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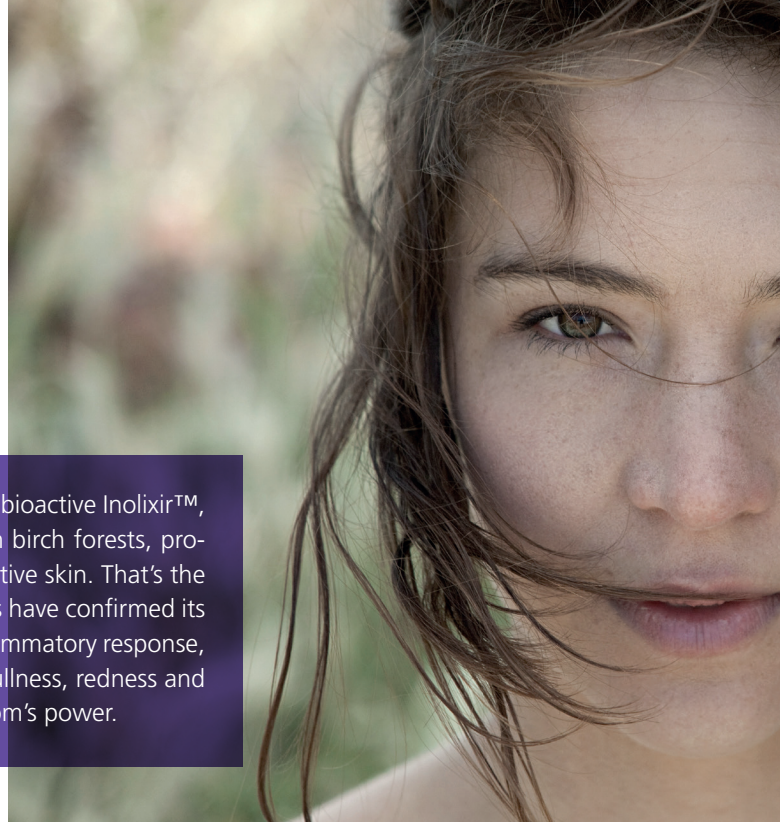
P. Moussou, S. Leoty-Okombi, C. Boury, F. Trombini, V. Andre-Frei

## A Shot of Well-being for Healthy Skin

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From a centuries-old superfood to a “magic” ingredient: BASF's latest bioactive Inolixir™, a 100% natural extract of the chaga mushroom native to Canadian birch forests, promotes skin health with fast and long-lasting efficacy for tired and sensitive skin. That's the feedback from trial participants – and various *in vitro* and *in vivo* studies have confirmed its efficacy. By fortifying the skin's protection system and reducing pro-inflammatory response, it delivers visible results on fine lines and dark circles, as well as skin dullness, redness and comfort. A special extraction method is key to harnessing the mushroom's power.



### Introduction

For at least half a millennium, people in Northern Eurasia have valued the chaga mushroom (*Inonotus obliquus*) for its various health benefits. Its nutritious conk – a dense black mass formed on the surface of birches and other host trees deep in Nordic forests [1, 2] – is traditionally consumed as a hot infusion in Eastern European countries, the Baltics and Russia to boost immunity and overall health [3, 4]. Thanks to the mushroom's unique biological properties, including antioxidant and anti-inflammatory characteristics, it can be used to treat stomach upsets and intestinal pain, satisfy hunger, and decrease tiredness. In North and Central Russian folk medicine, tinctures were also used for prophylaxis and for treating gastric disorders, and even cancer [5]. Based on its traditional uses in folk medicine and several scientific studies looking at its biological properties, the chaga mushroom has become a popular superfood in recent years. Online retailers stock powders and pills, tinctures and tea bags, capsules and even raw conks from the mushroom.

Now, BASF scientists have developed a novel bioactive ingredient from this remarkable mushroom: Inolixir™ (INCI: Glycerin (and) Water (and) Inonotus Obliquus (Mushroom) Extract) fortifies the skin's natural protection system by strengthening barrier function and the microvascular network – working on all fronts to return skin to a healthier condition. What's more, the active reduces the pro-inflammatory response thanks to its antioxidant and anti-inflammatory properties, with visible results on fine lines, dark circles, and skin dullness, redness and comfort.

### Subcritical water extraction

A novel extraction process was used to harness the chaga mushroom's biological activity in skin care: Subcritical water extraction (SWE) uses superheated, pressurized water as an extraction solvent, instead of organic solvents. Water is in a subcritical state when it is above boiling temperature (100°C) but below critical temperature (374°C), and at a high enough pressure to keep it in liquid state (see Fig. 1). Subcritical water differs from cooler water in polarity, surface tension, and viscosity, making it suitable for the efficient extraction of polar and less-polar phytochemicals (see Tab. 1) [6].

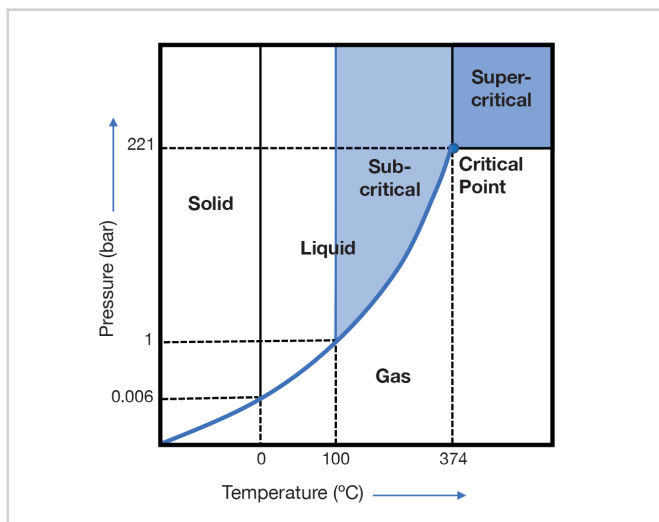


Fig. 1 Phase diagram of water as a function of temperature and pressure.

#### When the water temperature ↑

Dielectric constant ↓	Polarity ↓, similar to some organic solvents (e.g. MeOH at 200°C)
Surface tension ↓	Wetting of the raw material ↑
Viscosity ↓	Penetration inside the raw material and diffusion ↑

Tab. 1 Changes in extraction potential with the SWE process.



(involucrin x 4.9-fold), and epidermal cell cohesion as proven by an increased expression of the transmembrane proteins integrin  $\beta 1$  (+89%), claudin 1 (+20%) and occludin (+62%) (data not shown).

To measure the active ingredient's microvascular protection potential, BASF researchers proved that it stimulates collagen IV and VE-cadherin synthesis in endothelial cells. They also fed the proinflammatory cytokine TNF $\alpha$  into specially developed 3D reconstructed dermal sheets containing endothelial blood and lymphatic cells. The TNF $\alpha$  presence mimicked inflammatory stress, leading to an impairment of the microvascular network (measured by immunostaining of the microvascular wall structural protein CD31, see Fig. 3), whereas Inolixir protected the capillaries against stress-induced collapsing.

Finally, two hemi-face, double-blind, randomized, placebo-controlled clinical studies were performed to evaluate the benefits of the chaga extract for healthy skin.

An *in vitro* test proved the superiority of SWE compared to other common extraction methods for chaga. SWE was able to increase the polyphenols content by a factor of at least 1.6 compared to conventional water or hydroethanolic extraction (see Fig. 2A). At the same time, SWE demonstrates a better anti-inflammatory performance compared to hydroethanolic extract: SWE extract reduced the secretion of the pro-inflammatory signal protein IL-8 by human keratinocytes stimulated by a pro-inflammatory cocktail (see Fig. 2B).

Inolixir's efficacy on the skin has been proven in various *in vitro* and *in vivo* studies.

### A strengthened skin barrier with well-protected vessels

*In vitro*, the chaga extract showed skin-barrier strengthening properties: It stimulated keratinocyte differentiation

### A spa experience for tired skin

Thirty-two female volunteers aged 35 to 51 years took part in the first study. All of them were working, declared to be tired and stressed, and had dark circles under their eyes. They applied a facial cream containing Inolixir at 1% or a facial cream placebo twice daily for 28 days. The results of the study were compared

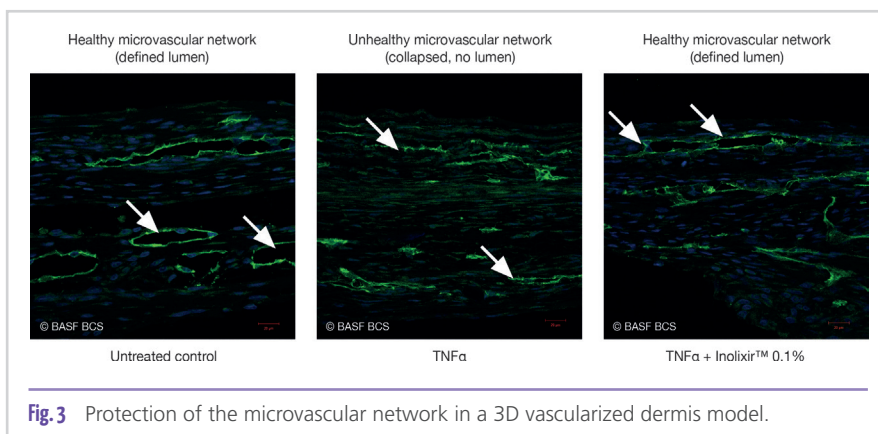


Fig. 3 Protection of the microvascular network in a 3D vascularized dermis model.

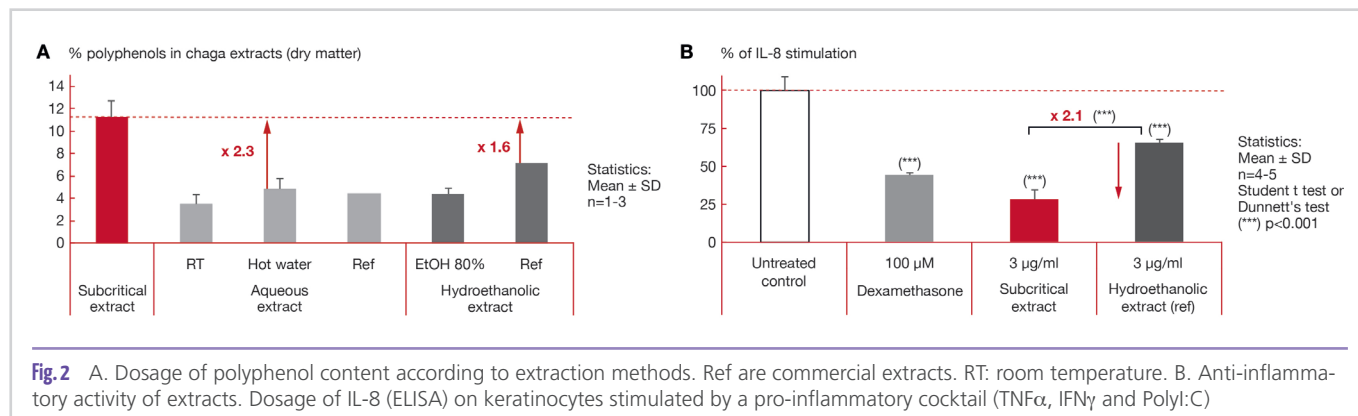


Fig. 2 A. Dosage of polyphenol content according to extraction methods. Ref are commercial extracts. RT: room temperature. B. Anti-inflammatory activity of extracts. Dosage of IL-8 (ELISA) on keratinocytes stimulated by a pro-inflammatory cocktail (TNF $\alpha$ , IFN $\gamma$  and PolyI:C)



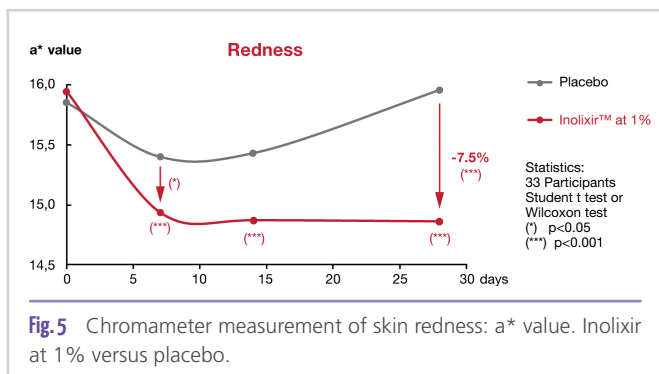


**Fig. 4** Illustrative picture showing improvement in signs of fatigue (fine lines) with Inolixir™.

with those obtained after a relaxing five-day spa cure including facial sauna and massages. A majority of the volunteers said their dark circles appeared less dark (78%) and less large (75%) after 28 days, that their skin was more beautiful (63%) after 28 days, and that their facial features were less tense after 7 and 28 days of application (72% and 75% respectively). All these self-assessments significantly outperformed the placebo and were globally similar to the relaxation spa experience (see Fig. 4).

### A wave of radiance and comfort for sensitive skin prone to redness

The second clinical trial involved 33 female volunteers aged 35 to 58 years with intolerant, reactive skin and facial redness. The efficacy of Inolixir at 1%, applied twice a day for 28 days, was evaluated by Chromameter measurement and self-assessment.



**Fig. 5** Chromameter measurement of skin redness: a\* value. Inolixir at 1% versus placebo.



**Fig. 6** Illustrative picture showing decrease in facial redness with Inolixir.

The active ingredient demonstrated a significant reduction in cheek redness (chromameter, a\* value) after one week compared to baseline (D0) and the placebo (Fig. 5 and Fig. 6). The redness did not appear again during the 28-day treatment, whereas no improvement was observed with the placebo over time. A similar significant improvement in skin brightness (chromameter, L\* value) was also observed. The volunteers themselves also noticed this improvement after 28 days: a significant majority agreed that their skin redness was reduced (73%), their skin felt soothed (88%), looked and felt healthier (85%) and was more comfortable (91%).

### Conclusion

The chaga mushroom's biological activity can be harnessed efficiently for skin care using eco-friendly subcritical water extraction technology. The new highly efficient extract Inolixir responds to the needs of personal care manufacturers to offer customers green, 100% natural solutions.

It offers genuine, visible benefits, promoting skin health with a dual action approach that delivers quick and long-lasting results for tired and sensitive skin.

### Formulations

Relaxing face cream (SC-FR-19-BC-50810) (see next page)

### References

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## Relaxing Face Cream | SC-FR-19-BC-50810-07



Phase	Ingredients	INCI	% by weight	Function
<b>A</b>	Emulgade® Sucro Plus	Sucrose Polystearate, Cetyl Palmitate	1.00	Emulsifier (O/W)
	Cetiol® LC	Coco-Caprylate/Caprata	7.50	Emollient
	Cetiol® Sensoft	Propylheptyl Caprylate	2.50	Emollient
	Cetiol® 4 All	Dipropylheptyl Carbonate	2.00	Emollient
	Lanette® O	Cetearyl Alcohol	4.00	Consistency agent
<b>B</b>	Water, demin.	Aqua	45.02	
	Preservative*		q.s.	Preservative
	Glycerin	Glycerin	0.70	Humectant
<b>C</b>	Water, demin.	Aqua	35.80	
	Rheocare® C Plus	Carbomer	0.20	Rheology modifier
<b>D</b>	Sodium Hydroxide (18% solution)	Sodium Hydroxide	0.28	pH Adjustment
<b>E</b>	Inolixir™ BC10079	Glycerin, Aqua, Inonotus Obliquus (Mushroom) Extract	1.00	Active ingredient

**Specifications**

pH value (23°C): 6.35

Viscosity (Brookfield; RVT; spindle TD, Helipath; 5 rpm; 23°C): 96 000 mPa s

**Performance**

Additional performance has not been evaluated

**Manufacturing Process**

1. Heat up phase A and B separately to about 80°C.
2. Add phase B to phase A whilst stirring.
3. After 2 minutes add phase C to phase A/B whilst stirring.
4. Stir slowly and start to cool to 50°C. Don't homogenize.
5. Add phase D and E one after another at a temperature below 40°C whilst stirring.
6. Continue to cool to room temperature whilst gently stirring.

**Stability test**

Stable 3 months at 4°C, RT, 40°C, 45°C

**Additional information**

Preservative system: Elestab™ 388 at 2.5%

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