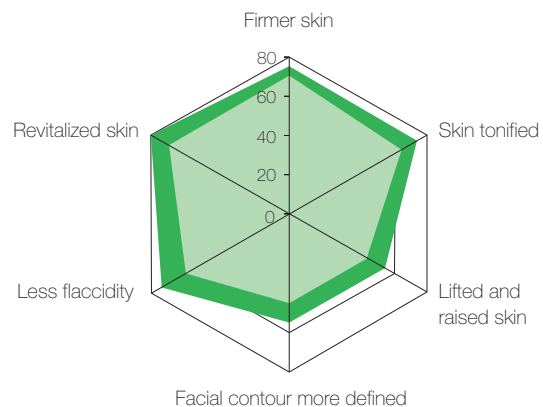


• Questionnaire results



■ Zirhafirm™
■ Placebo

Cosmetic applications

- Facial firming and modelling products
- Intensive treatments for mature and flaccid skin
- Firming and rejuvenating serums
- Body care products with firming complementary activity (anti-cellulite, slimming, post-pregnancy and bust products)
- Post-lifting cosmetic products

Technical specifications

• Zirhafirm 75060

PROPERTIES	Sinergic combination which improves cutaneous structure and cohesion, causing a firming effect. Strengthens the dermal-epidermal junction and its union structures. Recovers and maintains facial firmness, diminishes cutaneous flaccidity and redefines the facial oval
ACTIVE MOLECULES	Phytoecdysteroids (<i>Rhaponticum carthamoides</i>) Triterpenic derivatives (<i>Zizyphus spinosa</i>)
APPEARANCE	Liquid Light grey-brown color
SOLUBILITY	Soluble in aqueous solutions Insoluble in oil
RECOMMENDED DOSE	2 – 4%

Formulation

• Firming facial cream

	INCI / PCPC	% (w/w)
A	Cetearyl Alcohol, Ceteareth-25 Tocopheryl Acetate Isopropyl Myristate Butyrospermum Parkii (Shea Butter)	3,00 0,50 10,00 7,00
B	Aqua (Water) Disodium EDTA Potassium Sorbate Sodium Benzoate Sclerotium Gum Acacia Senegal Gum, Xanthan Gum Glycerin	68,20 0,10 0,30 0,30 0,50 0,75 5,00
C	Aqua (Water), Lactic Acid	0,20
D	Parfum (Fragrance)	0,15
E	ZIRHAFIRM™	4,00

Zirhafirm™

Restructuring, firming and repositioning active for a youthful look



Firming

ZIRHAFIRM™

Restructuring, firming and repositioning active for a youthful look

Zirhafirm™ activates the gene expression of the dermal-epidermal junction components and strengthens the intercellular unions. It increases skin firmness, elasticity and its resistance to harmful external factors.

Due to the intrinsic aging process, skin produces less structural components and its biomechanical properties get deteriorated (firmness and elasticity). At a facial level, this damage becomes worse because of sun exposure and terrestrial gravity, which pulls skin downwards deforming the facial oval.

Dermis and epidermis are tightly bound thanks to a complex structure called dermal-epidermal junction (DEJ). This union consists of lamina densa and lamina lucida. Lamina densa is composed of collagen IV (stabilizes) and laminins (anchorage glucoproteins). Lamina lucida reinforces the union between lamina densa and keratinocytes (epidermis) through laminins (laminin 5). Collagen VII from the dermis allows the union of this layer to the DEJ.

On the other hand, proteins like desmoplakin stabilize the unions among adjacent epidermal cells (desmosomes).

Zirhafirm™ is a synergic combination of triterpene derivatives of *Zizyphus spinosa* and phytoecdysteroids of *Rhaponticum carthamoides* which strengthens cellular union (desmoplakin), activates the generation of DEJ components (laminin, collagen IV and VII) and inhibits metalloproteinases. As DEJ and the extracellular matrix improve, intercellular communication gets re-established and keratinocytes and fibroblasts generate again the components for its union (positive loop).

The stronger the bondage between the layers is, the better skin resists flaccidity and sagging.

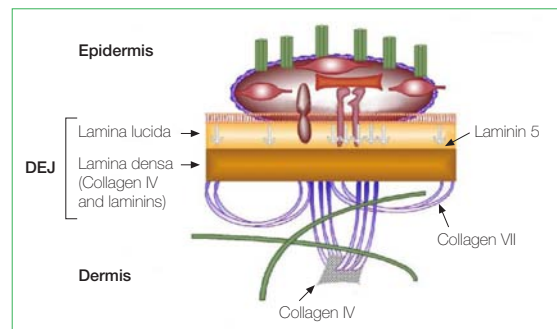
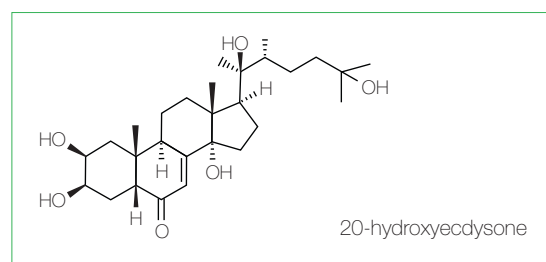
Zizyphus spinosa

- Spiny shrub from China
- Widely used in traditional medicine as a sedative
- Its seeds are rich in triterpenic derivatives like betulin, betulinic acid and jujubosides



Rhaponticum carthamoides

- Its common name is Maral Root
- Perennial species from South Siberia
- Extensively used in traditional medicine as energizer. Its roots are rich in ecdysteroids and mainly in 20-hydroxyecdysone



Keratinocytes and fibroblasts stimulate themselves to create the DEJ. When aging, the contact zone between layers diminishes, their cells do not detect each other and as a consequence, they do not create anchorage structures. This lack of communication generates a negative loop which accelerates even more the deterioration of the DEJ. Moreover, this process gets worse due to the action of enzymes like metalloproteinases (MMP), which deteriorate the extracellular matrix.

In vitro test, gene expression

Initial screening in a keratinocyte culture to detect natural actives with a modulating activity on DEJ and epidermal union genes.

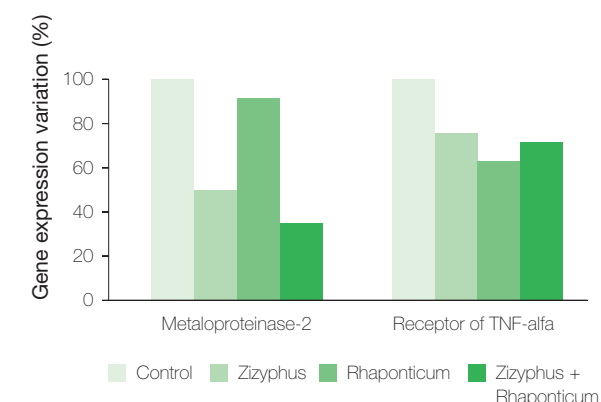
The phytoecdysteroids from *Rhaponticum* and the triterpenic derivatives from *Zizyphus* were the most effective actives versus control.

• Gene modulation in keratinocytes

	Gene activation	Gene inhibition
<i>Zizyphus</i> (triterpenic derivatives)	Desmoplakin Laminin 5 Collagen VII	Metalloproteinase 9
<i>Rhaponticum</i> (phytoecdysteroids)	Collagen IV Collagen VII	Metalloproteinase 9

The second step was a DNA array in fibroblasts. Effects of the relevant actives from the first screening were evaluated separately and also together (synergic activity). MMP2 gene encodes for a metalloproteinase (damages skin structural components) and TNFRSF1A gene codifies for the receptor of TNF-α, related to an additional MMP production.

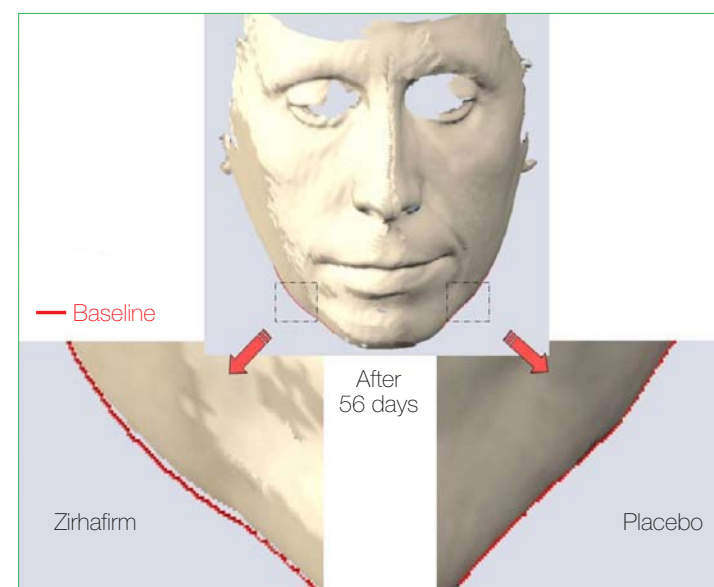
• Synergic effect on fibroblasts



In vivo test

- 20 volunteers, between 40-55 years old
- Experimental area: jaw-cheek zone
- Active formula (3% Zirhafirm™) on one half and placebo on the other
- Two daily applications, 56 days
- Measurements at D0 and D56, cutometry, FOITS (3D image) and questionnaire

• FOITS results



Results

- Firmness: enhances 14,3%
- Elasticity: increases 7%
- Improvement of the facial oval:
 - Maximum elevation: 22,5%
 - Recovering Top 60: 10%
 - Average enhancement of comparative volume: 4%
 - Comparative improvement of volume: 115 mm³
- Volunteer questionnaire:
 - Very positive opinion on the product and its effects
 - Results always much better than placebo