

Senolytics: Eliminating senescent cells

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The Alpine rose grows at high altitudes

One of the hallmarks of ageing is the accumulation of senescent cells.¹ Cellular senescence is a condition of irreversible cell cycle arrest, where the cells do not proliferate anymore, but are still viable and therefore 'not quite dead yet'. This led to them being characterised as 'zombie cells'. Importantly, senescent cells show an increased secretion of numerous different factors, such as matrix metalloproteinases, chemokines and inflammatory cytokines, which are termed senescence-associated secretory phenotypes (SASP).²

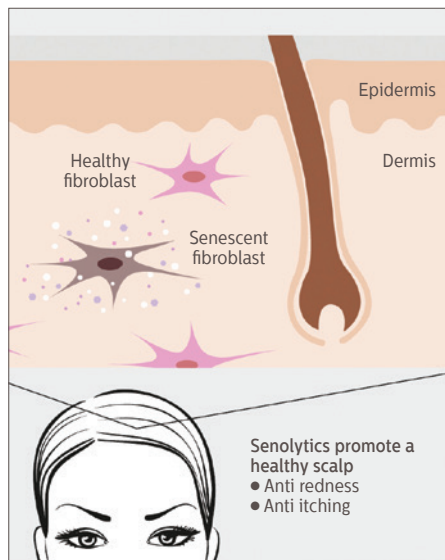


Figure 1: Scalp anatomy & skin senescence
Insert highlights senescent cells, present in the dermis of the scalp

This secretory behaviour in turn influences neighbouring cells, transforming them too into 'zombie cells', which further highlights the choice in analogy. SASP of senescent cells further contributes to chronic low-grade inflammation, also known as 'inflammaging', which is thought to accelerate the ageing process in the skin.³

To prevent inflammation and ageing of tissues, current strategies aim to eliminate the senescent cells by means of senolytics. These are compounds known to clear the zombie cells selectively while not negatively affecting healthy cells. Their use has previously been limited to preclinical or clinical studies on serious diseases, such as Alzheimer's disease, diabetes and pulmonary fibrosis.

The targeted elimination of senescent cells in the skin was, however, poorly investigated. In 2020, Mibelle Biochemistry pioneered a novel senolytic in skin care. We showed that the use of an Alpine rose extract can eliminate zombie cells from the skin, thereby reducing inflammation and promoting skin rejuvenation.⁴

Nature's senolytic

Alpine Rose Active is an organic purified extract of the leaves from the iconic Swiss Alpine rose (INCI: Rhododendron Ferrugineum Extract (and) Glycerin (and) Aqua). Due to its exposure at high altitude (up to 2,800 metres), this plant has developed impressive strategies to protect itself against stressful conditions and exposure to free radicals.

Mibelle Biochemistry collaborates with organic farmers in the Swiss Alps to harvest the Alpine rose leaves in a controlled and sustainable manner. This careful wildcrafting

ABSTRACT

Cellular senescence, a condition of irreversible cell cycle arrest, can lead to inflammation and accelerated ageing of tissues, including the skin. To prevent this, current strategies aim specifically to eliminate the senescent cells, or 'zombie cells', by means of senolytics. Sensitive scalps are a prominent problem and one potential treatment option is the use of senolytics in scalp care. As with the skin, zombie cells in the scalp can cause local inflammation, leading to dry and irritated scalps, so clearing these cells could promote a healthy scalp. An extract from the leaves of the Swiss Alpine rose was found to have strong senolytic activity in fibroblasts. In a placebo-controlled clinical study, the extract further demonstrated a significant soothing effect on sensitive scalps. This study firstly highlights the positive impact of senolytics in scalp health and care.

method not only ensures that the plants are fully regenerated, but also that the leaves are extracted from plants stressed by natural environmental factors, including steep temperature gradients and high UV irradiation.

The challenging environment stimulates the plants to synthesise protective metabolites to adapt to the disadvantageous conditions. *In vitro* studies have demonstrated Alpine rose extract's senolytic effect as it eliminates senescent cells while not negatively impacting on healthy cells. In clinical studies, the extract significantly reduced facial skin redness and increased the skin's elasticity.⁴

Taken together, this powerful natural extract can protect and rejuvenate the skin through its senolytic activity. Given these positive results on facial skin rejuvenation, we asked ourselves whether the compound could have similar benefits for the scalp.

Sensitive scalps: A common problem

The scalp is a specialised skin barrier, rich in blood vessels and with an abundance of hair follicles. As such, it provides effective protection against heat and water loss. A healthy scalp with a proper barrier function is further essential to prevent greying and possibly also to prevent hair loss.

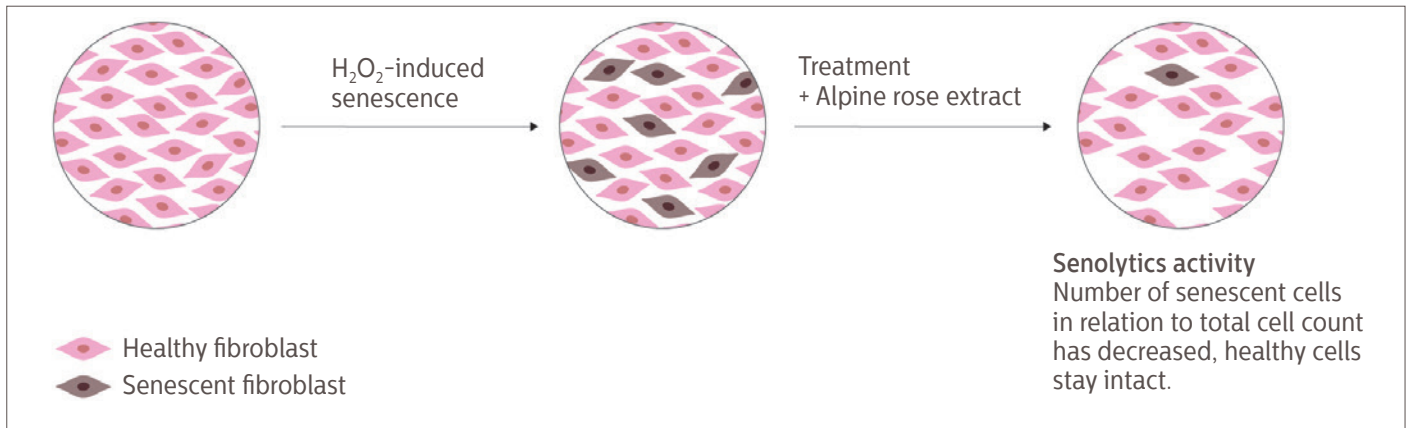


Figure 2: Graphical overview of senolytic assay

Senescence was induced in cultured fibroblasts with H_2O_2 , senescent cells are highlighted in brown. After treatment with Alpine rose extract, the number of senescent cells (brown) in relation to total cell count has decreased, while healthy cells (pink) remain viable and intact.

Despite an extensive microbiome supporting a healthy scalp, sensitive, dry and irritated scalps are a common problem today and are not currently acknowledged as an official skin disease. In a French epidemiological study, it was determined that 44% of the population studied, more frequently women (47.4% of women vs. 40.8% of men), suffered from a sensitive scalp. Therein the researchers also found a significant association between sensitive scalp and hair loss.⁵

Intriguingly, it was also noted that scalp sensitivity tended to increase with age.⁶ This evidence highlights the imminent need to focus our attention not only on the condition of sensitive scalps *per se*, but importantly also find effective treatment solutions.

A potential treatment option would be the use of senolytics in scalp care. As with the skin, zombie cells in the scalp can cause local inflammation and consequently lead to dry and irritated scalps. Clearing these cells could therefore promote a healthy scalp (Figure 1).

We designed an *in vitro* model where we could highlight the extract's prominent senolytic effect in clearing zombie cells from cultured stressed fibroblasts. In a clinical study, we tested the effect of alpine rose extract on sensitive scalps and found that treatment with a 1% Alpine rose extract had a significant soothing effect on sensitive scalps. This study highlights for the first time the positive impact of senolytics in scalp care and scalp health.

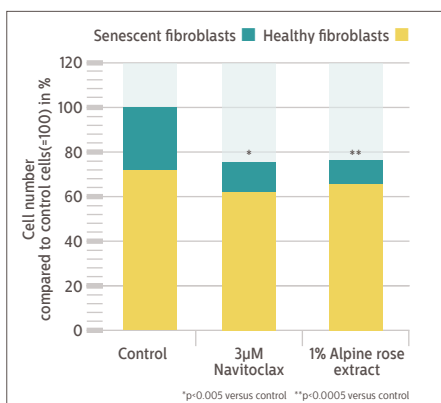


Figure 3: Senolytic activity of Alpine rose extract. Cell numbers of senescent and non-senescent cells are shown normalised to control cells. Premature senescence was induced with H_2O_2 .

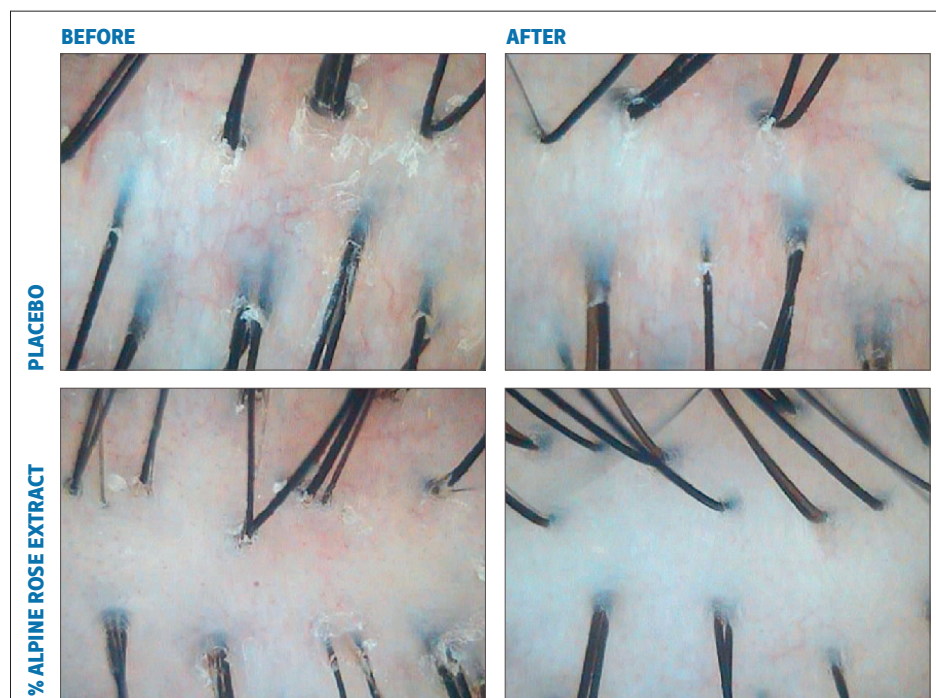


Figure 4: Volunteer before & after applying 1% Alpine rose extract serum once daily after hair wash for four weeks

Methods

To determine the extract's senolytic activity, normal human dermal fibroblasts were firstly treated with $500 \mu M$ of H_2O_2 for two hours to induce oxidative stress-induced premature senescence. The medium was then exchanged and the cells were cultured for three days to fully establish the senescent phenotype in a sub-population of the cells.

This mixed culture was then treated for 48 hours with either 1% Alpine rose extract or Navitoclax (supplied by Cayman Chemical), a known senolytic drug, or left untreated as a control. Following fixation with 2% formaldehyde and 0.2% glutaraldehyde, cells were stained with DAPI and the relative total cell number was determined by fluorescence measurement.

Senescence-associated β -galactosidase activity assay was performed according to a test described by Zhao *et al.* and a total of 400 cells were counted.⁷ Treatment efficacy was determined

by counting the β -gal-positive cells as a marker for senescence and calculating the percentage compared to the total cell number (Figure 2).

The scalp soothing effect of Alpine rose extract was evaluated in a double-blind, randomised, placebo-controlled clinical study. The study population consisted of 43 Asian subjects (34 female and nine male) aged from 23 to 59 years (mean = 44.3 years), all with sensitive scalps and scalp redness.

The subjects were divided into a test group (22 subjects), which received a serum containing 1% Alpine rose extract and a control group (21 subjects), which received a placebo serum without active ingredient. The Alpine rose extract was incorporated at 1% into a water-based alcoholic gel formulation, which is a standard formulation for cosmetic scalp care. Once daily for 28 days, after shampooing and drying the hair, 5 mL of test or control serum was applied on the scalp and massaged in for absorption.

Scalp redness was assessed via photo-grading with images taken using an Aramo TS from AramHuvis of Korea and evaluated by the investigator on a five-point scale. Scalp itching was evaluated by self-assessment on a five-point scale and the subjects further determined the product efficacy by means of self-questionnaires. These parameters were evaluated at baseline (before treatment) and after 28 days.

Results & discussion

When screening for a senolytic effect, a current challenge is to distinguish between senescence delaying actives, such as antioxidants that minimise the damage that may lead to senescence, and true senolytic activity.

To assess the latter, a senescent phenotype was initially established in cultured fibroblasts by treatment with H_2O_2 . After a further three days in culture allowing the senescent phenotype to be established, incubation with the potential senolytic active takes place.

After treatment, the number of senescent cells was quantified and compared to total cell number. Actives that eliminate senescent cells while not affecting healthy fibroblasts are considered to possess senolytic activity.

Treatment with 1% Alpine rose extract significantly reduced the number of senescent cells while the number of healthy fibroblasts remained unaffected. Compared to control cells, which had 28.1% senescent cells, treatment with Alpine rose extract reduced the percentage of senescent cells to 10.1%.

The effect was similar to a treatment with the known senolytic drug Navitoclax, which demonstrated a reduction of senescent cells to 12.3% (Figure 3). Therefore, it can be concluded that Alpine rose extract exhibits senolytic activity.

Scalp redness & itching

Some of the most common symptoms of sensitive scalps include scalp redness and sensations like scalp prickling and itching. We therefore evaluated these conditions and found that treatment with the Alpine rose extract for four weeks significantly decreased scalp redness compared to the placebo treatment.

The anti-redness effects were quite apparent as seen in the representative photographs in Figure 4. In comparison to initial conditions the scalp redness was overall decreased by 32.4% after four weeks (Figure 5). Furthermore, four weeks treatment with Alpine rose extract led to a statistically significant decrease in scalp itching by 64.3% (data not shown).

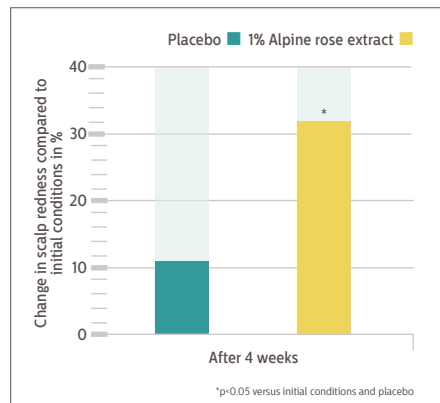


Figure 5: Quantified reduction in scalp redness. Scalp treatment with 1% Alpine rose extract compared to baseline and placebo control after 4 weeks

The positive effect of Alpine rose extract on irritated scalps was further evident from the self-questionnaire where 86.4% and 95.5% of volunteers described decreased scalp redness and improved scalp itching, respectively. A noteworthy 100% of volunteers reported an overall improved scalp condition (Figure 6).

Together, the results highlight that Alpine rose extract has a significant soothing effect on red or irritated scalps. Moreover, the use of a senolytic scalp serum resulted in a considerably positive consumer feedback. Therefore, such a treatment option has strong potential to be successfully implemented in general scalp care products.

To further meet current consumer demands, this senolytic active could potentially be combined with anti-dandruff actives in complementary formulations. The use of a senolytic scalp serum may yield benefits beyond anti-itching and anti-redness. Most recently it was demonstrated that improving scalp condition by means of antioxidants could significantly prevent hair shedding.⁸

As mentioned above, a significant association between sensitive scalps and hair loss has been detected in clinical studies.⁵ Indeed, the elimination of senescent cells from the epidermis was shown to increase proliferation within hair follicles.⁹ Furthermore, administering a senolytic peptide prevented age-related hair loss.¹⁰

Perhaps complementing hair care with senolytic scalp treatments can provide an uncomplicated and easy approach to supporting healthy scalps and preventing hair loss and greying. These compelling new possibilities should be further investigated, and one should further encourage scalp treatments in our day-to-day hair care.

Conclusion

The benefits of using Alpine Rose Active as a senolytic in scalp care is evident. This meets the current demands of senolytic skin care and beyond: it expands the use of Alpine rose extract to scalp and hair.

Actively pursuing a healthy scalp care with senolytics has great potential, not only in preventing inflammation but possibly even age-related hair loss. Therefore, formulations incorporating such senolytic actives, including Alpine rose extract, should be considered for future scalp care products.

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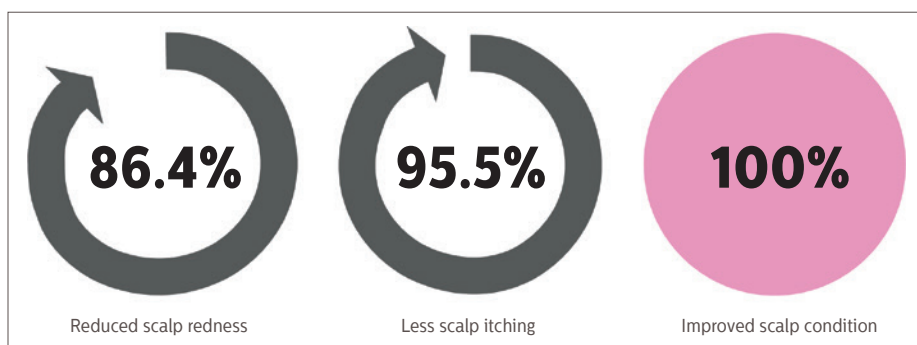


Figure 6: Reported scalp soothing effects