Nannoplankton bioactive for eye contour repair

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ABSTRACT

In the open ocean, marine life thrives in a fragile balance of crystalline, nutrient-poor waters. There, a micro-ecosystem known as nannoplankton flourishes by producing molecules such as polyphenols, saccharides, and enzymes that protect their cells from photodamage and marine pollution. MC Actives and Microalgae Solutions have developed OpenSee Phycoskin®, a marine nannoplankton extract for eye contour repair. The bioactive molecules of this marine ingredient improve skin integrity by addressing the problems induced by the natural ageing process and the impact of extrinsic factors that lead to thinning of the skin, accumulation of water and fat, and impairment of the skin's epidermal and dermal structures. Its amazing 360° effect reduces eye bags, wrinkles, drooping eyelids, and dark circles.

The health of the skin around the eye skin is influenced by a combination of intrinsic and extrinsic factors, both playing pivotal roles in determining its overall wellbeing. Intrinsic factors encompass genetic predispositions, ageing, and individual skin characteristics. As we age, the skin naturally loses collagen and elastin, leading to wrinkles and sagging around the eyes.

Extrinsic factors, on the other hand, involve external elements such as sun exposure, environmental pollutants, and lifestyle choices. Prolonged exposure to ultraviolet (UV) rays can accelerate the ageing process and contribute to fine lines and dark circles. Furthermore, lifestyle habits like smoking and poor nutrition can exacerbate skin damage.

The eye contour is a delicate area where skin deterioration is even more pronounced, leading to the appearance of undesirable external signs: drooping eyelids, lateral canthal lines (also known as crow's feet), eye bags and dark circles.

Drooping eyelids occur when upper eyelid margins scroll down. Causes include muscular and nervous issues, but poor skin integrity and ageing contribute to laxity.[1] There exists a conjunctive layer that works as a cover for muscles and connects them to other parts of the eye.

External and internal factors can cause the degradation of collagen and elastin, causing a loss of integrity in the dermal matrix. Moreover, ROS can act by attacking the epidermis and, hence, promoting a loss in the epidermic strength. This fact causes a general loss of integrity and elasticity that makes the eyelid to scroll down.

Lateral canthal lines, or crow's feet, are wrinkles formed on the lateral side of the eye that appear with age. Facial expression promotes the appearance of this characteristic due to the contractions of the lateral orbicularis oculi muscle. Conditions such as ptosis or skin weakness may accentuate the occurrence of lateral canthal lines.[2]

Eye bags are formed by swelling in the eye's orbits. They are caused by an accumulation of fluid around the eyes, which is related to the size of the fat layer known as sub-orbicularis oculi fat (SOOF).[3] They can be caused by various factors including ageing, medical conditions,[4] poor daily habits, and hormonal variations.

Dark circles are pigment macules located on the infraorbital areas. They are caused by diverse factors that include dermal melanin deposition,[5] post-inflammatory hyperpigmentation, periorbital edema or angiogenesis increase.

In some cases, prominent vessels are present due to a lack of collagen or fat, thin skin or a high density of vessels. Vascular endothelial growth factor A (VEGF-A) is known to promote blood vessel growth. As VEGF-A is expressed, angiogenesis, is promoted and pigmentation is augmented.[6]

There is a need to incorporate bioactive molecules into the skin system that aim to decrease or eliminate the causes of the ageing signs on the eye contour. Those molecules are antioxidants, collagen and elastin promoters, whitening agents, VEGF-A regulators molecules and moisturizers.

How marine molecules can restore eye-contour beauty

Nannoplankton, primarily referring to tiny phytoplankton, inhabits the vast expanse of the open sea, dwelling within the nekton marine environment. These minuscule organisms, often equipped with intricate calcium carbonate structures, form a crucial part of the ocean's microscopic community.

Endowed with an incredible ability to adapt, they have developed mechanisms to withstand the harsh effects of UV radiation in their sunlit habitat. Their resilience in oligotrophic conditions, characterized by nutrient-poor waters, showcases their adeptness in efficiently utilizing limited resources.

Despite their small size, these algae play a pivotal role in nutrient cycling, maintaining a delicate balance that sustains the larger marine

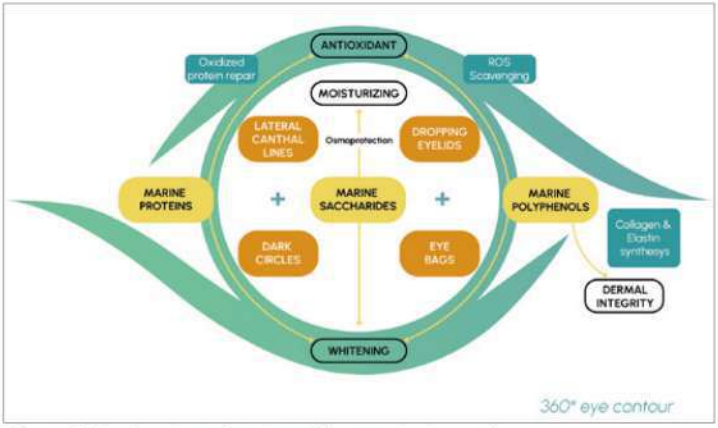

Figure 1: Mechanism of action of the marine ingredient

Protien	SAF
Phytase-like	1.072
Thioredoxin	0.448
Agmatinase	0.197
FAS1 domain	0.167
V-type proton ATPase subunit F	0.157
Ferredoxin-NADP(+) reductase	0.144
Thioredoxin y	0.124
40S ribosomal protein S4	0.123
Phosphoribulokinase	0.114
Adenosylhomocysteinase	0.110
Protein fucoxanthin chlorophyll a/c protein	0.108
Plant heme peroxidase family	0.103

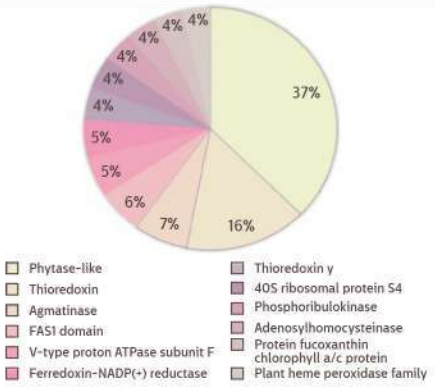

Figure 2: Proteomic profile of the marine ingredient. SAF: Spectral abundance factor

ecosystem. Furthermore, their presence serves as a vital buffer against the impact of marine pollutants, contributing silently but significantly to the health and equilibrium of the sea's ecological makeup.

By deepening the scientific understanding of nannoplankton, a myriad of molecules that perform vital functions has been identified in this micro-ecosystem and have apparently also been considered of interest for their bioactive potential applied to cosmetics to restore the skin, especially the signs of eye fatigue (Table 1). Let us see how some of these molecules work to combat the main signs of deterioration of the eye contour.

- Marine saccharides. All these molecules are considered to contribute to maintaining cell turgor.[7] As an example, L-fucose is able to act in: i) avoiding the degradation of hyaluronic acid,[8] and hence maintaining dermal matrix integrity; ii) MMPs inhibition process,[9] and, in consequence, preserving the structural molecules of the dermal matrix; and additionally, iii) regulating VEGF factors (A and B), preventing fluid accumulation (edema) and thus reducing the formation of eye bags and dark circles.[10]
- Marine proteins constitute functional molecules that work to maintain healthy skin. Inside this group, we find enzymes such as superoxide dismutase, well known for its antioxidant power due to the prevention of ROS formation.[11]
- Marine polyphenols are small bioactive molecules composed of phenolic hydroxyl groups,[12] which work in an integrated system acting as ROS scavengers, such as p-coumaric acid,[13] but also as whitening agents.[14]

Skin integrity and moisturizing molecules like mentioned above are crucial for combating eye signs caused by natural ageing, thinner skin, fat accumulation, and degeneration of epidermal and dermal structures.

Applying blue biotechnology, we create a new 360º eye-contour marine ingredient that possesses an impressive molecular richness, containing specific compounds to fight against the eye contour problems, as highlighted in Table 1.

A novel 360° eye-contour marine ingredient

The mechanism of action of OpenSee Phycoskin®, hereafter known as the marine ingredient, which is aimed at restoring the beauty of the eye contour, is based on the presence of three groups of molecules: saccharides, polyphenols, and proteins.

Together these act to achieve a full cosmetic effect: (i) antioxidant, (ii) moisturizing, (iii) dermal integrity preservation and (iv) whitening, through different mechanisms of action leading to the reduction of dark circles, eye bags, lateral canthal lines, and drooping eyelids (Figure 1).

A molecular complex of the marine ingredient exhibits a multifunctional effect to combat eye contour fatigue signs by working on different mechanisms of action. We now detail the three main groups of molecules

Molecule Group	Subgroup	Compound	Cosmetic Efficacy	Mechanism	Main Target	References
Saccharides	Monosaccharides	Glucose*	Well-ageing	Osmoprotection	Lateral canthal lines/ drooping eyelids	7
		L-fucose	Well-ageing	Hyaluronidase inhibition	All	8
				MMPs inhibition	All	9
			Whitening	VEGF A & B regulation	Dark circles/eye bags	10
	Disaccharides	Trehalose	Well-ageing	Procollagen synthesis	All	20
			Antioxidant	ROS scavenging/enzyme inhibition	All	
	Polysaccharides	Chrysolaminarin	Antioxidant	ROS scavenging	All	21
		Sulfated polysaccharides	Well-ageing	Enhances epidermal cells junctions	All	16
Proteins	Enzymes	Superoxide Dismutase*	Antioxidant	ROS prevention	All	11
		Thioredoxin*	Antioxidant	Protein reduction	All	17
	FSA-1 domain protein*		Whitening	Angiogenesis inhibition	Dark circles/eye bags	18
	Mycosporine-like amino acids (MAAs)	Mycosporine-glycine	Antioxidant	Prevent lipid peroxidation	All	22,23
		Asterin-330	Antioxidant	Prevent lipid peroxidation	All	
		Porphyra-334	Antioxidant/Well-ageing	MMPs inhibition/ collagen and elastin synthesis	All	
		Shinorine	Antioxidant/Well-ageing	Prevent lipid peroxidation	All	
Phenols		p-coumaric acid	Antioxidant	ROS scavenging	All	13
			Whitening	Tyrosinase inhibition	Dark circles	14
		4- hydroxybenzaldehyde*	Antioxidant	ROS scavenging	All	15
			Whitening	Angiogenesis inhibition	Dark circles/eye bags	
		Gallic acid*	Antioxidant	ROS scavenging	All	16
			Well-ageing	MMPs & IL-6 inhibition/Collagen & elastin synthesis	All	

Table 1: Molecules found in nannoplankton with cosmetic efficacy to fight against signs around the eye. *Molecules found in the marine ingredient

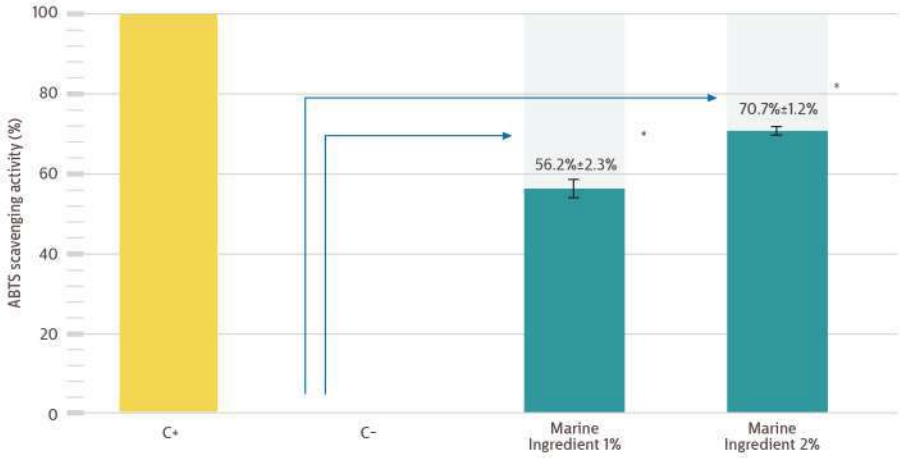

Figure 4: Antioxidant capacity expressed as ABTS scavenging activity of the marine ingredient at 1% and 2%. Preliminary results. p>0.05; one-way ANOVA

found in the ingredient, and their mechanism of action (Figure 1).

- 1. Marine saccharides molecules form hydrogen bonding with water, and retain it, increasing the cell size, which leads to cell turgor increase. Polysaccharides acts as a film, by protecting the skin layers, preventing the trans-epidermal water loss (TEWL). So, marine saccharides found in the marine ingredient can act as a solution producing a plumping effect on lateral canthal lines and drooping eyelids.
- 2. Polyphenol system is integrated by two main phenolic compounds: 4-hydroxybenzaldehyde (4-HB) and marine gallic acid (MGA). They accomplish three main functions: antioxidant (scavenging ROS),[15,16] whitening, and skin integrity maintenance. 4-HB, found in our ingredient, act as a whitening agent by inhibiting angiogenesis,[15] that is the formation of new blood vessels and causes skin problems such as dark circles, as explained in the introductory part of the article. Moreover, MGA modulates MMP-1 production and increases elastin and procollagen type I, protecting the dermal matrix.[16]
- 3. Marine protein complex: several proteins have been found in the marine ingredient (Figure 2). One remarkable molecule has been identified in this group: thioredoxin. Thioredoxin is an enzyme that is able to reduce oxidized proteins, by acting as antioxidant.[17] FSA-1 domain protein has been also detected in the marine ingredient. This protein is able to inhibit the angiogenesis process, reducing dark circles and eye bags.

In vitro collagen type I production

This marine ingredient was assessed in an immunofluorescence test to analyses the collagen type I production at different concentrations. Preliminary results showed that 0.3% of the marine ingredient caused a significant increase of collagen type I, as can be observed in Figure 3. As fluorescence increases, collagen production increases.

In vitro elastin production

The marine ingredient was also tested in terms of elastin production. Preliminary results offered an outstanding increase in elastin of 72.2%±10% at only 0.3% concentration.

These results dilucidated the well-ageing potential of the marine ingredient, by preserving the main structures of the dermal matrix (collagen and elastin).

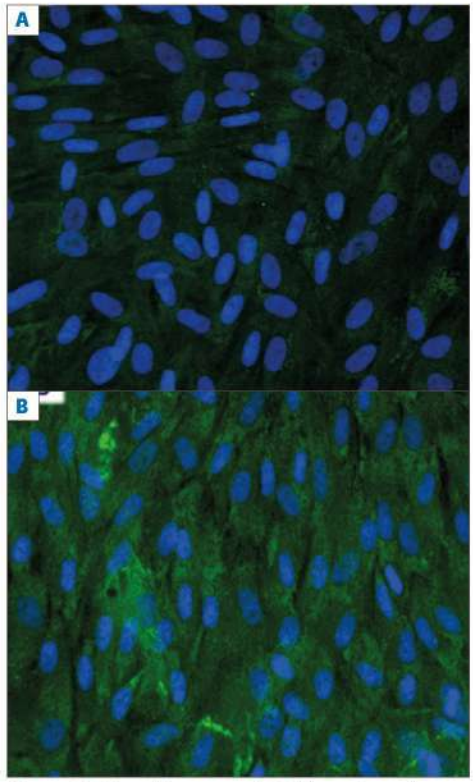

Figure 3: Visual increase of Collagen type I production. A: 0% marine ingredient; B: 0.3% marine ingredient

In vitro antioxidant capacity

Preliminary results of the ABTS assay[19] showed that the marine ingredient offered an antioxidant capacity of 56.2%±2.3% at 1% and an impressive antioxidant power of 70.7%±1.2% at 2% (Figure 4).

In vivo double-bind clinical study

The cosmetic efficacy of the marine ingredient was assessed in a double-blind *in vivo* study with 36 volunteers (31-63 years old) for 28 days.

Figure 5: *In vivo* study of the marine ingredient at 3%. Visual effect on eye bags wrinkles reduction after 28 days. Images processed with Image J software

Phase	Ingredients	INCI	Contents (%)
A	Water	Aqua	q.s.
	Butylene Glycol	Butylene Glycol	3.00
	Glycerin	Glycerin	2.00
	Sodium Benzoate, Potassium Sorbate	Sodium Benzoate, Potassium Sorbate	0.80
	Visconat U®	Cyamopsis Tetragonoloba Gum, Xanthan Gum, Lecithin, Agar, Sclerotium Gum	0.60
	Caprylyl glycol	Caprylyl glycol	0.20
B	Isostearyl Isostearate	Isostearyl Isostearate	5.00
	Shea butter	Butyrospermum parkii butter	5.00
	Argan kernel Oil	Argania spinosa kernel oil	4.00
	Coco-Caprylate/Caprate	Coco-Caprylate/Caprate	3.00
	Glyceryl Stearate	Glyceryl Stearate	2.00
C	Marine Ingredient	Aqua, Populus tremuloides bark extract, Plankton extract	3.00
	Hydrolyzed collagen	Hydrolyzed collagen	1.00

Table 2: Example of formulation of an eye contour with the marine ingredient. Procedure: Combine phase A and homogenize using a homogenizer. Heat phase B until all components are completely melted. Add phase B to A and emulsify using homogenizer until a homogeneous mixture. Add ingredients of phase C below 40°C one by one into phase A+B. Adjust pH to 5-6

The marine ingredient at 3% decreased wrinkle length by 40.6%±13.2% (Figure 5), data supported by the outstanding increase in elasticity of 39.9% in crow's feet area (Figure 6A) and 29.1% in eye bags after 28 days (Figure 6B).

The marine ingredient increased moisture from day 0 to day 28, 10.7% in crow's feet area (Figure 7A) and 16.5% in eye bags area (Figure 7B). The above results highlight that the marine ingredient is a potent well-ageing.

Additionally, the marine ingredient was shown to reduce dark circles by 25.6%±11.7%. Moreover, it helped to reduce the angle formed by eye bags by 10.6%±4.6% (Figure 8), and lifted eyelids in a 10.9%±5.4%.

Tips for formulators

The marine ingredient is composed of a blend of polyphenols, proteins and saccharides extracted from nannoplankton as an upcycled ingredient from a green and environmentally friendly downstream process. This 360º eye contour ingredient is water-soluble, 100% natural origin content (ISO 16128), and Vegan and COSMOS-approved.

The marine ingredient is easy to formulate together with other natural ingredients to produce an eye contour end product that covers marketing claims such as: well-ageing to reduce crow's feet, eye bag wrinkles, eye bag area and improved antioxidant action.

It also offers benefits such as eyelid tensor effect; firming and lifting thanks to the moisturizing effect and elasticity increase; and whitening, reducing dark circles. An example of eye contour formulation is shown in Table 2.

Conclusions

OpenSee Phycoskin® is composed of four key molecules: the polyphenols 4-hydroxybenzaldehyde and marine gallic acid, and the proteins thioredoxin and FSA-1 domain protein, which together have been shown to cover multiple claims related to eye contour:

- Exhibited high antioxidant capacity
- Increased collagen type I and elastin at only 0.3% dosage
- Increased elasticity and moisture
- Reduced wrinkle length by 40.6%±13.2%
- Reduced dark circles by 25.6%±11.7%
- Reduced eye bag's area by 10.6%±6%
- Exhibited eyelid tensor effect by 10.9%±5.9%

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References to this article are available at: https://bit.ly/49n77K7

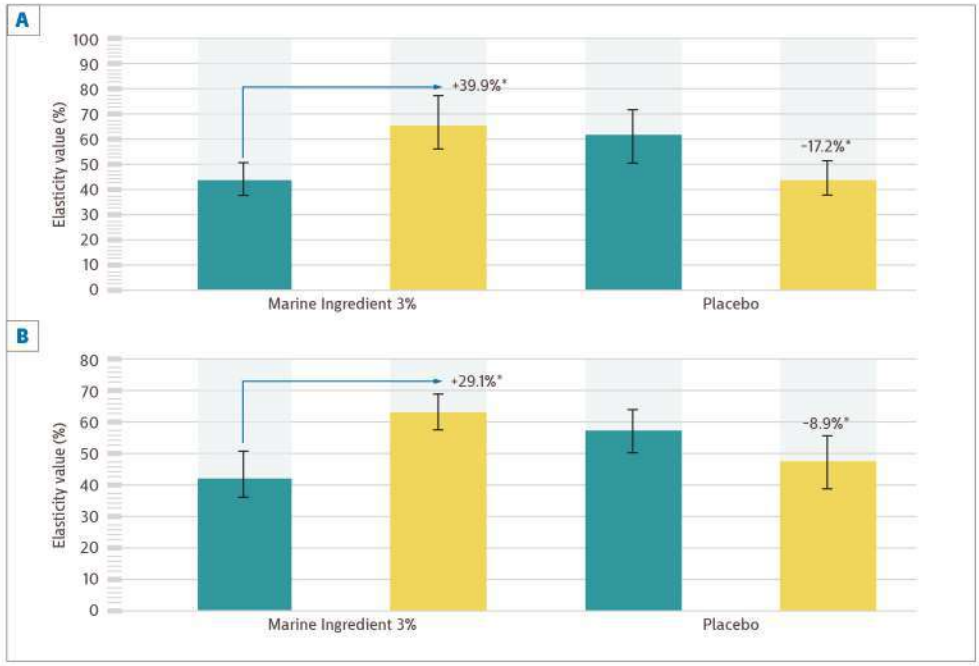

Figure 6: Elasticity value (%) increase. *In vivo* study. Doble-blind test with 36 volunteers (31-63 years old). Marine ingredient applied twice a day at 3% for 28 days. A: crow's feet area; B: Eye bags area. P>0.05; one-way ANOVA

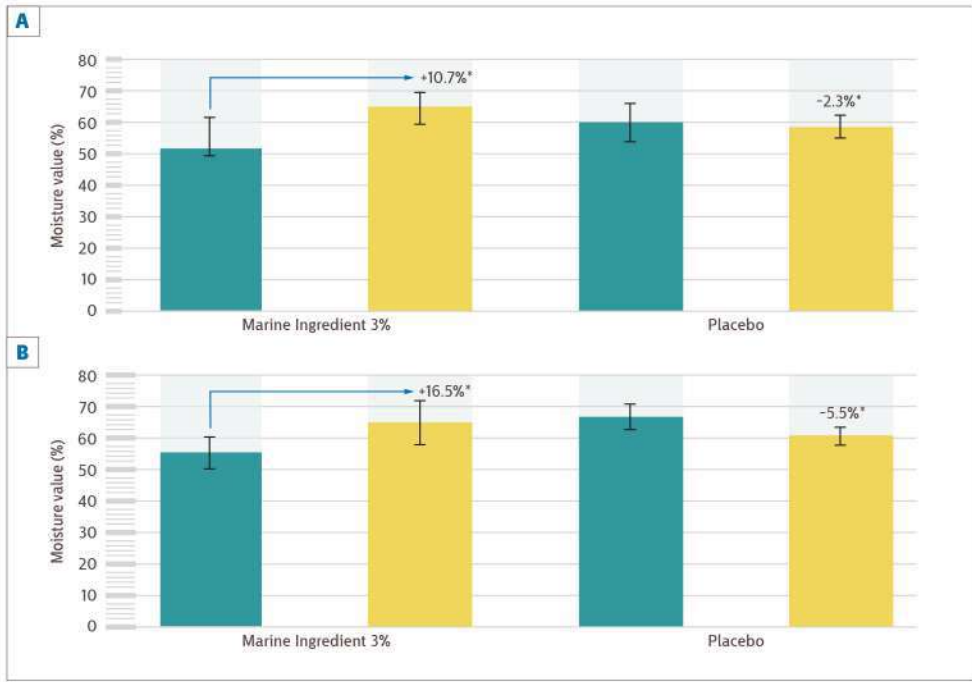

Figure 7: Moisture value (%) increase. *In vivo* study. Doble-blind test with 36 volunteers (31-63 years old). Marine ingredient applied twice a day at 3% for 28 days. A: Crow's feet area; B: Eye bags area. P>0.05; one-way ANOVA

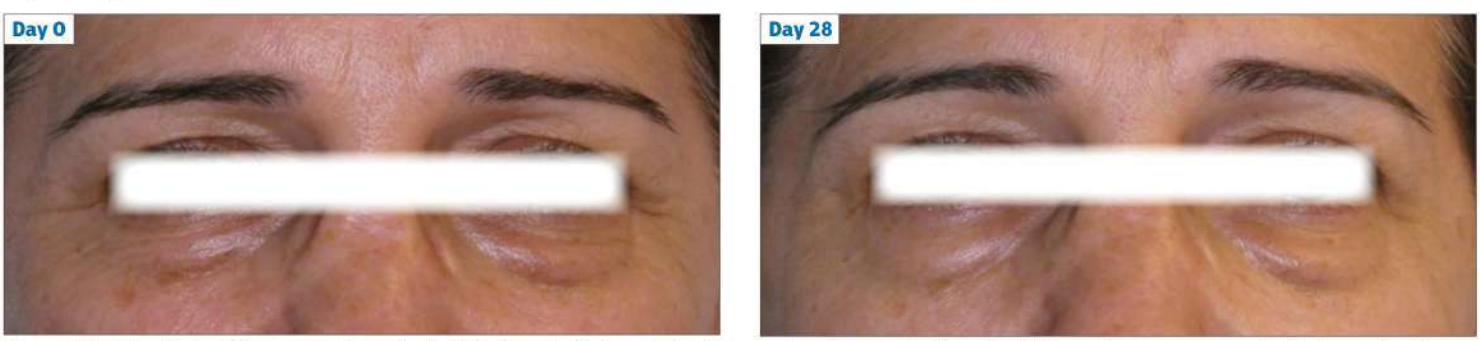

Figure 8: *In vivo* study of the marine ingredient at 3%. Visual effect on dark circles and eye bags reduction after 28 days. Images processed with Image J software