

CLINICAL DATA – ALBATRELLUS OVINUS EXTRACT

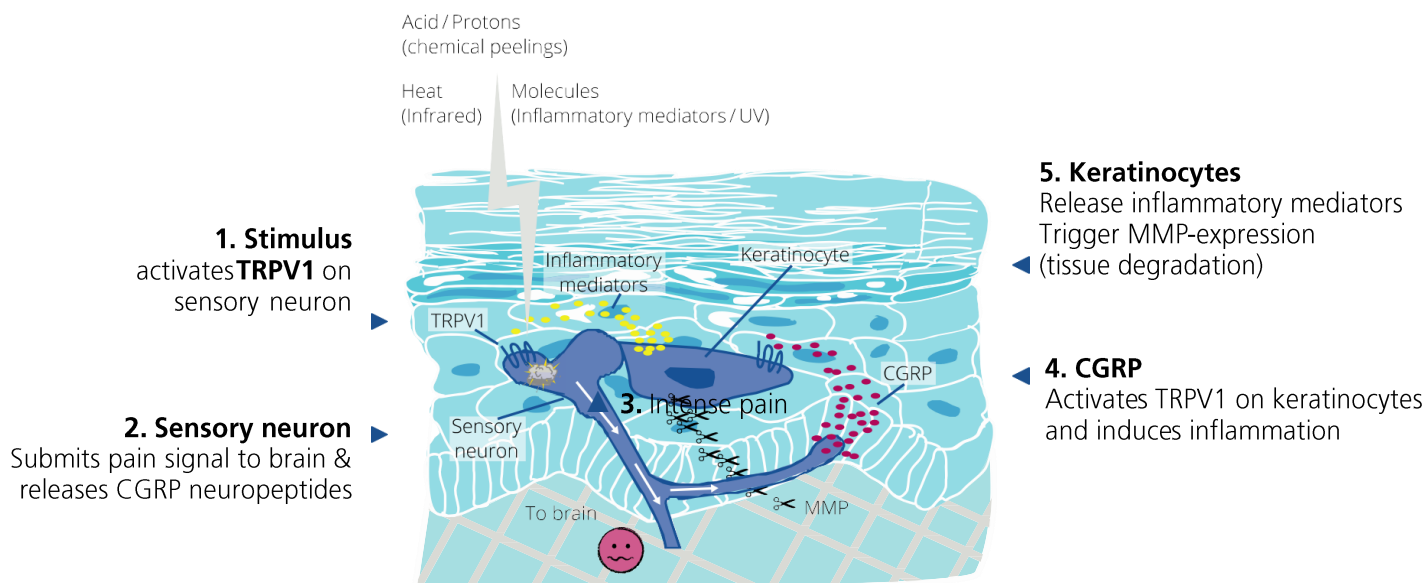
Albatrellus ovinus extract

- Neuro-soother from edible mushroom *Albatrellus ovinus*
- Blocks the pain receptor TRPV1, a mediator of skin hypersensitivity and premature skin ageing
- Calms overstressed skin
- Prevents thermal ageing: premature skin ageing induced by heat stress

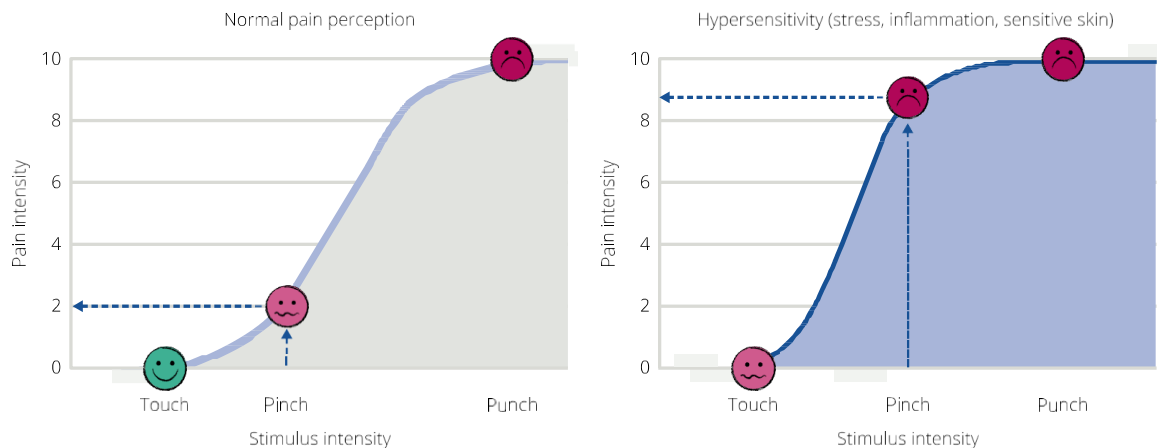
TRPV1– neurosensory of uncomfortable sensations and inflammation

- Activated by heat stress (> 42 °C), protons (acids, pH < 6.5), endogenous molecules (inflammatory mediators), exogenous molecules (e.g. capsaicin, geraniol, citronellol, retinoids)
- Expressed in epidermal sensory neurons and keratinocytes
- Starting point for skin-nerve communication to induce inflammation

A heated debate between nerve and skin - Activation of TRPV1 starts crosstalk and induces inflammation



TRPV1 – a key factor for epidermal hypersensitivity



▲ Normal condition
No stimulus / no TRPV1 activation – no discomfort
Small stimulus / TRPV1 activation – mild discomfort

▲ Inflammation triggers over-reactivity of TRPV1:
No stimulus / TRPV1 activation – discomfort
Small stimulus / TRPV1 activation

TRPV1 inhibition as a cosmetic strategy against overstressed skin

- Sensitive skin / atopic eczema – skin discomfort and rash
- Sunburn - itchiness
- Shaving / epilation – burning sensations
- Emotional stress / nervousness – facial stinging
- Sleep restriction – increased pain perception
- Fragrance allergens / whitening substances / retinoids – hypersensitivity
- Chemical peeling – skin prickling
- Laser / IPL hair removal – pain and itching
- Senile pruritus – itchiness without obvious cause

TRPV1 inhibition as an anti-ageing concept against heat-induced “thermal ageing”

Thermal ageing – a novel concept

- TRPV1 activation due to heat stress (IR / sun)
- Promotion of matrix degradation and premature skin ageing
- Laptop thighs: modern skin disorders of digital natures due to heat stress

TRPV1 inhibition - an effective anti-ageing strategy

- Prevention of thermal ageing
- Protection against laptop thighs
- Protection against IR-radiation

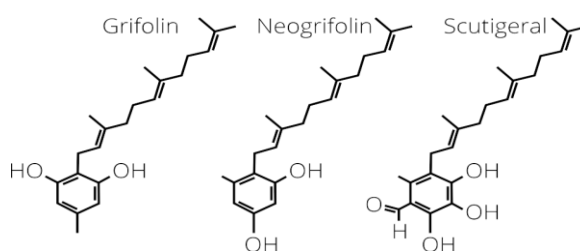
Albatrellus ovinus - The Northern truffle

- Common names are sheep polypore, Schafsporling, polypore des brebis
- Grows in coniferous forests in Northern Europe and North America
- Edible mushroom, mainly used in the food industry

- *Albatrellus ovinus* extract: Extract from the fruiting bodies in propanediol

Active compounds in *Albatrellus ovinus* - grifolin, neogrifolin, scutigeral – the skin analgesics

- Prenylated phenols
- Two-tier strategy
 - Grifolin and neogrifolin directly block TRPV1
 - Scutigeral induces receptor desensitisation by constant low-level activation



Active compounds in *Albatrellus ovinus* extract - Stability of grifolin, neogrifolin and scutigeral

Method:

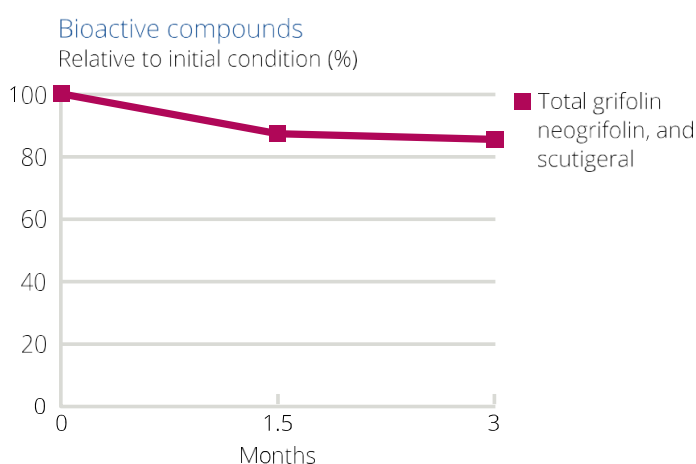
Storage test at 40°C and HPLC analysis

Implementation:

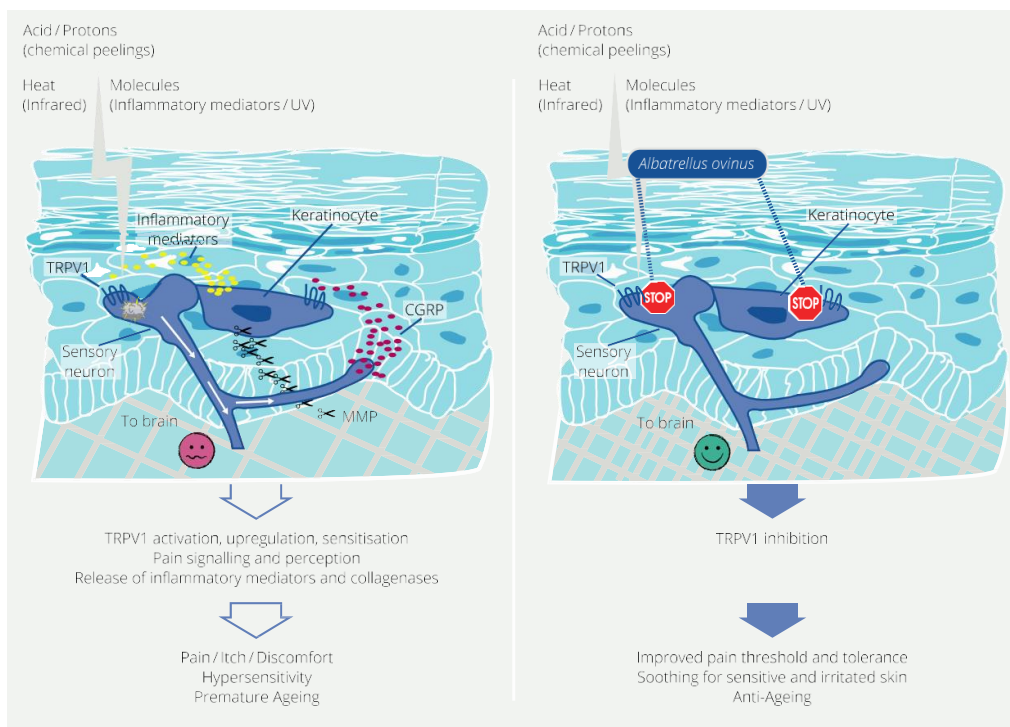
Determination of grifolin, neogrifolin, scutigeral by HPLC

Result:

Compounds account for 20% of dry mass. Good temperature and light stability



Mode of action- Skin soothing and anti-ageing due to TRPV1 inhibition



***Albatrellus ovinus* extract - The Neuro-Balm from A to Zen**

Scientifically confirmed effects

In-vitro studies

- Grifolin, neogrifolin and scutigerol show excellent affinity to TRPV1
- Grifolin functionally blocks the TRPV1 receptor
- Grifolin counteracts neuron-driven inflammation

In-vivo studies

- *Albatrellus ovinus* extract reduces discomfort and irritation
- *Albatrellus ovinus* extract protects from heat stress and soothes hyperalgesic skin
- *Albatrellus ovinus* extract prevents IR-induced barrier damage



Grifolin, neogrifolin and scutigerol show excellent affinity to TRPV1 (*in-vitro* study)

Test design: Biochemical assay

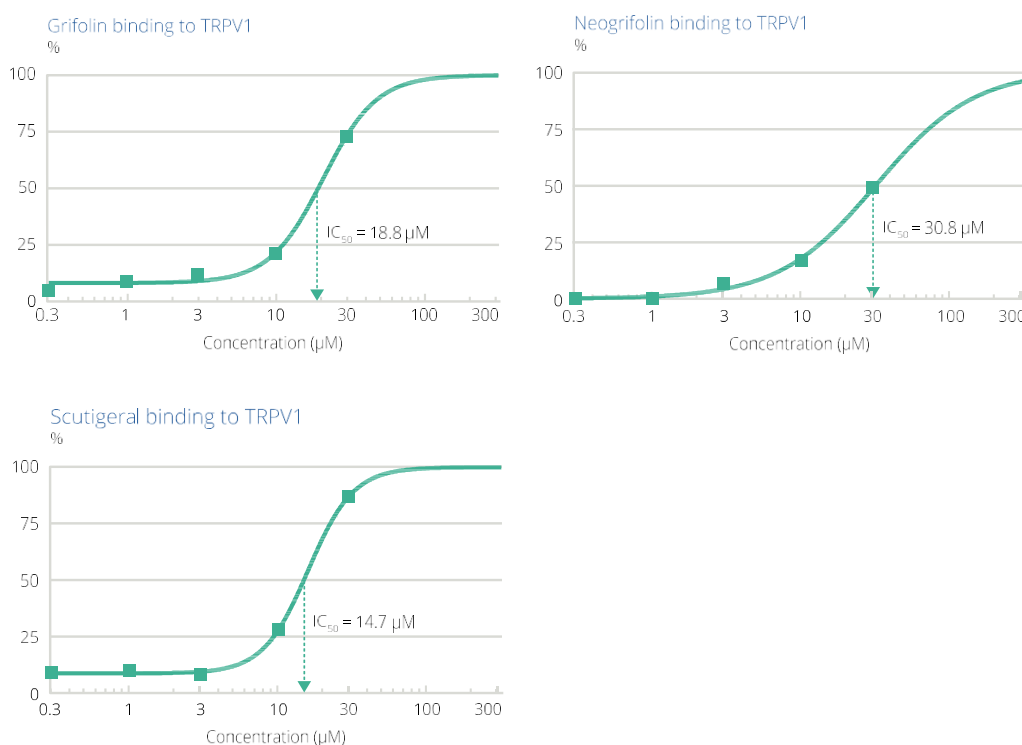
Test items: Measurements were done in duplicates

Test formulations: TRPV1 channels were incubated with 0.2nM of the radioactively labeled reference binder resiniferatoxin (radioligand) in the presence of increasing test substance concentrations.

Application period: 60 minutes at 37 °C

Primary endpoint: 50% inhibitory concentration (IC_{50}): It represents the concentration of a test substance that is required to inhibit the binding of the reference binder by half. It is a measure of how potent an inhibitor is. The smaller the IC_{50} ; the more potent the substance. IC_{50} values and response curves were calculated, by non-linear, least squares regression measurements analysis.

Scutigeral, grifolin and neogrifolin block the binding site of the TRPV1 receptor



Grifolin functionally blocks the TRPV1 receptor (*in-vitro* study)

Test design: Patch-clamp assay

Test subjects: CHO-cells stably expressing TRPV1 channels. N = 4

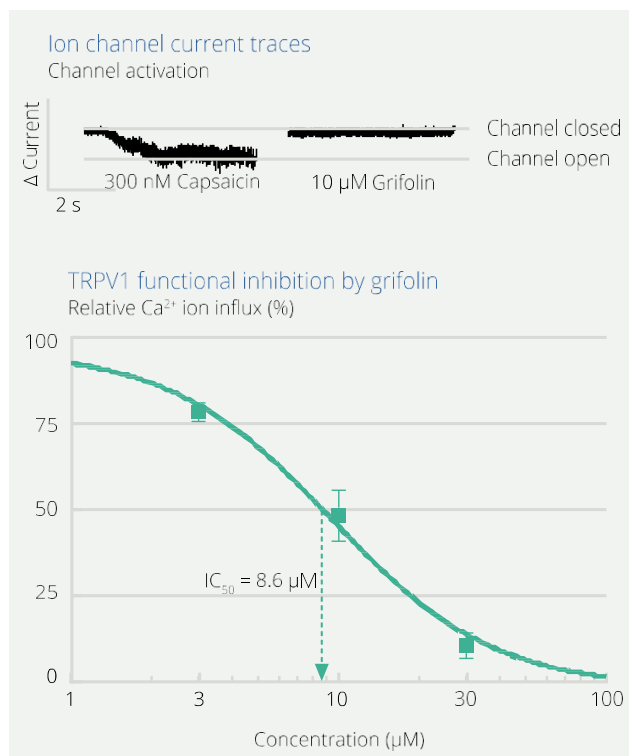
Test formulations: Cells were incubated with Activation test: 300 nM capsaicin or 10 µM grifolin and Inhibition test: 300 nM capsaicin with 0, 3, 10 and 30 µM grifolin.

Application period: Real-time recording for 5s.

Primary endpoint: Membrane current amplitudes. Amplitudes with capsaicin / grifolin were related to values obtained by capsaicin / vehicle.

Secondary endpoint: IC_{50} : see previous experiment for details.

Grifolin inhibits TRPV1 already in low-molecular range



Capasaicin triggers TRPV1 channel opening and induces current. Grifolin has no effect.

Grifolin reduces neuronal responsiveness by blocking the TRPV1 channel.

Grifolin counteracts neuron-driven inflammation (*in-vitro* study)

Test design: Quantification of CGRP released by nerve cells.

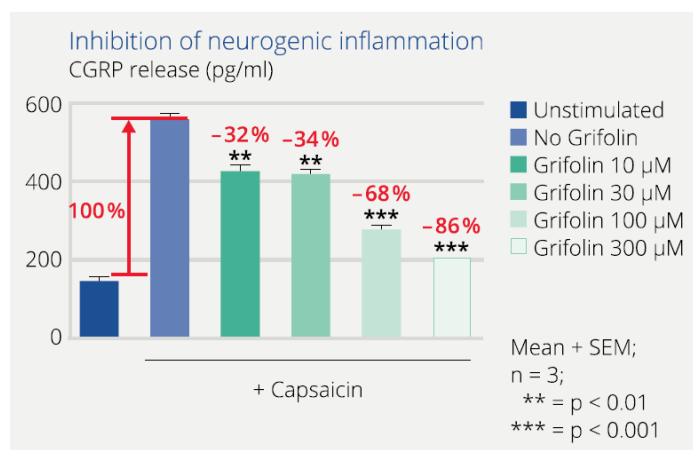
Test subjects: Sensory neurons co-cultured with normal human epidermal keratinocytes.
N = 3.

Test formulations: Cells were pre-incubated with 0, 10, 30, 100 and 300 µM grifolin followed by stimulation with 300 nM capsaicin or nothing (unstimulated).

Application period: 30 minutes pre-incubation, followed by 30 minutes stimulation.

Primary endpoint: CGRP release.

Grifolin reduces the irritation of nerve endings



***Albatrellus ovinus* extract reduces discomfort and irritation (in-vivo study)**

Test design: Double-blind, placebo-controlled, randomised, crossover study.

Test subjects: 21 female subjects, Caucasian skin, positive skin response to topical application of capsaicin (stingers), 21 – 56 years (average 36.2).

Test formulations: Emulsion containing 0% *Albatrellus ovinus* extract (placebo)
Emulsion containing 3% *Albatrellus ovinus* extract

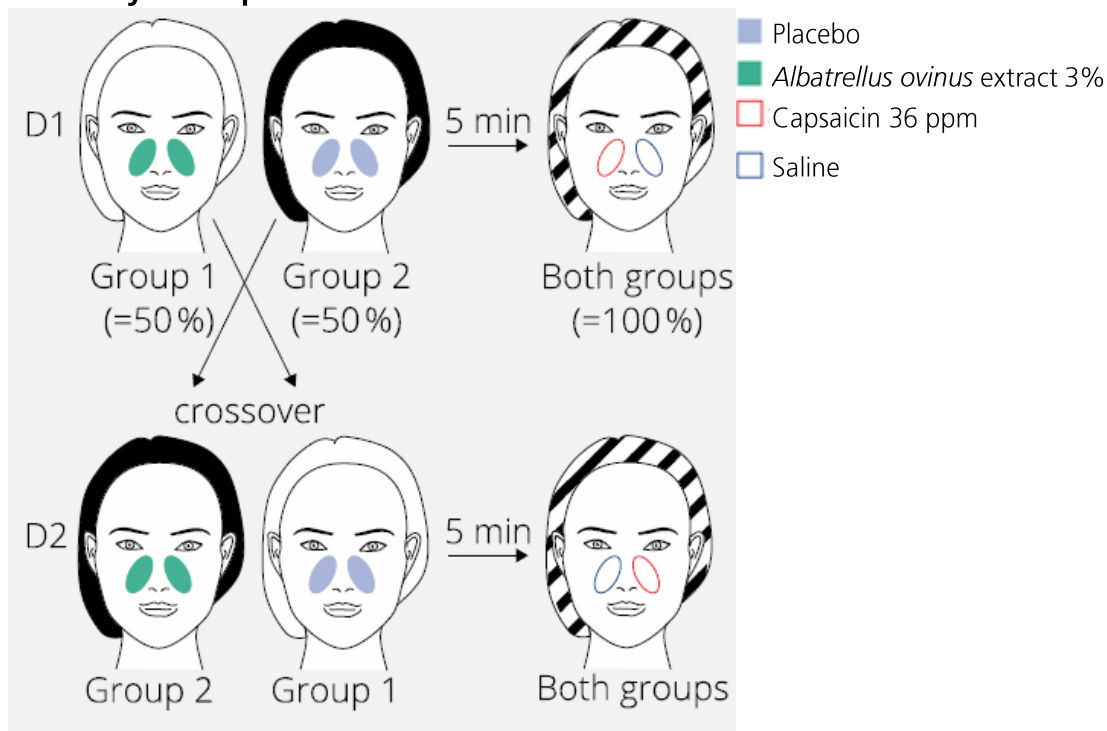
Application area/period/frequency: Nasolabial fold/half of the subjects applied *Albatrellus ovinus* extract on day 1 and placebo on day 2, whereas the other half applied the placebo on day 1 and *Albatrellus ovinus* extract on day 2 on the whole face. Five minutes after the application of the test formulations, one nasolabial fold was irritated with 36 ppm capsaicin solution, while the other side was treated with saline solution.

Primary endpoints: Stinging, burning and tingling sensations: 3 minutes after product application the volunteers graded the intensity of discomfort, according to the following scale:

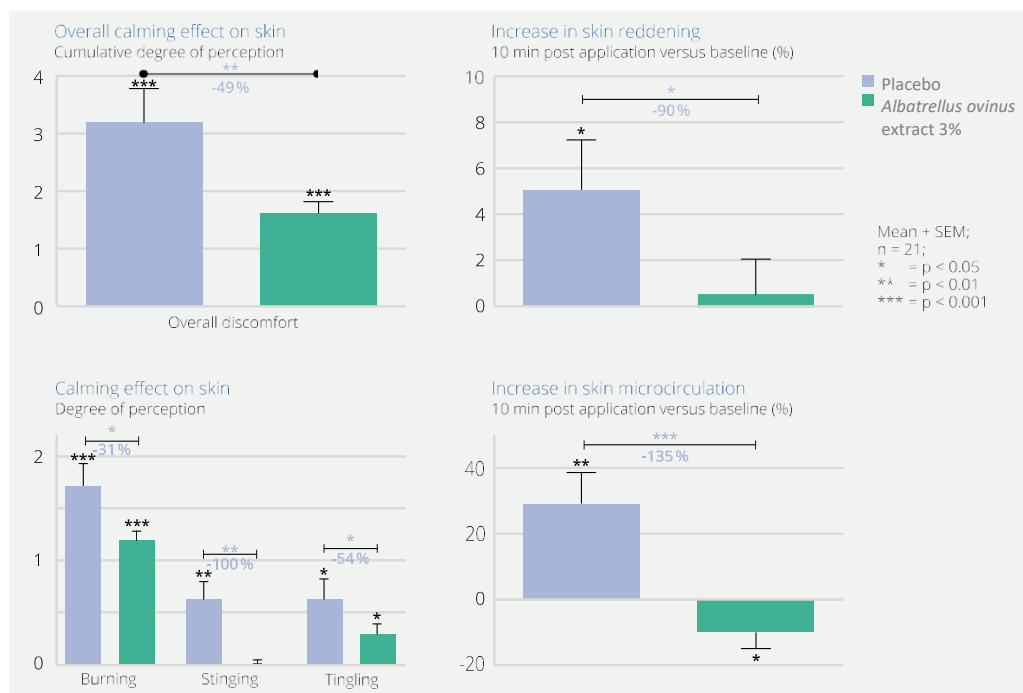
- 0 = no perception
- 1 = light perception
- 2 = moderate perception
- 3 = significant perception

Secondary endpoints: Skin reddening: evaluation of a-value. Skin microcirculation: evaluation of blood flow. Measurements were done before and after (10 minutes after) capsaicin irritation.

Crossover study - Setup



***Albatrellus ovinus* extract quickly calms stressed skin, reduces reddening and microcirculation**



***Albatrellus ovinus* extract protects from heat stress and soothes hyperalgesic skin (in-vivo study)**

Test design: Double blind, placebo-controlled, randomised

Test subjects: 8 male, 9 female subjects, Caucasian skin, 26 – 63 years (average 43.7)

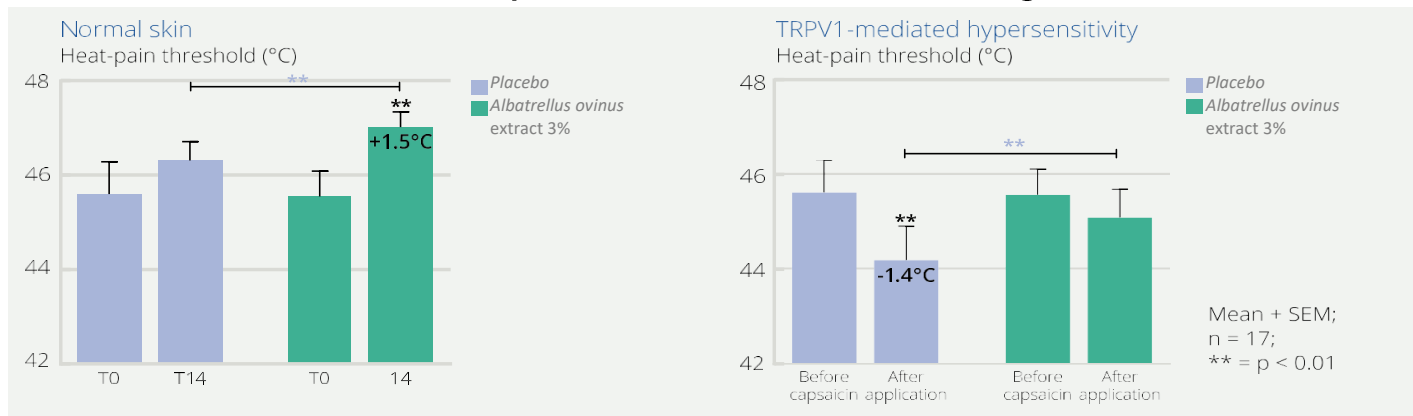
Test formulations: Emulsion containing 0% *Albatrellus ovinus* extract (placebo) and Emulsion containing 3% *Albatrellus ovinus* extract.

Application area/period/frequency: Forearms/Placebo was applied on one arm, *Albatrellus ovinus* extract on the other arm/14 days / twice daily; ABC cream (0.75 mg/g capsaicin) once at day 14, 30 minutes after last test formulation application.

Primary endpoints: Heat-pain threshold: TSA II neurosensory analyser.

Secondary endpoints: Heat-pain threshold on chemically induced hyperalgesic skin.

***Albatrellus ovinus* extract protects normal & irritated skin against heat stress**



Test subjects: 2 male subjects, Caucasian skin, 36 and 44 years

Test formulations: Emulsion containing 0% *Albatrellus ovinus* extract (placebo)
Emulsion containing 3% *Albatrellus ovinus* extract

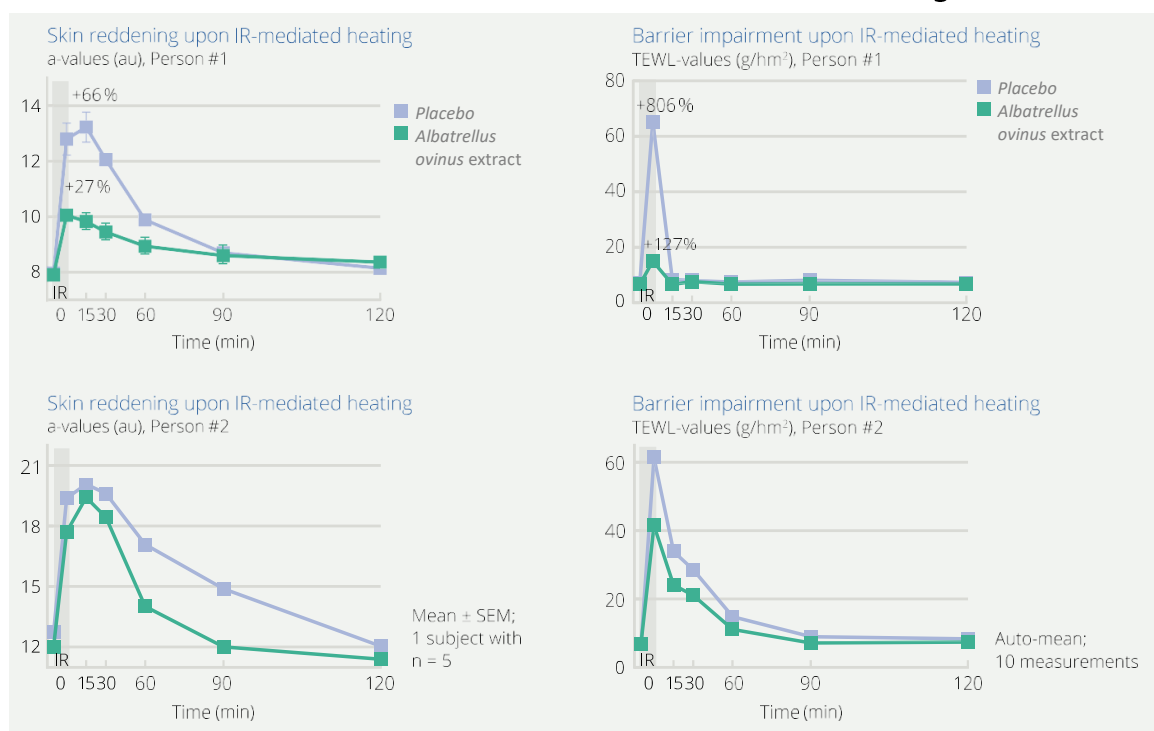
Application area/Period/Frequency: Forearms. Placebo was applied to one area and *Albatrellus ovinus* extract to an adjacent area (Ø 2 cm). 30 minutes later the skin was exposed to a standard broad spectrum IR radiator (Philips Infrared PAR38 150W) at ~200 mW/cm² for 10 minutes. This IR dose is equivalent to ~80 min European midday sun. Single application.

Primary endpoints: Skin reddening: Evaluation of a-value.

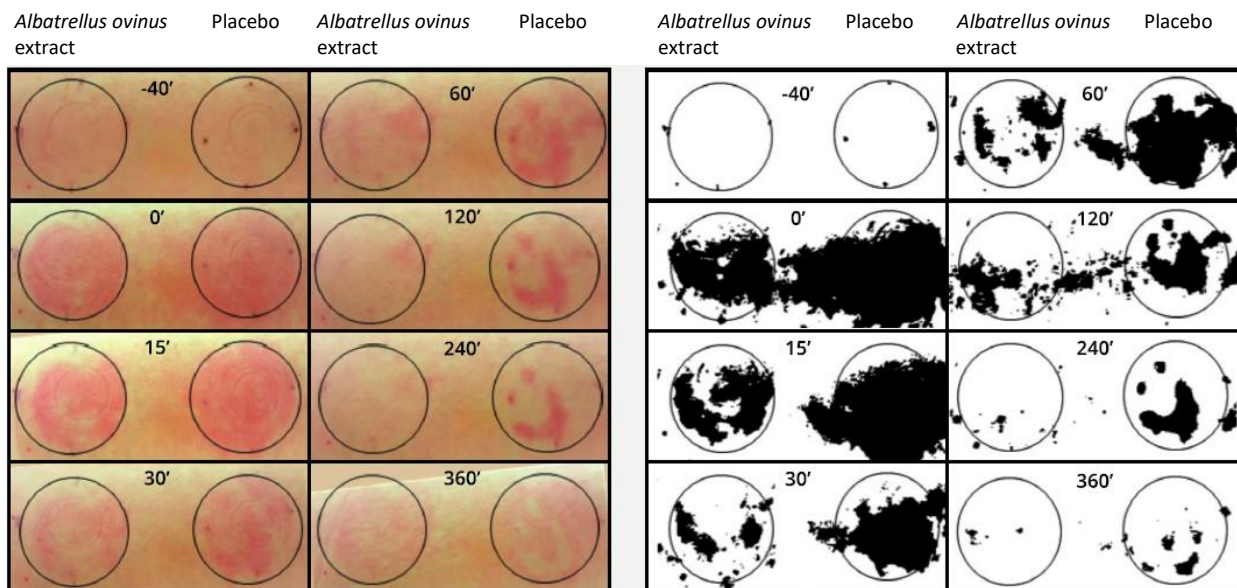
Skin disturbance: Evaluation of TEWL.

Secondary endpoints: Photographic images for illustrative purposes.

***Albatrellus ovinus* extract attenuates IR / heat-induced reddening reactions**



Inhibition of TRPV1 – A novel concept of IR protection



***Albatrellus ovinus* extract (in-vivo study)**

Test design: Double-blind, placebo-controlled, randomised, crossover study.

Test subjects: 30 female subjects, Caucasian skin, positive skin response to topical application of capsaicin (stingers), 18 – 70 years (average 46.6).

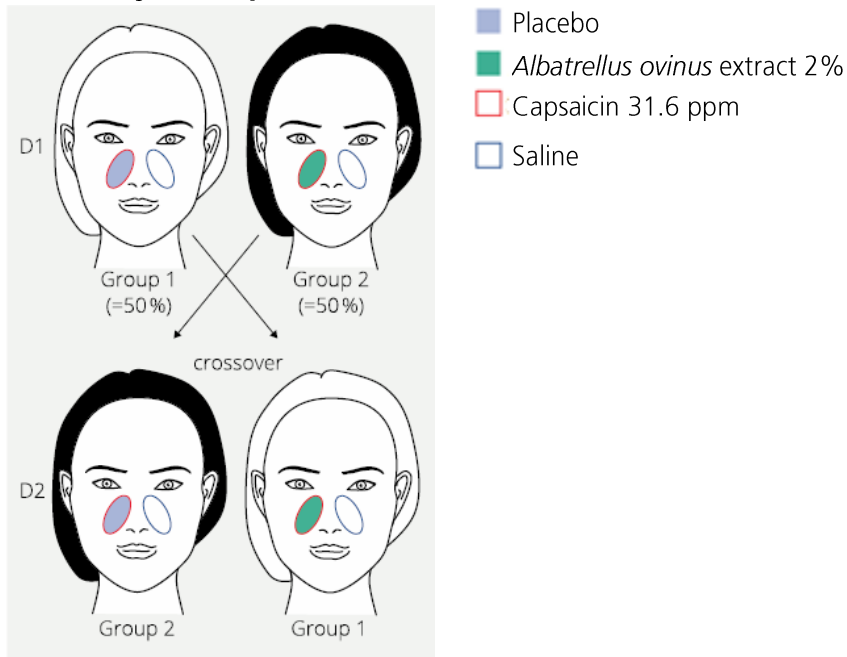
Test formulations: Emulsion containing 0% *Albatrellus ovinus* extract (placebo) and 31.6 ppm capsaicin. Emulsion containing 2% *Albatrellus ovinus* extract and 31.6 ppm capsaicin.

Application area/period/frequency: Nasolabial fold/half of the subjects applied *Albatrellus ovinus* extract on day 1 and placebo on day 2, whereas the other half applied the placebo on day 1 and *Albatrellus ovinus* extract on day 2 on the whole face. In each case, test emulsions were applied to one nasolabial side, while the other side was treated with saline solution.

Primary endpoints: Stinging, burning and tightness sensations: 3 minutes after product application the volunteers graded the intensity of discomfort, according to the following scale:

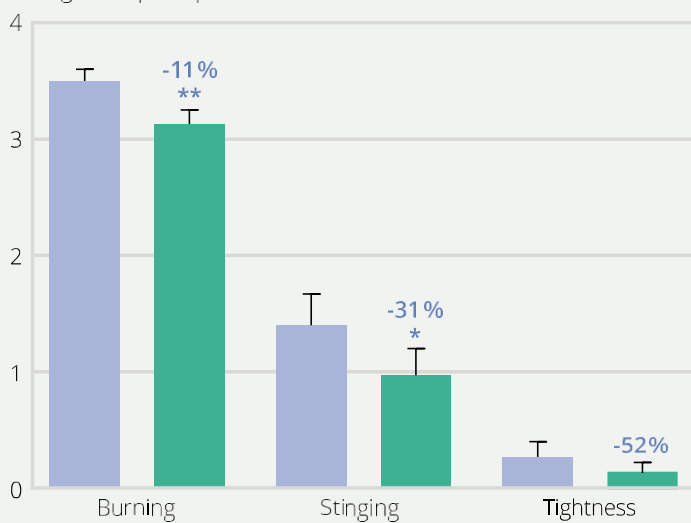
- 0 = no perception
- 1 = minimal perception
- 2 = slight perception
- 3 = moderate perception
- 4 = strong perception

Crossover study - Setup.



Albatrellus ovinus extract calms skin irritated by harsh formulation ingredients

Calming effect on skin
Degree of perception



Overall calming effect on skin
Cumulative degree of perception

