

Flawless skin via Swiss stone pine extract

■ Franziska Wandrey, Daniel Schmid, Fred Züllli – Mibelle Biochemistry, Switzerland



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A flawless, even looking skin is a desire of many but it is not easily achieved since there are numerous causes for skin tone unevenness. Several different stresses that our skin encounters daily can lead to skin redness and age spots. Oxidative stress caused by reactive oxygen species (ROS), generated by pollution, UV light or internal ageing processes, leads to the activation of inflammation which in turn leads to skin redness.¹ Exposure to UV light upregulates the matrix metalloproteinase-1 (MMP-1), a collagen degrading enzyme,² and additionally causes excess melanin production that results in age spots. Sensitive skin is especially prone to redness, as it reacts more strongly to external stimuli. The reason is often an exaggerated activation of TRP receptors in the skin. These receptors are predominantly expressed in neurons but also present in keratinocytes and fibroblasts. TRP receptors sense chemicals, temperature and pH and translate them into an appropriate response by promoting the release of neuromediators that induce pain, itching and inflammation.³ An overactivation of these receptors would lead to constant inflammation and local redness in the skin. All these mentioned effects of stresses on the skin are naturally exacerbated through ageing and lack of sleep since the skin is not able to sufficiently regenerate.

Therefore, there is a strong need for a cosmetic active that protects from several of these stresses to yield a more even skin to look refreshed and radiant.

A protectant molecule found in the wood of Swiss stone pine

Pinus cembra, which is also known as Swiss stone pine, is a species of pine tree that grows in the Alps and the Carpathian Mountains of central Europe. It symbolises high mountains as it typically grows at altitudes of 1,500 - 2,500 metres where winters are particularly long and harsh.

The Swiss stone pine is an extremely resistant tree as it survives temperatures as low as -50°C as well as strong wind exposure. For centuries, the wood from Swiss stone pine has been used for carving sculptures thanks to its tender

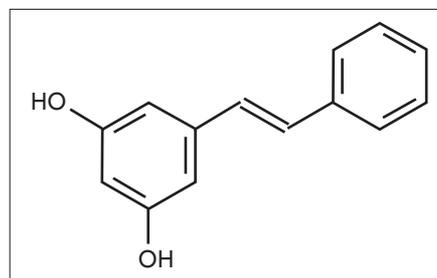


Figure 1: Schematic representation of the pinosylvin molecule.

Abstract

A novel cosmetic active, an extract from Swiss stone pine wood that contains high amounts of the resveratrol-like molecule pinosylvin, was tested for its effect on skin reactivity to external stresses. Studies demonstrated that Swiss stone pine extract inhibits the function of the pain receptor TRPV1 and reduces the production of inflammatory markers upon oxidative stress. Swiss stone pine extract also protects skin collagen by inhibiting the UVA-induced MMP-1 production. Additionally, treatment with Swiss stone pine extract reduced local redness and age spots on the face, which led to a visible increase in the overall skin tone evenness for a radiant appearance.

characteristics. It is also particularly used to make beds, sleeping room furnishing as well as pillow fillings. Interestingly, research has revealed that the wood of Swiss stone pine is conducive to relaxation and deep sleep phases as the heartbeat frequency is reduced when sleeping in a Swiss stone pine bed. Swiss stone pine wood and needles contain the molecule pinosylvin, a stilbenoid similar to resveratrol (Fig 1). Its function is mainly to protect the tree from fungal infections as it possesses potent antibacterial and antifungal activities, more potent than resveratrol against certain types of yeast.⁴ The pine produces more pinosylvin upon encountering various external stresses such as UV light irradiation,⁵ high ozone concentrations, infection as well as wounding.⁶ Many scientific studies have been conducted with pinosylvin to investigate its numerous positive effects in mammalian cells. It was shown that pinosylvin neutralises free radicals by upregulating heme oxygenase-1⁷ and reduces the amount of intra- and extracellular ROS.⁸ Furthermore, pinosylvin reduces inflammatory responses and inflammatory gene expression.⁹ Notably, it is able to modulate TRP pain receptor

channels and thus inhibits subsequent inflammation.¹⁰⁻¹¹ This makes pinosylvin an ideal candidate to counteract inflammation resulting from various stresses especially in sensitive skin that reacts strongly to environmental influences.

A concentrated extract of the wood of Swiss stone pine containing pinosylvin (Pinolumin/INCI: Pinus Cembra Wood Extract (and) Alcohol (and) Pentylene Glycol (and) Aqua / Water) was used to investigate the protective effects on skin cells and its ability to reduce redness and age spots for a more even skin tone.

Materials and methods

CGRP release in neurons

Sensory neurons were seeded and cultivated in 96 well plates for seven days. Culture medium was then removed and replaced by assay medium containing or not (control) Swiss stone pine extract at different concentrations and the cells were pre-incubated for 30 minutes. Following pre-incubation, capsaicin was added at 6 different concentrations (0.01, 0.03, 0.1, 0.3, 1, 10 µM) and the cells were incubated for 30 minutes. A non-stimulated control was performed in parallel. The presence of CGRP in the supernatants was determined by ELISA.

Inhibition of IL-8 and PGE2 release

Human keratinocytes were cultured in 96-well plates for 24 hours. The cell culture medium was then removed and replaced by culture medium containing or not (control) Swiss stone pine extract at different concentrations or the reference compounds (10⁻⁷ M dexamethasone for the IL-8 release assay and 10⁻⁶ M indomethacin for the PGE2 release assay) and the cells were pre-incubated for 24 hours. Afterwards, the medium was replaced with culture medium containing the inflammatory inducer phorbol myristate

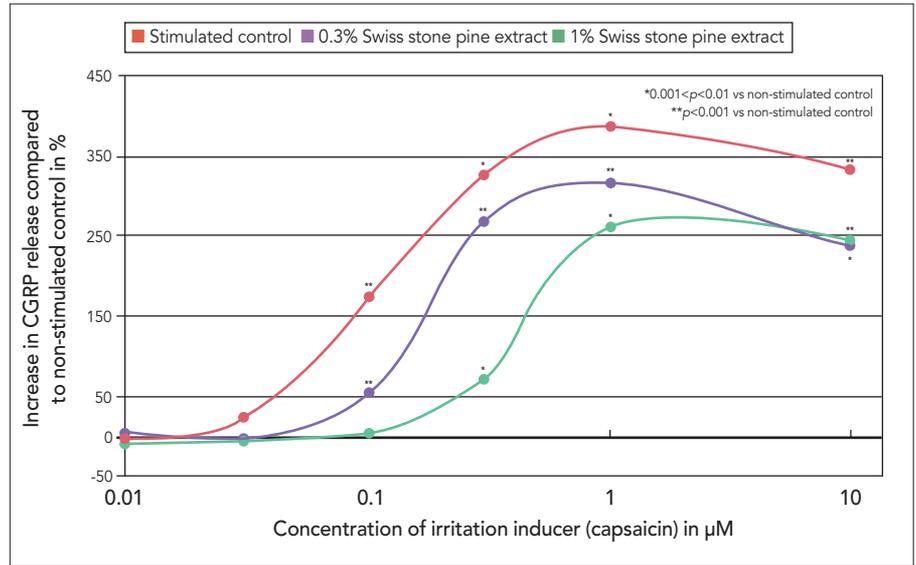


Figure 2: Swiss stone extract inhibits the release of CGRP by neurons upon irritation with capsaicin.

acetate (PMA; 0.1 µg/ml) and containing or not (control) Swiss stone pine extract or the references and the cells were further incubated for 24 hours. A control without inducer was performed in parallel (non-stimulated control condition).

At the end of incubation, the quantities of IL-8 and PGE2 in culture supernatants were measured using specific ELISA kits

Inhibition of UVA-induced MMP-1 release

Human dermal fibroblasts were cultured in 96-well plates for 24 hours. The cell culture medium was then removed and replaced by culture medium containing or not (control) Swiss stone pine extract at different concentrations and the cells were pre-incubated for 24 hours. The medium was then replaced by irradiation medium and cells were irradiated with UVA (15 J/cm²) using a SOL500 Sun Simulator with a H1 filter (Dr. Hönle AG, Germany). Following irradiation, the medium was

replaced by culture medium containing or not (control) Swiss stone pine extract at different concentrations and the cells were further incubated for 48 hours. A control without irradiation was performed in parallel (non-irradiated control). At the end of incubation, MMP-1 release in culture supernatants was measured using a specific ELISA kit.

Clinical study to assess skin colour in age spots

In a double-blind, placebo controlled clinical study, twenty-two women with age spots who were aged from 43 to 63 years (mean age: 54.3 years) applied a 2% Swiss stone pine extract emulsion on one half of their faces and the corresponding placebo on the other half of their faces twice a day for a period of two months. Skin lightness (L*) and redness (a*) were determined on selected age spots by using a Spectrophotometer CM-700d (Konica Minolta, Japan).

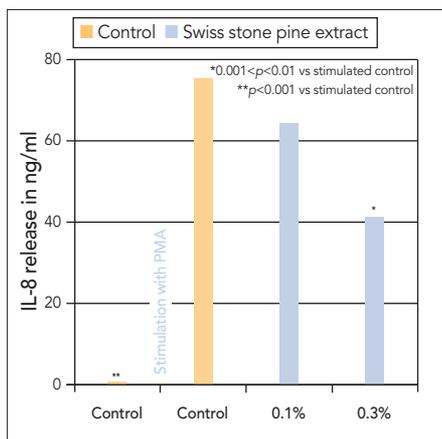


Figure 3: Reduction of the release of IL-8 by keratinocytes upon stimulation with PMA by treatment with Swiss stone pine extract at different concentrations.

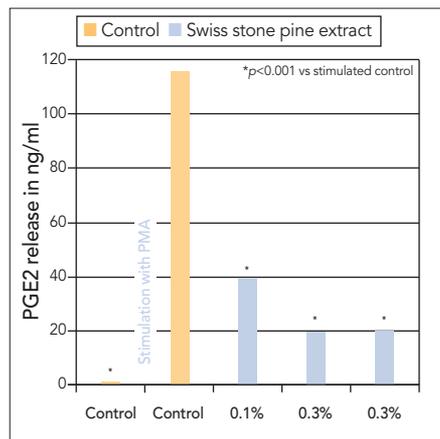


Figure 4: Reduction of the release of PGE2 by keratinocytes upon stimulation with PMA by treatment with Swiss stone pine extract at different concentrations.

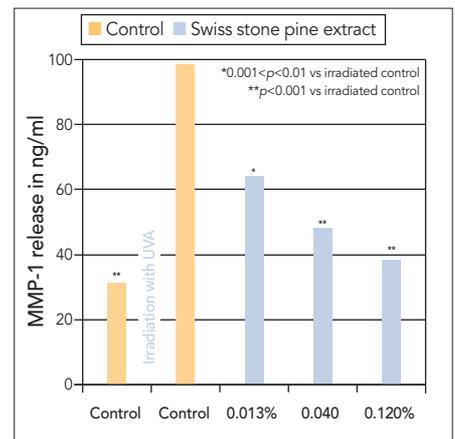


Figure 5: Reduction of the release of MMP-1 by fibroblasts upon irradiation with UVA by treatment with Swiss stone pine extract at different concentrations.

Clinical study to assess skin tone homogeneity

In a double-blind, placebo controlled clinical study, forty-one women aged from 32 to 65 years (mean age: 52.6 years) were separated into two groups comprising twenty and twenty-one volunteers respectively. A 2% Swiss stone pine emulsion was applied twice daily to the faces of one group for a period of 56 days, the same treatment was carried out on the second group by using the corresponding placebo emulsion. The heterogeneity of the complexion was analysed by measuring the skin lightness (L^*) and the skin colour parameters (a^* and b^*) on 24 selected locations on the face using a Spectrophotometer CM-700d (Konica Minolta, Japan) and calculating the standard deviation of these measurements. Additionally, photographs of the faces were taken using a Visioface device (Courage+Khazaka, Germany) to document visible effects.

Results and discussion

Swiss stone pine extract inhibits neuro-inflammation

The ability of Swiss stone pine extract to modulate the activation of sensory neurons was evaluated in a cell culture model that mimicked an irritation of the nerve endings through activation of the TRPV1 receptor. TRPV1 is a member of the TRP receptor family and reacts to low pH, certain chemicals as well as high temperatures.

In this study, sensory neurons were irritated with capsaicin, a TRPV1 receptor agonist, in the presence or absence (control) of Swiss stone pine extract.

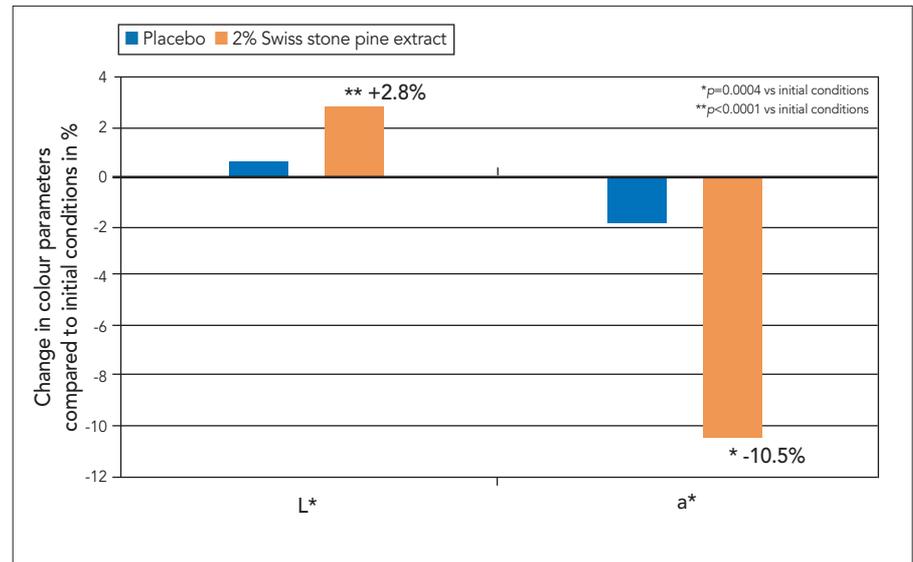


Figure 6: Reduction of skin redness and lightness in facial age spots after treatment for 56 days with a cream containing 2% Swiss stone pine extract.

Irritated neurons react through releasing the neuropeptide calcitonin gene related peptide (CGRP), which leads to vasodilation, histamine release and subsequently the activation of inflammatory pathways. Treatment with Swiss stone pine extract reduced the capsaicin-induced CGRP release in a significant and dose-dependent manner (Fig 2). This indicates that Swiss stone pine extract inhibits the activation of the TRPV1 receptor upon neuronal irritation, which suggests that it has a soothing effect.

Anti-inflammatory activity of Swiss stone pine extract

To investigate the anti-inflammatory effect

of Swiss stone pine extract, keratinocytes were treated with the oxidative stress inducer phorbol myristate acetate (PMA), which leads to an increased production of the inflammatory markers IL-8 and PGE2. Treatment with Swiss stone pine extract reduces the inflammation response upon PMA-induced oxidative stress in a dose-dependent manner (Fig 3, 4).

Protection of collagen from UVA-induced destruction

When skin gets damaged by UV radiation, the expression of collagen-destroying enzymes, such as MMP-1 is upregulated.

To test whether Swiss stone pine extract is able to protect collagen, fibroblasts were

irradiated with UVA light in the presence or absence of Swiss stone pine extract and MMP-1 release was measured. Results showed that Swiss stone pine extract inhibited the UVA-induced MMP-1 release in a significant and dose-dependent way (Fig 5), which indicates a protective effect of skin collagen.

Reduction of redness and age spots

As Swiss stone pine extract is able to reduce irritation and inflammation *in vitro*, and molecules similar to pinosylvin have exhibited whitening activity, a clinical study was performed to determine the anti-redness and lightening activity of the active ingredient. After two months of application of 2% Swiss stone pine extract, skin lightness (L*) significantly increased in age spots while skin redness (a*) was significantly reduced (Fig 6). The effect was observed in 95% and 77% of the volunteers, respectively.

Overall improvement of skin tone evenness

To investigate whether, in addition to the reduction of age spots, Swiss stone pine extract evens out the skin tone on the whole face, another clinical study was carried out to assess skin tone homogeneity. For this, two groups of volunteers applied either an emulsion containing 2% Swiss stone pine extract or the corresponding placebo emulsion on their whole face for two months. The heterogeneity of their complexion was analysed by choosing 24 selected locations across the face and measuring on each area the skin lightness (L*), redness (a*) and yellowness (b*). The standard deviation of the 24 measurements was determined as a marker for skin tone homogeneity. The smaller the standard deviation, the more homogeneous is the skin tone. Treatment with 2% Swiss stone pine extract increased the skin tone homogeneity of all three colour values compared to placebo (Fig 7). The improvement of skin complexion was also visible in before and after photographs (Fig 8).

Conclusion

Treatment with Swiss stone pine extract containing the constitutive protectant molecule pinosylvin reduces irritation and inflammation induced by chemicals, oxidative stress and UVA. Therefore, Swiss stone pine extract offers a multi-targeted soothing approach for sensitive skin. Furthermore, treatment with Swiss stone pine extract reduces facial redness and age spots and thus results in a more homogeneous and radiant skin. PC

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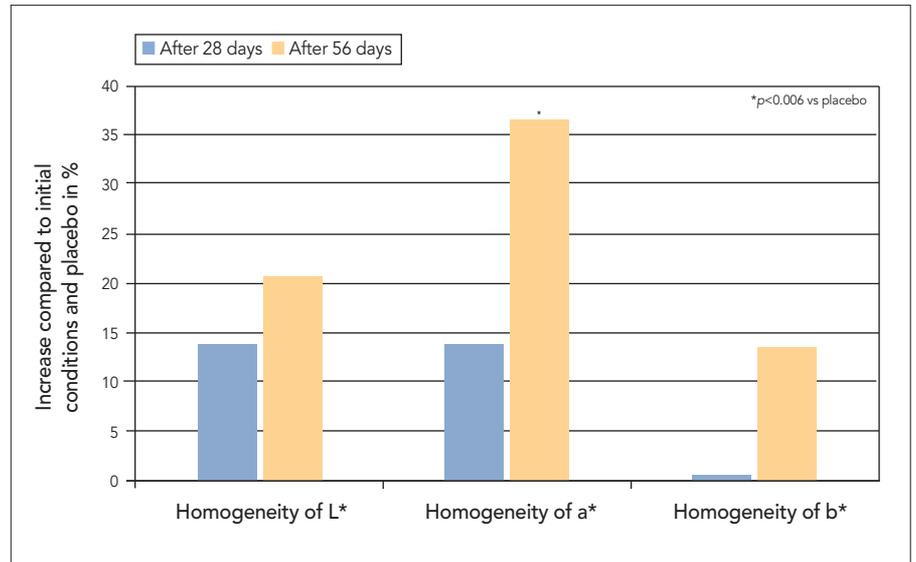


Figure 7: Improvement of skin tone homogeneity after treatment with a cream containing 2% Swiss stone pine extract.

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Figure 8: Pictures of a volunteer before and after treatment with a cream containing 2% Swiss stone pine extract for 56 days.