

Senolytic science: plant extract for well-ageing

Dr Leithe Budel, François Paul – DSM-Firmenich



Skin ageing continues to be consumers' top concern. However, a new survey commissioned in November 2023 by DSM-Firmenich shows that consumers representing four of the world's largest personal care markets are looking for healthy ageing or well-ageing solutions that holistically tackle all their skin concerns, such as skin sagging, wrinkles, the loss of skin elasticity and firmness, skin roughness, a dull complexion, and age spots, which lead to a loss of self-confidence (Figure 1).

Consumers do not want to be reminded that they are ageing, and for more than 50% of skin care users, "anti-ageing" has a negative connotation; however, the ageing consumer would welcome a scientifically supported breakthrough that brings new life to their skin.

Senolytic science – a breakthrough

Senolytic science is a cutting-edge discipline that is moving toward infinite well-ageing. As cells age or become damaged by ultraviolet rays, oxidants, toxins, and pollutants, they can do one of two things when repair is not possible. They either enter an irreversible stage known as senescence or undergo an orderly form of cell death known as apoptosis.

When skin cells enter senescence, they begin to damage the surrounding tissue. Senescent cells no longer divide and produce the key

proteins collagen and elastin poorly.

Furthermore, they also secrete damaging factors. Cells that enter proper apoptosis, on the other hand, are eliminated without causing harm to the organism. As more and more destructive senescent cells, which are also known as zombie cells, build up over time, they play an increasingly larger role in the characteristic signs of skin ageing.

Extensive research shows that it is these zombie cells that cause the impaired tissue function that leads to many of the clinical signs of ageing, such as wrinkles and fine lines, skin thinning, the loss of elasticity, irregular pigmentation, dryness, hair loss, and hair greying. The conclusion of these studies is that to achieve powerful rejuvenation, these zombie cells must be removed.^{1,2}

Senolytic science investigates methods that induce the removal of senescent cells through programmed cell death, as well as ways to deactivate the survival pathways that allow senescent cells to persist.

By targeting these mechanisms, senolytics aim to address a fundamental driver of tissue ageing. In a promising study by a Dutch research group in 2017, it was shown that senolytic activity can dramatically improve age-related changes.

The Dutch study demonstrated that

ABSTRACT

Senescence cells are seen as fundamental drivers of tissue ageing. Senescent 'zombie cells' accumulate with age and cause damage through damaging secretions. Eliminating these cells is a revolutionary approach to restoring youthful skin. Research by experts in ageing has revealed that *Epilobium fleischeri* extract is a natural botanical senolytic (a substance that can selectively induce the death and removal of senescent cells). The experiments discussed in this paper show that *Epilobium fleischeri* extract can selectively clear senescent cells from the skin. *In vitro* evidence demonstrates the targeted elimination of senescent fibroblasts by *Epilobium fleischeri* extract, which is mediated by bioactive flavonoids. *In vivo* studies have shown the reduced expression of senescence biomarkers, improved collagen production, and dramatic improvements in multiple signs of skin ageing. In clinical trials, elasticity and tonicity were improved by 18% after two months of using a cream with 3% *Epilobium fleischeri* extract as compared with a control group using a placebo cream.

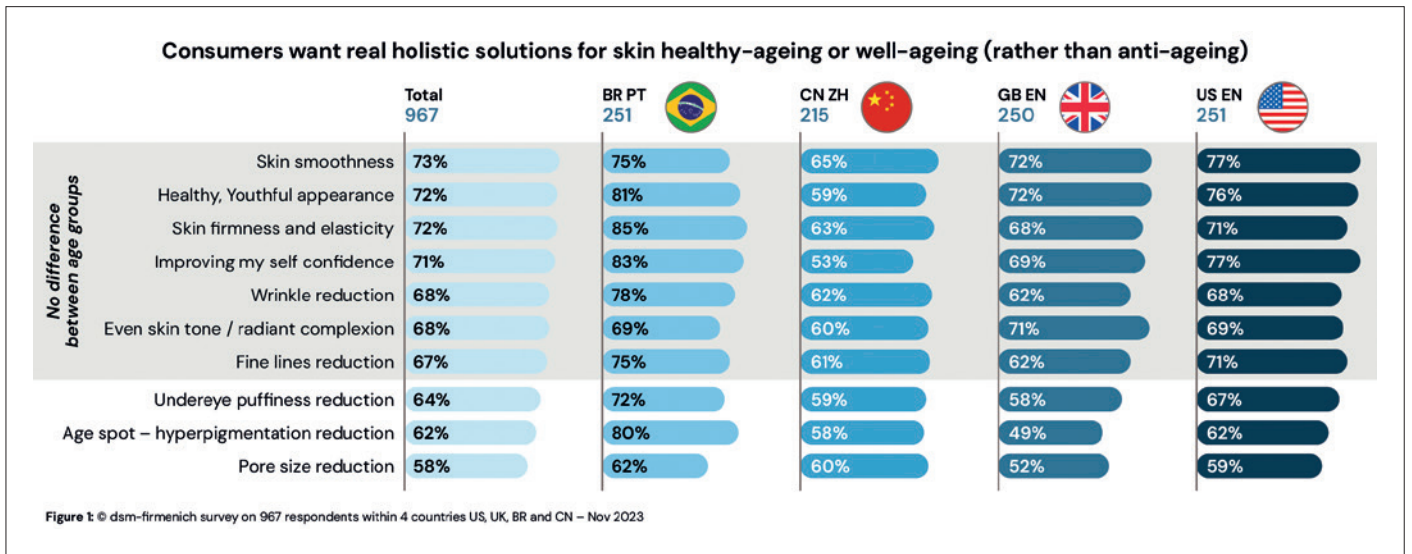


Figure 1: Results of DSM-Firmenich survey on 967 respondents within 4 countries US, UK, BR and CN

eliminating senescent cells in their test models was possible after age-related health decline had begun and that in this way, living research models were restored to a more youthful state. These amazing results in living subjects highlight the potential of using senolytics to treat the signs of and even reverse ageing.³

Senescent zombie cells damage tissue

Senescent cells produce high levels of reactive oxygen species, which impair cells and tissues. They also secrete a complex mix of damaging bioactive factors that are part of the senescence-associated secretory phenotype (SASP).

For example, the SASP contains pro-inflammatory cytokines (e.g., interleukin 1 (IL1) and interleukin 6 (IL6)), chemokines, growth factors, proteases (e.g., MMP1 and MMP3), and other substances. The various components of the SASP stimulate inflammation through immune cell proliferation and migration.

They can also modulate, suppress, or help zombie cells evade the body’s defensive

immune system and so resist natural removal processes. These SASPs weaken and damage surrounding cells, but more importantly, they can begin a snowball effect, causing the surrounding cells to also become senescent and thus produce yet more SASPs.

Key markers of senescence

The irreversible arrest of the cell cycle that defines senescence is controlled by complex pathways that suppress proliferation-associated genes. Two key regulatory proteins involved in the pathways stopping cell division are p16 (CDKN2A) and p21 (CDKN1A).

Given their roles in inducing cell cycle arrest, p16 and p21 serve as useful biomarkers for senescence. Additionally, the absence of Ki-67, a protein involved in cell division, provides another senescence marker.

The combined presence of p16 and p21 and the lack of Ki-67 are used as definitive indicators of senescent cells. Senescence-associated beta-galactosidase (SA-β-Gal) is a marker of senescent cells that can distinguish between senescent cells and healthy non-

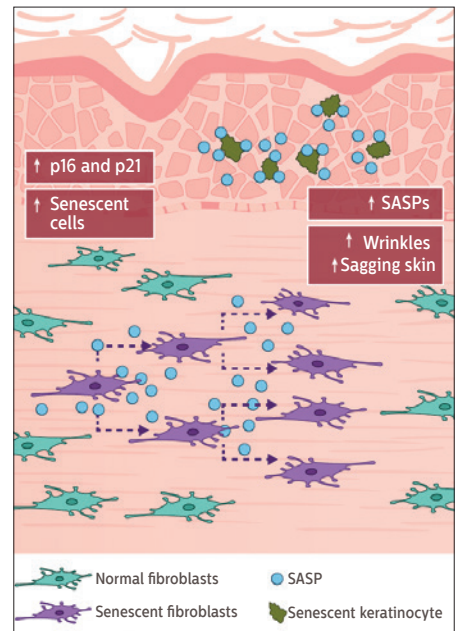


Figure 2: p16 and p21, key markers of senescence

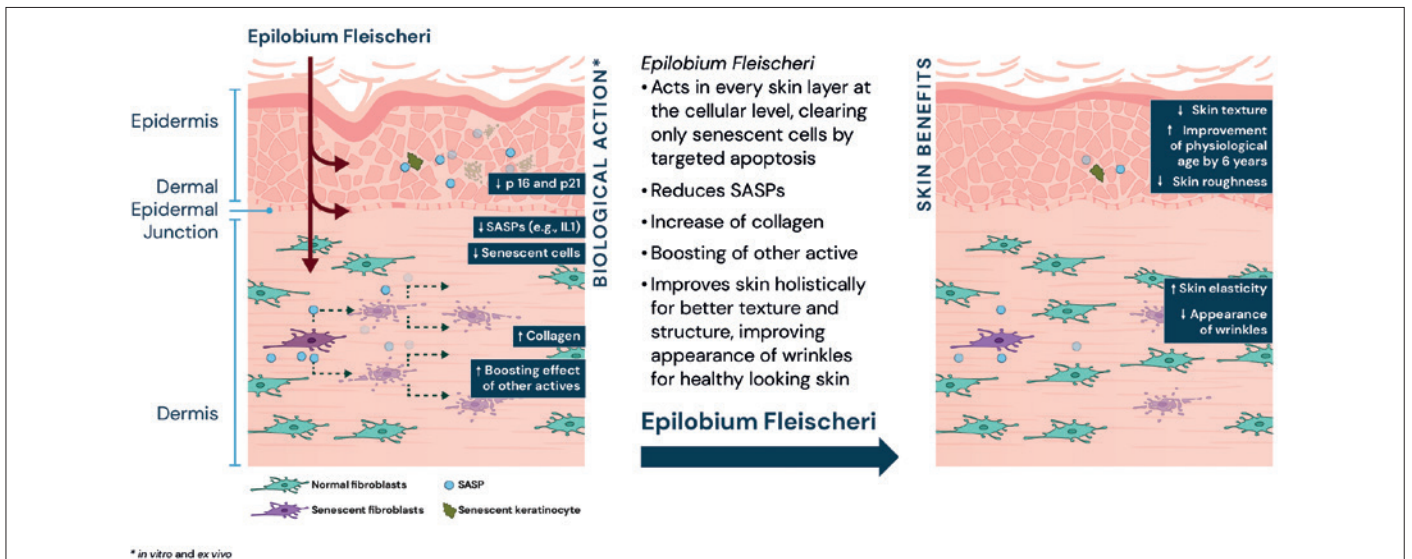


Figure 3: The mode of action of *Epilobium Fleischeri* dry extract

proliferating, quiescent cells, such as those involved in tissue repair and normal cell turnover.

Figure 2 illustrates the self-propagating cycle of senescent fibroblasts accumulating in the dermis, secreting SASPs, and triggering senescent keratinocytes to build up in the epidermis. This snowballing of senescent cells and SASPs ultimately results in more wrinkles and sagging skin and can be investigated by measuring the increased expression of the senescence markers p16 and p21.

Epilobium fleischeri extract

It required the combined screening and analytical skills of senescence experts and personal care researchers at DSM-Firmenich, in Switzerland, to discover and develop DSM-Firmenich's first 100%-natural, true senolytic bioactive, which is named Eterwell™ Youth (patent pending).

Epilobium fleischeri, a rare alpine willowherb, is sustainably grown and harvested in July, when its flower buds first appear. The leaves and stems are gently extracted in ethanol and dried, following the principles of green chemistry.

Epilobium fleischeri extract is standardized based on its flavonoid content, which is calculated as myricitrin, via ultra-high-pressure liquid chromatography (UHPLC). Using *in vitro* and *ex vivo* models of aged skin, *Epilobium fleischeri* extract has been shown to act on the epidermis, dermal-epidermal junction, and dermis at the cellular level.

In clinical studies, *Epilobium fleischeri* extract reduced the appearance of wrinkles, helped smooth the skin, improved skin texture and structure, and increased skin elasticity to restore healthy-looking skin.

The mode of action of Epilobium fleischeri extract

Epilobium fleischeri extract acts at the cellular level, clearing only senescent cells through targeted apoptosis in both the dermal and epidermal layers of the skin. Consequently, damaging SASPs are reduced, and collagen

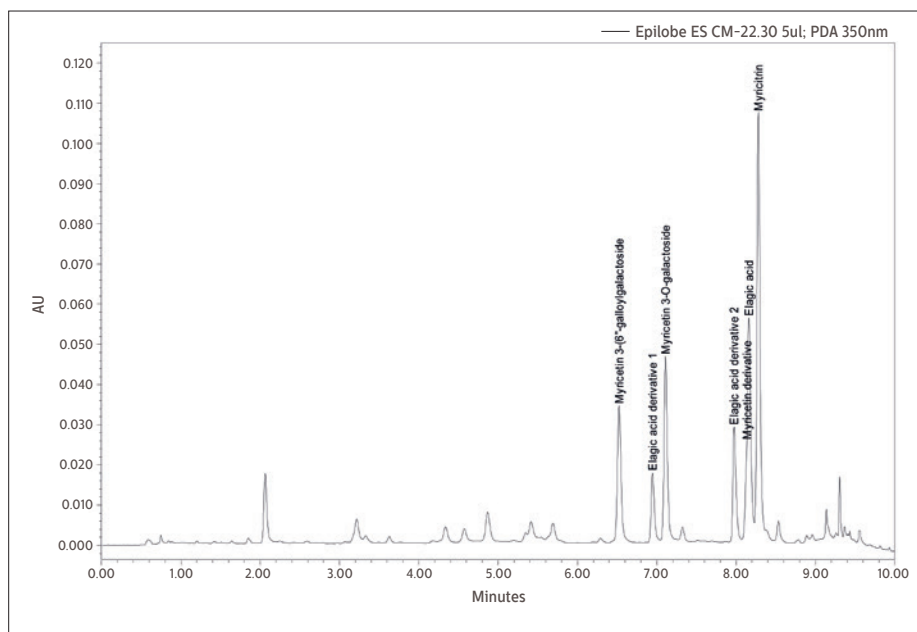


Figure 4: UHPLC chromatogram of *Epilobium Fleischeri* Leaf/Stem Extract showing flavonoids

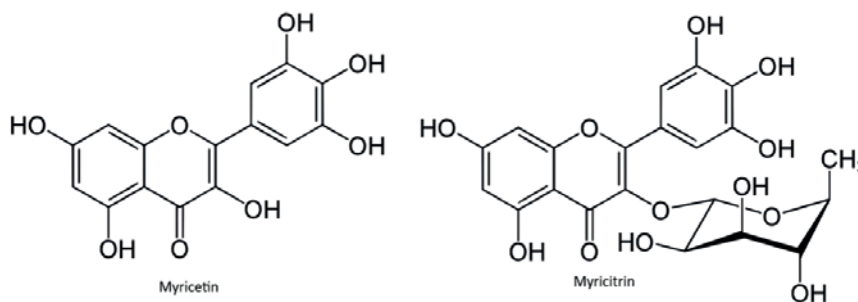


Figure 5: Myricetin and Myricitrin, the 3-O-rhamnoside of Myricetin

production is increased, which promotes skin recovery.

With the reduction of senescent cells, the skin's normal cellular activity returns and is boosted. The skin structure improves, reducing wrinkles and producing skin that looks and feels healthier (Figure 3).

Flavonoids, the source of senolytic activity

The UHPLC chromatogram (Figure 4) shows that myricitrin is the dominant flavonoid among the many present in *Epilobium fleischeri* dry extract.

In addition to having antioxidant, anti-inflammatory, and antimicrobial activities, myricitrin, the 3-O-rhamnoside of myricetin, is demonstrably senolytic.⁴

Myricitrin is structurally similar to fisetin and quercetin, which along with the chemical navitoclax, also have senolytic activity.⁵ The structures of myricetin and myricitrin are shown in Figure 5.

Senolytic activity

Flow cytometry, using senescence-associated beta-galactosidase as the marker to gauge the % of senescent cells, was used to demonstrate the senolytic activity of *Epilobium fleischeri* dry extract and myricitrin *in vitro*.

A mix of 70% normal dermal fibroblasts and 30% hydrogen-peroxide-induced senescent dermal fibroblast cells were subject to concentrations of *Epilobium fleischeri* dry extract of 0.00002–0.0010% and concentrations of myricitrin of 1–25 μM.

As the graph in Figure 6 shows, with both *Epilobium fleischeri* dry extract and myricitrin,

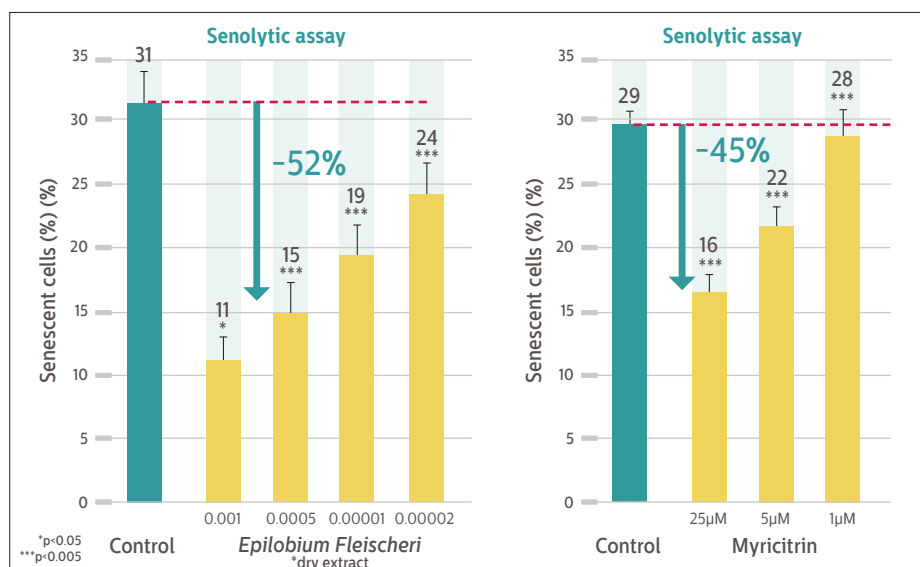


Figure 6: Senolytic activity of both *Epilobium Fleischeri* dry extract and myricitrin

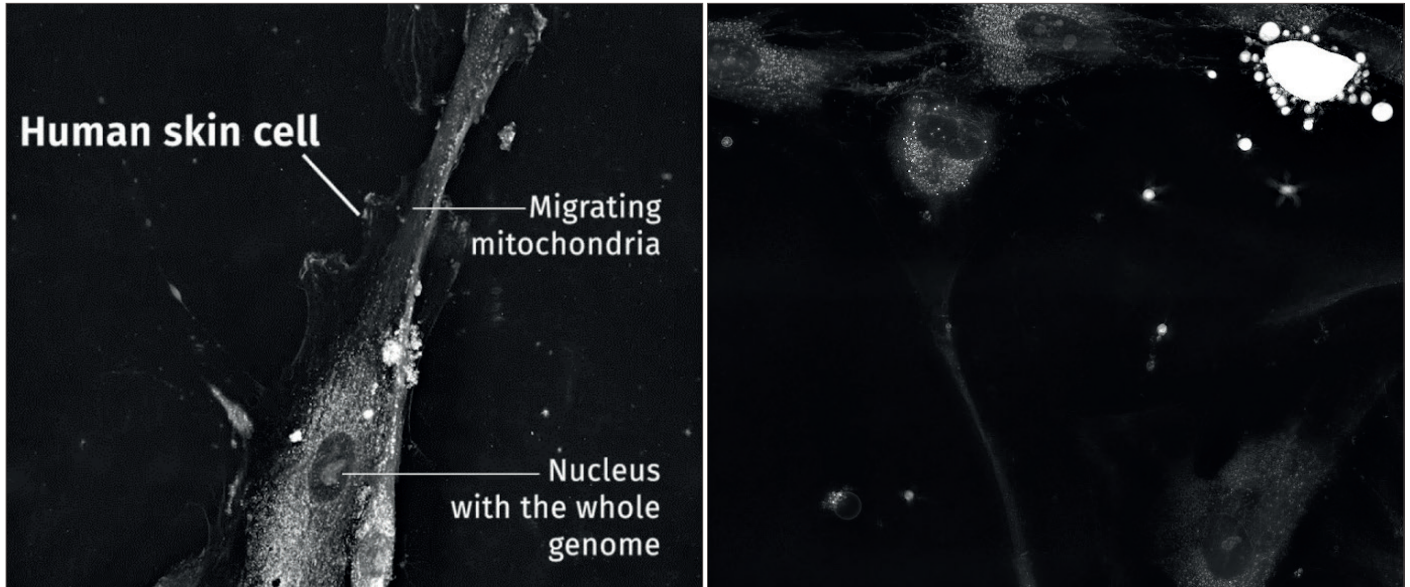


Image 1: Human skin cells with the presently described technology (left) and those undergoing apoptosis (right) using holotomography

there were significant dose-related responses. As little as 0.0005% *Epilobium fleischeri* dry extract reduced senescent fibroblasts by 52%. This exciting result compares very well with the results for pure myricitrin, 25 µM of which reduced senescent fibroblasts by 45%.

Visualizing apoptosis in real time

Holotomography is an innovative microscopy method that enables the clear imaging of living cells in their natural environment. Just as CT scans use differences in X-ray absorption to visualize the inside of the body, holotomography microscopy uses variations in the refractive index as a form of imaging contrast to view unaltered living cells and tissues.

This groundbreaking technique provides detailed views of cellular structures and activities in real time and in their native contexts, overcoming the limitations of traditional microscopy approaches that rely on fixatives, dyes, and other interventions that in themselves, can change cell structures. Image 1 shows human skin cells with the presently described technology (left image) and those undergoing apoptosis (right image) using holotomography.

The cell nucleus, with its nuclear material and migrating mitochondria, can be clearly seen in the left image. The right image reveals similar detail, but in this image, one zombie cell has shrunk because it has entered apoptosis.

In Image 2, this outstanding technology is used to visualise the effects of *Epilobium fleischeri* extract on senescent cells in an *in vitro* model designed to mimic aged skin in real time.

The aged skin model consisted of 50% normal fibroblasts and 50% senescent fibroblasts. At the 32-hour stage, a senescent cell is clearly visible. Just 30 minutes later, the same senescent cell can be seen to have entered apoptosis and is considerably smaller.

After another 2.5 hours, a second adjacent senescent cell clearly shows signs of shrinking,

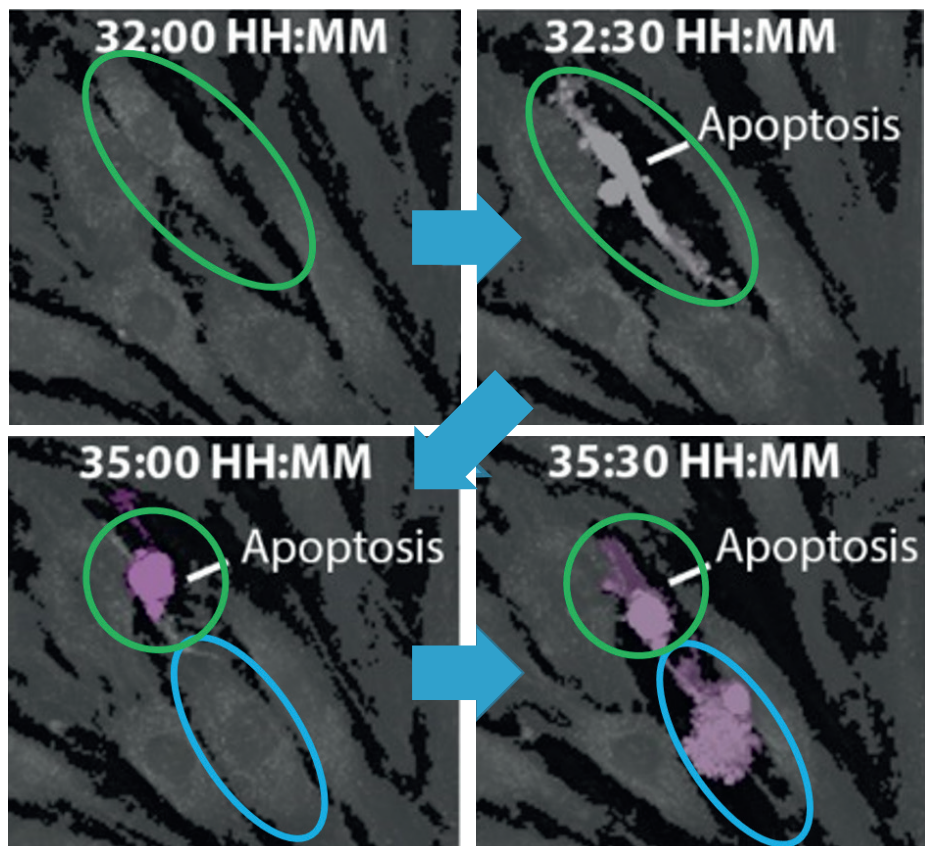


Image 2: Holographic microscopy images as living human senescent fibroblast cells enter apoptosis after treatment with *Epilobium Fleischeri* Leaf/Stem Extract

and 30 minutes later, both senescent cells are in the later stages of apoptosis and removal.

When holotomographic images from experiments run over 48 hours are subject to AI-based analysis (Figure 7), the 100% normal fibroblasts treated with 0.0005% *Epilobium fleischeri* dry extract remain healthy.

However, when the aged skin model consisting of 50% normal fibroblasts and 50% senescent fibroblasts is treated with 0.0005% *Epilobium fleischeri* dry extract, a dramatic 200% increase in cell death is seen as

compared to the control. This demonstrates, in real time, that *Epilobium fleischeri* extract is very effective in reducing senescent cells, while leaving normal cells healthy.

Collagen boosting

The expression of collagen I was assayed via an *in vitro* test using human fibroblast cells (50% normal and 50% senescent). When the fibroblast cells were pretreated with *Epilobium fleischeri* dry extract and compared to the untreated fibroblast cells (control), there was

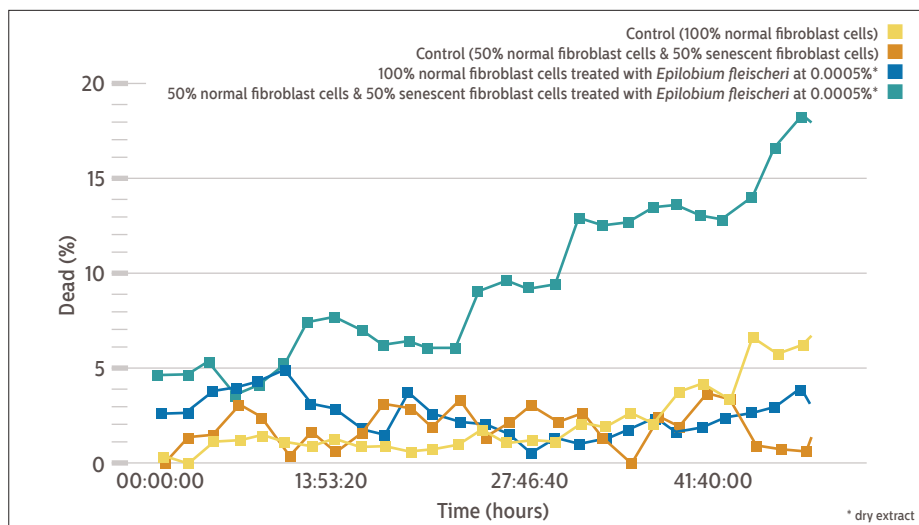


Figure 7: AI Analysis of holotomographic images of an *in vitro* aged skin model showing % dead cells over time, after treatment with *Epilobium fleischeri* dry extract.

a 94% increase in native collagen I expression (Figure 8).

Senolytic activity on whole tissue

Key markers of senescence confirmed that *Epilobium fleischeri* extract exhibits strong senolytic activity on senescent cells in tissue, as well as in culture.

First considering the results for p21, which is a protein that is essential in inducing cell cycle arrest, as shown in Figure 10, when skin explant tissues from a 60-year-old female were treated with *Epilobium fleischeri* dry extract at concentrations of 0.005%, 0.024%, and 0.072%, there was a significant reduction in p21 expression.

Notably, at 0.024%, *Epilobium fleischeri* dry extract decreased p21 by 46%, which was similar to the reduction due to 50 µM navitoclax, a known senolytic drug that inhibits apoptosis. When the SASP marker IL1 was investigated, a 62% reduction was observed in explant tissue treated with 0.072% *Epilobium fleischeri* dry extract, which was far greater than the reduction due to navitoclax (Figure 9).

Also, the expression of Collagen I, which is a marker for anti-ageing, increased by an impressive 44% in a skin explant treated with 0.024% *Epilobium fleischeri* dry extract

(Figure 9). Taken together, these results confirm that senolytic activity is the basis for the dramatic improvement in explant skin structure following *Epilobium fleischeri* extract treatment.

The micrographs of histologically stained cross-sections of tissue taken from a 10-day-old untreated skin explant (control) and from a 10-day-old skin explant that had been treated with *Epilobium fleischeri* dry extract (treated) are shown in Image 3.

The *Epilobium fleischeri* dry extract treatment was as follows: on the first day, the skin explant from a 60-year-old female volunteer was sampled and treated with various *Epilobium fleischeri* dry extract concentrations (0.005%, 0.024%, and 0.072%).

Then, on days 3, 5, and 7, the *Epilobium fleischeri* dry extract was reapplied, and the skin explants were finally sampled on day 10. Based on the two micrographs shown in Image 3, no toxic effects could be seen after ten days of application.

However, the immunostaining of p16 and p21 in the epidermis explant on day 10 confirms that increasing concentrations of *Epilobium fleischeri* dry extract reduced its intensity such that at 0.072%, *Epilobium fleischeri* dry extract reduced p16 by 19%

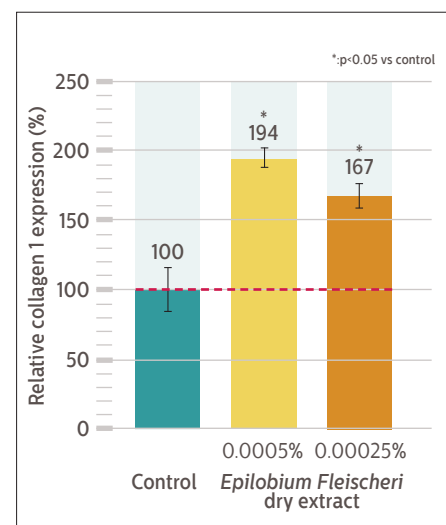


Figure 8: Collagen I expression following treatment with *Epilobium fleischeri* dry extract

(Figure 10) and, at 0.024%, it reduced p21 by 27% (Figure 11), further confirming that *Epilobium fleischeri* extract can reduce senescent cells, allowing aged skin to regain a more youthful structure.

Clinical study

Clinical study is being carried out with subjects applying a cream containing 3% *Epilobium fleischeri* extract. In one *in vivo* double-blind study, which ran for three months, two groups of 25 female volunteers with mixed skin ethnicities (skin phototypes II to V) aged between 45 and 60 years (the average age was 55 years) applied a cream twice daily.

Their skin elasticity was measured using a cutometer and photography was used to show fine lines and wrinkles around the eye (e.g., crow's feet). Skin elasticity and tonicity (Figure 12) had improved by 18% after two months of using the cream with *Epilobium fleischeri* extract as compared with a control group using a placebo cream.

The immediate bounce (R1), which indicates the skin's ability to recover after extension, was improved by 18%. The skin's resilience (i.e., its net elasticity (R5)) had improved by 19%, and an 18% improvement was seen in skin firmness.

Using the cream with *Epilobium fleischeri*

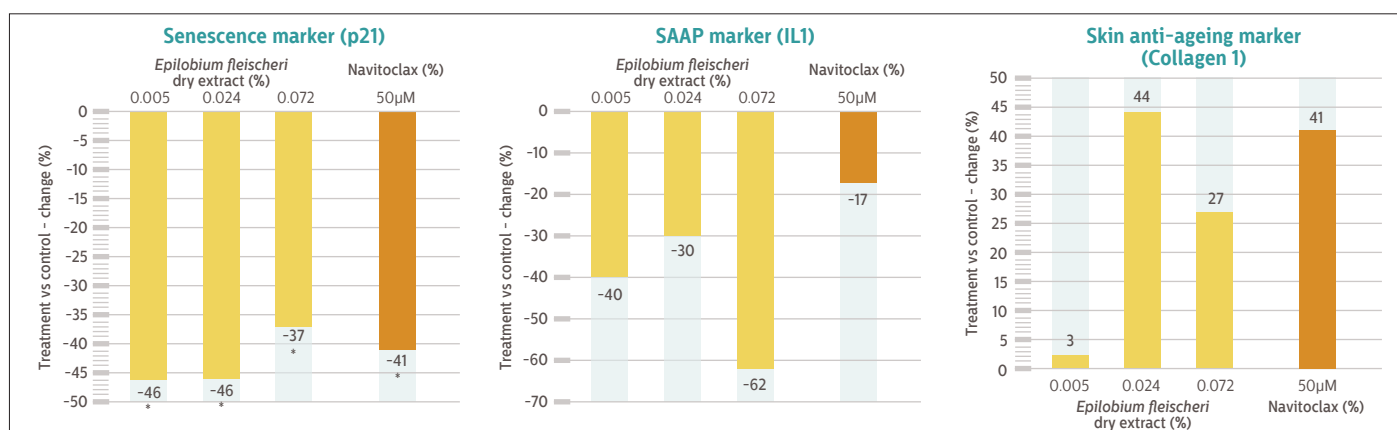


Figure 9: The senolytic and anti-ageing effect of *Epilobium fleischeri* dry extract treatment on *ex vivo* 60-year-old female skin

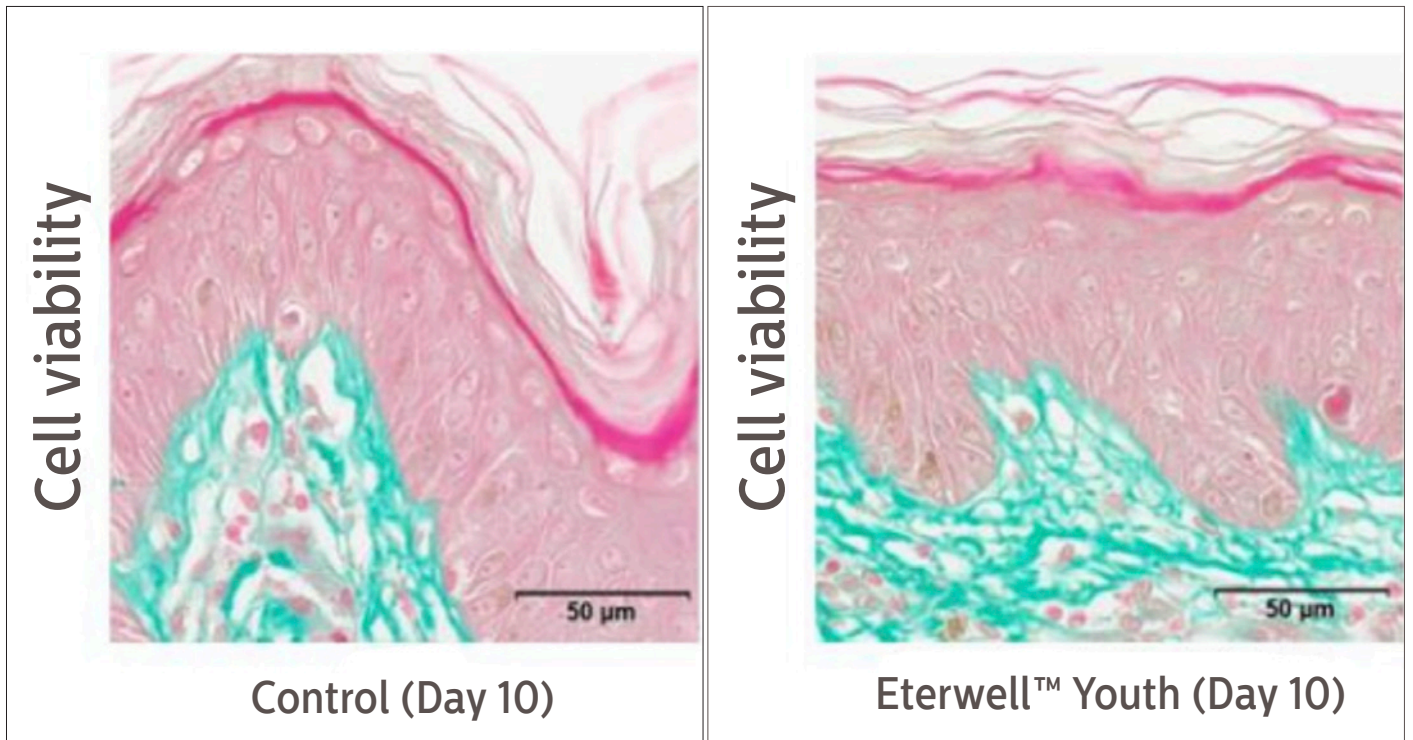


Image 3: The effect of *Epilobium fleischeri* dry extract treatment on a skin explant taken from a 60 year-year-old female volunteer

extract reduced wrinkle volume and the depth of crow’s feet as compared to using the placebo cream. These improvements can be clearly seen after two months in the before and after photos of a 58-year-old volunteer (Image 4).

Sustainability

Because *Epilobium fleischeri* extract is an organic extract of a rare alpine plant growing on ancient glacier moraines, it is highly important that it is ethically produced, with a positive social impact and a low environmental impact. *Epilobium fleischeri* is harvested three times per year, which allows for very efficient use of the land.

Organic cultivation and sustainable land use are optimised to ensure that this Cosmos, Natrue-certified ingredient, is preserving Swiss alpine biodiversity. The cultivation of *Epilobium fleischeri* follows regenerative farming practices that are aligned with the Union

Ethical BioTrade’s (UEBT) regenerative farming, which goes beyond responsible sourcing and ethical practices and focuses on improved soil restoration, water purification, the maintenance of biodiversity, and enhanced climate resilience.

There is a fair-for-life, Fairtrade-certified transparent and traceable supply chain from seed to bioactive, ensuring responsible sourcing. Also, DSM-Firmenich’s ethical relationships with small stakeholders guarantee a minimum price for farmers, which is fixed before harvest. With production requiring less than 3 kg of CO₂eq/kg, *Epilobium fleischeri* extract has a low carbon footprint from cradle to gate. This and applying green chemistry principles to a gentle extraction process using organic ethanol limits its environmental impact.

Conclusion

Recent research in senolytic science shows that by eliminating senescent skin cells, the

skin can become healthier and more youthful. The accumulating burden of senescent zombie cells and their inflammatory, tissue-damaging secretions is now recognized as a root cause of many of the characteristics of skin ageing, including sagging skin, wrinkles, the loss of skin elasticity and firmness, skin roughness, and a dull complexion.

The exciting data presented here and the more impressive results of *in vivo* studies on skin texture, skin structure, and wrinkles that are soon to be published demonstrate that *Epilobium fleischeri* extract acts as a natural senolytic, selectively inducing apoptosis only in senescent fibroblasts. This targeted removal of zombie cells allows the skin to regain youthful function and a youthful appearance.

In vitro and *ex vivo* studies confirm the senolytic activity of *Epilobium fleischeri* extract. When a cream with 3% *Epilobium*

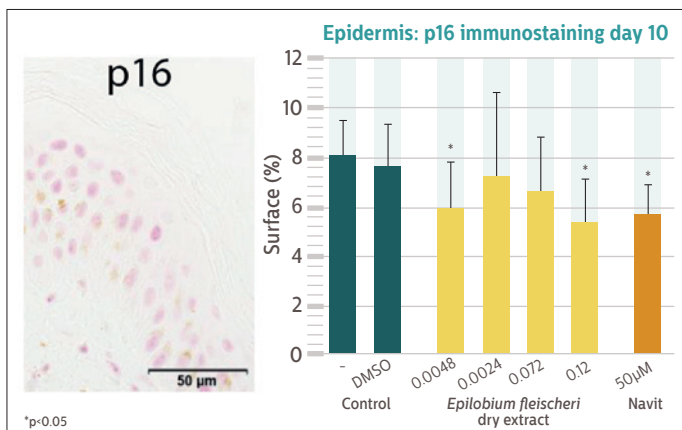


Figure 10: Histological immunostain intensity for p16, on day 10 following *Epilobium fleischeri* dry extract treatment on explant tissue taken from a 60-year-old-female skin

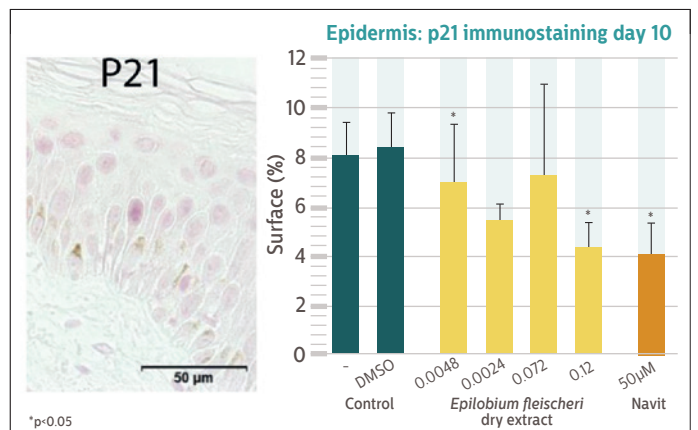


Figure 11: Histological immunostain intensity for p21, on day 10 following *Epilobium fleischeri* dry extract treatment on explant tissue taken from a 60-year-old-female skin

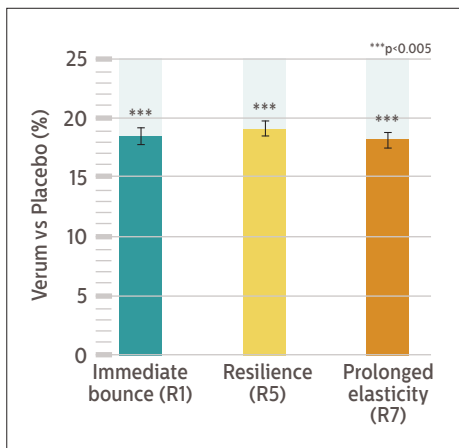


Figure 12: Improvement in skin elasticity 2 months following *Epilobium fleischeri* extract treatment

fleischeri extract was applied, clinical testing showed dramatic improvements in wrinkles, elasticity, and firmness.

The senolytic properties of this sustainable, environmentally friendly botanical extract and its clinical effectiveness in holistic skin rejuvenation make *Epilobium fleischeri* extract one of the most exciting next-generation healthy-ageing actives.

Harnessing senolytics represents a paradigm shift in anti-ageing skincare. The *Epilobium fleischeri* extract leads the way in translating cutting-edge senolytic science



Image 4: Reduction of crow's feet wrinkles on a 58-year-old volunteer after 3 months following *Epilobium fleischeri* extract treatment

into efficacious consumer products that safely restore youthful, healthy skin by targeting fundamental ageing mechanisms at the cellular level.

PC

References

1. Scudellari M. To stay young, kill zombie cells. 2017. *Nature*. Vol. 550, p448–450
2. Wyles SP, Carruthers JD, Dashti P, Yu G, Yap JQ, Gingery A, Tchkonja T, Kirkland J. Cellular senescence in human skin aging: Leveraging senotherapeutics. *Gerontology*. 2024. Vol. 70 (1), p7–14
3. Baar MP *et al*. Targeted apoptosis of senescent cells restores tissue homeostasis in response to chemotoxicity and aging. *Cell*. 2017. Vol. 169, p132–147
4. Zhu ML, Zhang PM, Jiang M, Yu SW, Wang L. Myricetin induces apoptosis and autophagy by inhibiting PI3K/Akt/mTOR signalling in human colon cancer cells. *BMC Complement. Med. Ther.* 2020 Vol. 20(1), p209
5. Kirkland JL, Tchkonja, T. Senolytic drugs: from discovery to translation. *Journal of Internal Med.* 2020. Vol.288(5), p518–536