated in collaborations the phytochemistry and screened the biological activities of the Alpine Rose. The presence of a large number of interesting compounds could be determined in alpine rose leaves as taxifolin and antioxidant and antiviral activities were discovered.
Mibelle Biochemistry decided to develop its own cultivations of Rhododendron Ferrugineum in the Swiss Alps in collaboration with a specialized institute. The supply is therefore not dependant on wild harvesting and respects the sustainability of this slow-growing plant.

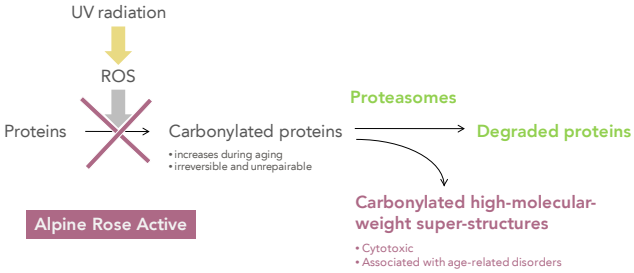


Fig 1: Alpine Rose Active Inhibits the Formation of Carbonylated Proteins

Results and Discussion

Study: Protection against Protein Oxidation (in vivo)

Oxidative stress was evaluated in vivo by measuring the carbonyl protein content in human skin after exposure to UVA radiation. The suction blister technique was used to perform this measurement. Skin blisters are produced with a vacuum device on the forearm and then the blister fluid is collected. It represents the interstitial fluid of the skin and can be used for analysis of skin biomarkers.
The test products (cream with 2% Alpine Rose Active and placebo cream) were applied twice daily for 14 days on the inner side of the forearms of 12 volunteers aged from 40 to 54. After the last product application, the test sites were irradiated with 10 J/cm2 UVA-light.
Subsequently, suction blisters were induced, the suction blister fluids were collected and their content of protein carbonyls was analyzed with an ELISA-kit.

Results showed that the carbonyl protein content was significantly reduced in the suction blister fluid of the test site treated with Alpine Rose Active compared to the placebo-treated skin area, indicating a protective effect against the oxidative stress induced by UVA (Fig. 2).

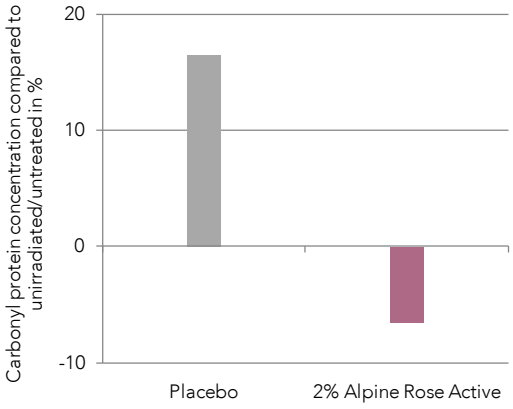


Fig 2: Inhibition of Carbonyl Protein Formation

Study: Antiviral Effect against Herpes Simplex (in vitro)

Oral herpes is a viral disease caused mainly by herpes simplex virus type 1 (HSV-1). The visible symptoms of this infection are cold sores which infect the face and mouth.

The general antiviral effect of Alpine Rose Active was tested on Vero cell cultures (epithelial kidney cells). The Vero cells were seeded in plates. The virus was preincubated with different concentrations of Alpine Rose Active and then added to the cell monolayer. To evaluate the anti-viral activity of Alpine Rose Active, the incubated cells were stained with crystal violet, a reagent that only interacts with living, not-infected cells. Thus, infected cells form plaques in the violet monolayer that can be counted and are an equivalent for the anti-viral activity of an active.

Results showed that Alpine Rose Active strongly inhibited the activity of herpes simplex virus type 1 (HSV-1) (Fig. 3).
In order to understand the mechanism of Alpine Rose Active, an adsorption assay with an immune-fluorescence staining was performed.
Vero cells were cooled down to reduce the permeability of their cell walls. Then the virus was pre-incubated or not with 0.02% Alpine Rose Active and added to the Vero cell cultures. After incubation, the Vero cells were washed. To investigate the capacity of the virus to adhere to the cell membrane, viruses were stained with a specific antibody.
Microscope analysis showed that Alpine Rose Active strongly inhibited the adherence of the HSV-1 virus on cells, meaning that the antiviral effect of Alpine Rose Active against herpes relies on its capacity to prevent the adhesion of this virus on cells (Fig. 4).

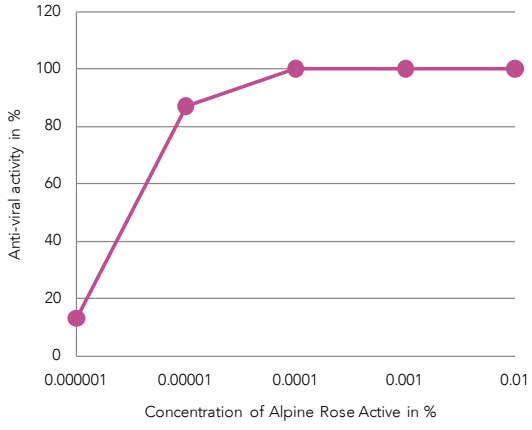
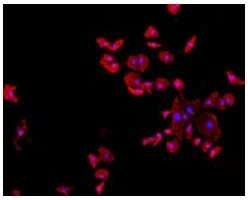
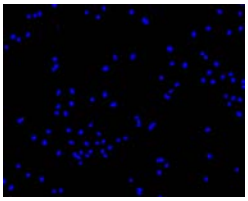


Fig 3: Inhibition of HSV-1 Activity



Cells incubated with virus (HSV-1) that adhere to the surface of the cells (control).



Cells incubated with viruses pretreated with **0.02% Alpine Rose Active** → no adherence to cells

Viruses in red (HSV-1 Cy3 staining),
cell nuclei in blue (DAPU staining)

Fig 4: Alpine Rose Active Inhibits Adhesion of the virus

Conclusion

Alpine Rose Active is a Ecocert certified extract of organic alpine rose leaves, which inhibits the formation of carbonylated proteins and protects the skin against herpes occurrence.
The activity of Alpine Rose Active was demonstrated in several in vitro and in vivo studies.
The extracts inhibits the carbonylation of cutaneous proteins, an irreversible, and age-related oxidative process induced by reactive oxygen species.
Alpine Rose Active strongly inhibited the adherence of the HSV-1 virus on cells, meaning that the antiviral effect of Alpine Rose Active against herpes relies on its capacity to prevent the adhesion of this virus on cells.
Alpine Rose Active can thus protect skin proteins against damage induced by oxidative stress as well as reinforce its defense against pathogens.
This innovative active based on the extremophile Alpine Rose can be used in various cosmetic applications as anti-aging formulas, photo-aging prevention, sun care products, protective skin care and lip contour treatments.

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