Cyanidium caldarium is an amazing type of micro alga. Evolved approximately 1.3 billion years ago, these algae represent one of the oldest forms of life on our planet. The unicellular red alga belongs to the extremophilic microorganisms, which are able to survive under extreme conditions. During its evolution Cyanidium caldarium has adapted to extreme habitats and grows between pH 0.05 and 5 and up to 60°C. Growth under such hostile conditions requires biosynthesis of special substances, and one of these bioactive substances produced by Cyanidium caldarium is gamma aminobutyric acid (GABA). GABA is a non-proteinogenic amino acid and functions in the central nervous system as a neurotransmitter. It is found in animals and many plant forms. The production of GABA by an alga has not been reported previously and is rather surprising. It is assumed that GABA influences internal proton pumps and thereby maintains the essential intracellular pH value of 6.6 to protect cellular components from the acidic stress of the environment. In addition, GABA is said to stimulate hyaluronic acid and collagen synthesis in fibroblasts and enhances the survival rate of dermal fibroblasts when exposed to oxidative stress.³ GABA is also known as a functional food ingredient and as an active ingredient in the cosmetic industry. It is applied in the context of anti-wrinkle and skin smoothing products. GABA is described as possessing anti-inflammatory activity.² Inflammatory processes are also known in the context of chronological ageing, which is referred to as ‘inflammaging’ (inflammation-induced ageing). Recently, it was described that age-associated inflammation inhibits epidermal stem cell function.³

Another stress response mechanism relates to the production of osmoprotectants or compatible solutes. These are low molecular weight organic compounds from different natural product classes like sugars, amino acids and their derivatives. These molecules accumulate in cells and balance the osmotic gradient between the cell’s surrounding and the cytosol. Due to their protective properties against extreme environmental stress they are also referred to as extremolytes. Such substances often show high protective potential with respect to cellular macromolecules, for instance DNA. Cyanidium caldarium synthesises extremolytes like prolin, lysine and ornithin and their derivatives to survive under extreme temperatures and very low pH levels.

Besides the above mentioned properties, Cyanidium algae contain dermatologically valuable proteins and polyphenols, which

**ABSTRACT**

During the chronological ageing process epidermal skin stem cells become less effective, meaning that the renewing and repairing activity of the epidermis is reduced. Moreover, fewer elastic fibres are synthesised, thereby inducing a progressive loss of skin elasticity.

The standardised, COSMOS certified Cyanidium caldarium red algae extract, unique in its capability to produce gamma aminobutyric acid (GABA) with proven combined activity on epidermal stem cells and elastic fibres, clearly retains youthful skin appearance and reduces the signs of chronological ageing.

![Figure 1: Suggested working mechanism of Cyanidium caldarium extract.](image1)

![Figure 2: Colony forming efficiency (CFE) of epidermal progenitor cells after application of Cyanidium caldarium extract.](image2)
were shown to delay the natural ageing process.

The micro alga *Cyanidium caldarium* was found in a broad screening process of numerous micro algae. The most promising candidates out of this research were selected and cultivated. Depending on their growth properties, some of these micro algae were further explored for potential cosmetic activity. Finally, *Cyanidium caldarium* was the most promising alga that was extensively further examined regarding biological and physiological properties on the skin.

The strain that is used for the production of the *Cyanidium caldarium* extract was isolated on the Sunda Islands in Southeast Asia from Mount Lawu fumaroles on the island of Java.

Production of the bioactive algae extract is a natural and eco-friendly process from biorenewables. After cultivation, the algae cells are processed by a proprietary mild extraction method followed by a filtration step enriching the bioactive compounds.

*Cyanidium caldarium* extract was found to protect and maintain epidermal stem cell capacity for a rejuvenated skin activity. On a molecular level, it boosts elastic fibres leading to highly supple skin and a reduction of skin elasticity fatigue (Fig. 1).

**In vitro studies**

Different in vitro studies were carried out to analyse the effects of *Cyanidium caldarium* extract on a biological level. Commercially available epidermal keratinocyte progenitor cells were used as cell culture model to study the effects on epidermal stem cells. Dermal fibroblasts were used to evaluate dermal effects.

**Colony forming efficiency**

The capacity to form a colony in cultivation is a characteristic trait of stem cells. They can make identical copies of themselves as well as generating specialised cells. They can form colonies representing a mixed population, consisting of stem cells, transient amplifying cells (cells in an intermediate state) and differentiating cells. This capacity is called ‘colony forming efficiency (CFE)’ and can be measured when cultivating the cells in vitro. The number of colonies formed is a value for the concentration of vital progenitor/stem cells. It gives an indication for maintaining stem cell characteristics.

The colony forming efficiency of epidermal progenitor cells was analysed after application of *Cyandium caldarium* extract in different concentrations and compared to a vehicle control and *Malus domestica* as well as *Solar vitis* extracts as market products.

To analysis the colony forming efficiency (CFE), the cells were cultured with a low seeding density (800 cells/25 cm²) in presence of *Cyanidium caldarium* extract for 8 days. End concentration of *Cyanidium caldarium* extract in the medium was 30 ppm or 100 ppm (relative to dry matter content). *Malus domestica* and *Solar vitis* extracts were tested in concentrations recommended by the manufacturer. A cultivation without active ingredient, only with medium, served as vehicle control. All cultivations were performed as three biological triplicates. After treatment of the keratinocyte progenitor cells colony forming efficiency (CFE) was analysed as a major readout (Fig. 2).

The results of this study clearly show that *Cyanidium caldarium* extract helps the epidermal stem cells to maintain their characteristics. Therefore, *Cyandium caldarium* extract will significantly support and protect the maintenance of epidermal stem cell activity.

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**Figure 3:** Affinity histochemistry, hyaluronic acid (top) and paxillin (bottom) staining.

**Figure 4:** Fine lines expressed as roughness parameters after 8 weeks of application, calculated relative to vehicle treated skin (significance: **p<0.1 vs. vehicle).**
Fibroblast activity and interaction

In addition to epidermal stem cell effects, the activity of *Cyanidium caldarium* extract was also evaluated on fibroblasts.

Primary human dermal fibroblasts were cultivated in Dulbecco’s Modified Eagle Medium (DMEM) with different concentrations of fetal calf serum (FCS) depending on the cultivation step and 1% penicillin/streptomycin at 37°C and 5% CO₂. For the study, cells were seeded at a density of 1.5 x 10⁵/well into 24-well-plates and the serum starved for 24 hours. Subsequently, cells were stimulated by addition of 250 ppm *Cyanidium caldarium* extract (relative to dry matter content) applied in media containing 2% FCS. A cultivation without active ingredient, only with media, served as control (vehicle). [3H]-Thymidine (1 µCi/mL) was added for the last six hours of the stimulation. [3H]-Thymidine incorporation was normalised to total cellular protein (BioRad, Munich) as readout of DNA synthesis.

**Affinity cytochemistry of pericellular and intracellular HA**: Cells were fixed in 3.7% formaldehyde/PBS, 70% ethanol and 5% glacial acetic acid, all v/v). Following rinsing with PBS, cells were permeabilised by 0.1% Triton X-100 in PBS for 10 minutes. After additional rinsing with PBS, cells were stained for HA using a biotinylated HA-binding protein followed by streptavidin-FITC in PBS containing 1% bovine serum albumin. As controls, cells were digested with Streptomyces hyal to prior to staining, which abolished HA staining (data not shown). Imaging of the cells was performed using a Zeiss imager microscope and a 63x objective.

**Immunocytochemistry of Paxillin**: Cells were fixed in 3.7% formaldehyde/PBS. Following rinsing with PBS, cells were permeabilised by 0.1% Triton X-100 (Sigma-Aldrich) in PBS for 10 minutes. This protein represents a key component of primary HA in the ECM. An increased production of HA and paxillin staining could be observed (not shown).

An increased production of HA and general ECM structure support as well as improved interaction of cells with the ECM in the dermal layer could be shown, as demonstrated by increased paxillin staining. This protein represents a key component of focal adhesions, mediating cell anchoring in the ECM.

Therefore, it can be claimed that *Cyanidium caldarium* extract stimulates fibroblast activity leading to more dynamic dermal behaviour.

**In vivo studies**

**Elasticity study – inner forearm**

Nineteen volunteers (male and female, aged between 33 and 59 years) received an O/W formulation containing 1% *Cyanidium caldarium* extract (250 dry matter content), 21 panellists received the formulation containing 5% ppm *Cyanidium caldarium* extract (1250 dry matter content) and 20 panellists received the formulation with 2% *Malus domestica* extract as a market reference product. Twenty panellists received the formulation without an active ingredient (vehicle). Test formulations were applied twice daily for eight weeks on the inner forearm in a randomised test design. Prior to application and after eight weeks, skin elasticity and skin surface parameters were determined. Skin elasticity measurements were conducted using a Cutometer.

In Figure 4 reduction of fine lines is presented after normalisation to the vehicle. It can be seen that *Cyanidium caldarium* extract improves the skin structure by reducing fine lines after 8 weeks of application compared to the vehicle treatment.

Improving skin elasticity requires the active ingredient to ‘work’ in the dermal parts of the skin. To achieve positive effects in the dermis, higher concentrations of *Cyanidium caldarium* extract are required.

Both skin elasticity parameters that describe the remaining deformation of the skin after stretching are significantly reduced by application of 5% *Cyanidium caldarium* extract compared to vehicle treatment (Fig. 7).

R1 describes the ability of the skin to...
bounce back after stretching and R4 describes skin elasticity fatigue. *Cyanidium caldarium* extract is able to improve both parameters leading to supple skin.

**Biopsy and anti-wrinkle study – gluteal region and face**

A placebo controlled study enrolling 20 healthy volunteers (female and male) from 50 to 77 years of age was performed in two parts.

For the biopsy part, the volunteers applied once daily in the morning 2 mg/cm² of different O/W test formulations containing either no active ingredient (vehicle), 1% or 5% *Cyanidium caldarium* extract or 0.1% retinol (anti-ageing standard, 0.2% of a commercial 50% Retinol solution were used) on the upper medial quarter of the gluteal region using a randomised test design. After eight weeks’ treatment 6 mm punch biopsies were taken from the four different testing areas and gene expression analysis was carried out.

The second part of the study was performed on the face. The volunteers applied the O/W formulation without an active ingredient on one part of the face and the formulation containing 5% *Cyanidium caldarium* extract on the other side (half-side test design) once daily in the morning for a period of eight weeks.

Before and after the treatment phase digital images of the face were taken, and an image analysis was performed using a VISIA-CR facial imaging system (Canfield Scientific, Inc., NJ, US). Furthermore, visual expert grading was performed. Dermatologists graded the degree of wrinkle reduction after eight weeks on a 5-grade scale (1=low wrinkle appearance, 5=high wrinkle appearance) compared to the beginning of the study.

In the first part of this study, relative gene expression was analysed after eight weeks of application of different test formulations with *Cyanidium caldarium* extract and retinol compared to the vehicle formulation out of punch biopsies from the gluteal region of the volunteers (Fig. 8).

The results show an increased gene expression of relevant stem cell markers at 5% *Cyanidium caldarium* extract, pointing out that *Cyanidium caldarium* extract maintains epidermal stem cell characteristics. Furthermore, the elastin boosting activity of *Cyanidium caldarium* extract which was already observed in vitro could be confirmed as the elastin gene expression was approximately two-fold upregulated after eight weeks application of 5% *Cyanidium caldarium* extract compared to untreated skin.

In addition, the volunteers applied two test formulations on the face during this study. Wrinkle reduction was analysed after the application period.

Figure 8 shows results from expert grading, where wrinkle formation was evaluated on a 5 grade scale before and after the application of either the vehicle formulation on one side of the face or the formulation with 1250 ppm *Cyanidium caldarium* extract on the other side.

The investigation of the experts shows that a significant reduction in the appearance of wrinkles could be observed after eight weeks treatment with 5% *Cyanidium caldarium* extract. This is also visible in the image analysis with the VISIA-CR device (Fig. 9).

The wrinkle reduction after treatment with *Cyanidium caldarium* extract can be seen in the pictures on the left side, and is further illustrated using the VISIA-CR analysis software (right side; green lines reflect wrinkle structures on the face).

**Conclusion**

Overall it was shown that *Cyanidium caldarium* extract has a broad biological activity. The extract boosts elastic fibres and the overall ECM network resulting in a highly supple skin and diminished skin elasticity fatigue. Epidermal stem cell function is maintained, and stem cells are protected by the algae extract which provides rejuvenation of skin cell activity. In summary, application of a cosmetic product which contains *Cyanidium caldarium* extract leads to a youthful appearance and delays chronological ageing.

**References**