

# GATULINE® AGE DEFENSE<sup>2</sup>



PRO-ACTIVE PROTECTION AGAINST TIME  
AND ENVIRONMENTAL AGGRESSION



The walnut tree, able to live up to 400 years, is an example of a real challenge to aging. Over the centuries, it has protected the secret of its long life.

Romans were already familiar with walnut oil and used it for its positive effect on convalescence. They also boiled walnut leaves to treat eczema, blepharitis and attacks of indigestion.

Much later, the nutritive and energetic benefits of the walnut fruit were discovered, attributed not only to the fruit's lipidic fraction, but also to its high content of proteins, glucids, mineral elements and polyphenols.

The "noix de Grenoble" is now revealing all its secrets, so well kept up to now in its protective cockle-shell. This specific variety of walnut has a very familiar gentle yet spicy taste and a unique, plumpy shape.

From the groves overlooking the Alps, fed by glacier water and mountain air, the walnut has fascinated Gattefossé: we extracted and concentrated its hydrophilic active ingredients from the green walnut:

## GATULINE® AGE DEFENSE<sup>2</sup>

Pro-Active protection against time and environmental aggression

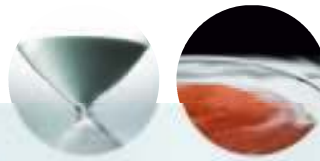
Extracted from "noix de Grenoble", Gatuline® Age Defense<sup>2</sup> brings to the skin **phytic acid, polyphenols, proteins, mineral salt and vitamins.**

Here is an active which protects the cells antioxidant molecular pool, while offering biostimulating properties.

This **patented active** helps the skin keep its **heritage of youth.**

Moreover, struggling against unavoidable **chronological aging**, it smoothes the complexion and brightens the skin, providing a look of youth and firmness.





## THE COMPLEX ANSWER TO CUTANEOUS AGING

It is necessary to briefly review the primary biological reactions involved in skin aging.

Modifications of the cutaneous tissue occurring over time have **two origins**:

- **Extrinsic aging**, also called photoaging or oxidative stress, related to external aggression, such as ultraviolet radiation, environmental pollutants and heavy metals...
- **Intrinsic aging**, also called chronological aging, referring to a genetically programmed processes.

These cutaneous tissue modifications take place both in the dermis and in the epidermis.

## EXTRINSIC AGING AND OXIDATIVE STRESS

Continuously subjected to UV radiation and other forms of aggression, the skin is the center of a high concentration of **Reactive Oxygen Species (ROS)**.

- In the normal functioning of the cell, ROS participate in regulation systems; the intracellular redox system is balanced by endogenous defense mechanisms, either enzymatic or chemical.
- In some cases, typically upon exposure to UV radiation and increasing amounts of chemical pollutants, the quantity of ROS in the cells sharply increases. The saturated antioxidant pool then fails to neutralize the excess ROS; this process is referred to as "**oxidative stress**".

Over the short term, an excess of ROS results in dramatic modifications of certain biological compounds (DNA, proteins and lipids), affecting cell function so much that it cannot survive. This triggers a process of programmed cell death called apoptosis, resulting in the elimination of damaged, **dead cells**.

Simultaneously, the generated free radicals activate specific transduction paths, triggering the release of epidermic mediators that cause the development of local inflammation.

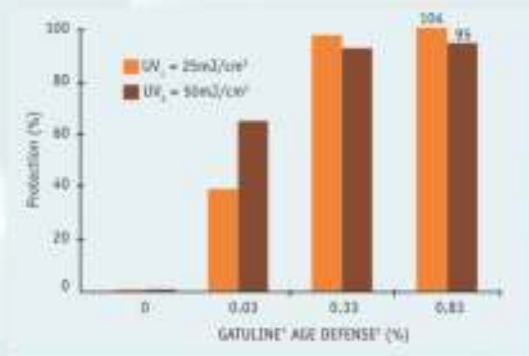
From a histological point of view, extrinsic aging leads to a **destruction of the tridimensional network, directly responsible for the deterioration of cutaneous mechanical properties**.

The net result is that skin appears to be rough, thicker, irregularly pigmented and marked with fine lines and deeper wrinkles.

### GATULINE® AGE DEFENSE<sup>2</sup> PROTECTS THE NATURAL ANTIOXIDANT POOL OF THE CELLS, *IN VITRO*...

The aim of this test is to show that Gatuline® Age Defense<sup>2</sup> protects the antioxidant pool of the cells. This test was performed on normal human keratinocytes by measuring the intracellular oxidation rate following UVB exposure. The cellular oxidation rate was assayed by fluorimetry.

#### Protection against oxidative stress

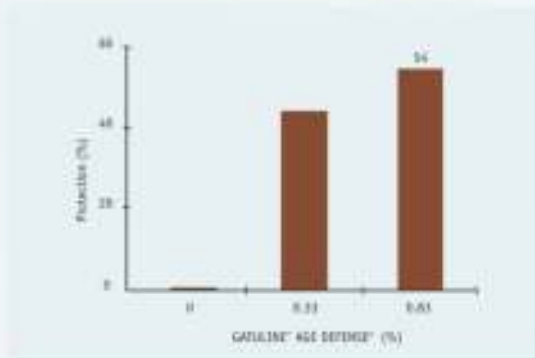


Under the conditions of this study, it was shown that Gatuline® Age Defense<sup>2</sup> protects the cells against oxidative stress, dose-dependently, achieving complete protection from 0.83%.

### ... GATULINE® AGE DEFENSE<sup>2</sup> THEREFORE PREVENTS UV-INDUCED APOPTOTIC PROCESS

The protective activity against UVB-induced apoptosis was evaluated on normal human keratinocytes via the determination of cellular DNA fragmentation.

#### Protection against UVB-induced apoptosis



This test indicates that Gatuline® Age Defense<sup>2</sup> lowers the amount of apoptotic cells. At 0.83%, 54% protection can be noted (dose-dependent).

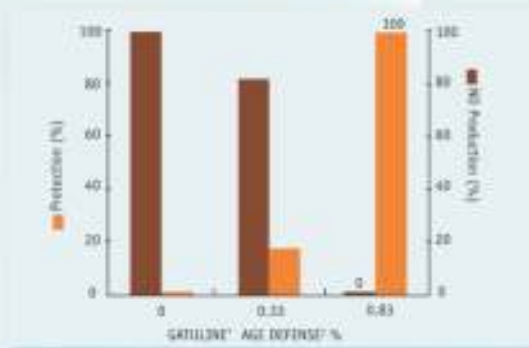


### ... GATULINE® AGE DEFENSE® PREVENTS THE FORMATION OF PRO-INFLAMMATORY MEDIATORS

Protective activity was measured on normal human keratinocytes by measuring UVB-induced nitric oxide (NO) production, using Griess reactive.

This mediator is known to play a complex role in the modulation of inflammation, and is also thought to be involved in the apoptotic process.

#### Anti-inflammatory action



At 0.83%, it is clear that Gatuline® Age Defense® completely inhibits NO production (dose-dependent).

### IN VIVO TEST: PROTECTION OF THE NATURAL ANTIOXIDANT POOL OF THE CELL

The effect of Gatuline® Age Defense® on skin peroxide formation was evaluated after exposure to mixed UVA/UVB simulating the sun. The study was performed on 10 people over 4 days (day<sub>2</sub> to day<sub>1</sub>).

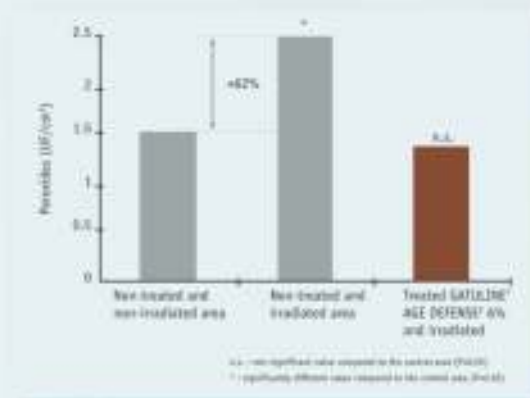
- At Day<sub>2</sub> and Day<sub>1</sub>, all panelists received a normalized quantity of a gel containing 6% Gatuline® Age Defense® applied to an area of skin on their back.
- At Day<sub>0</sub>, the treated zone was irradiated, then the gel was applied again at T0, T2h, T4h and T6h.
- At Day<sub>1</sub>, the rate of peroxide formation was assayed after strippings, with a fluorescent probe.

The study was made in comparison with a non-treated and non-irradiated zone, as well as with a non-treated and irradiated zone.

Gatuline® Age Defense<sup>2</sup> used at 6% in a gel, facilitates a return to a basal level of cutaneous peroxide demonstrating its complete protective action.

Gatuline® Age Defense<sup>2</sup> helps to prevent oxidative stress, one of the major causes of skin aging.

#### Anti-peroxidation in vivo



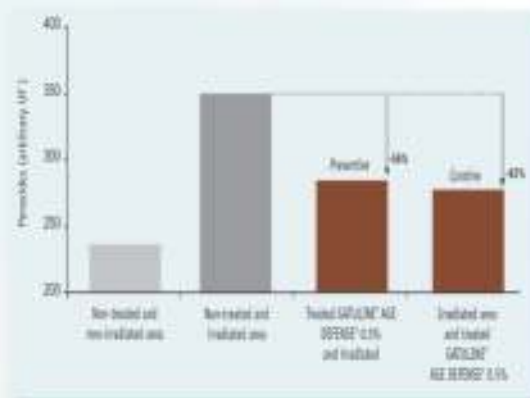
## CAPILLARY PROTECTION

Furthermore, an ex vivo test on 10 different hair locks confirmed the significant preventive and curative effect of a 0.5% aqueous solution of Gatuline® Age Defense<sup>2</sup> on UV-induced peroxidation.

- Dosage of peroxides with fluorimetric measurements after UVA/UVB irradiation
- Topical application of Gatuline® Age Defense<sup>2</sup> (0.5% in aqueous solution) before or after irradiation.

#### Anti-peroxidation on hair-locks

Gatuline® Age Defense<sup>2</sup> helps to protect the capillary fibers against the deleterious effects of UV, due to its double anti free radical activity: preventive and curative.



Gatuline® Age Defense<sup>2</sup> protects cutaneous tissue and also hair from environmental aggressions such as UV, pollutants and heavy metals.



## CHRONOLOGICAL AGING

Chronological aging implies a slowing down of cellular functions. The capacity of keratinocytes to proliferate decreases with time, and fibroblasts synthesize less extracellular matrix.

Simultaneously, the balance between protease enzymes (Matrix Metalloproteinases, or MMP) and their inhibitors is disrupted, leading to changes in the skin's mechanical properties.

As a result, chronological aging contributes to thinner, flaccid skin marked with numerous lines.

### GATULINE® AGE DEFENSE® BOOSTS THE PROLIFERATION OF HUMAN KERATINOCYTES

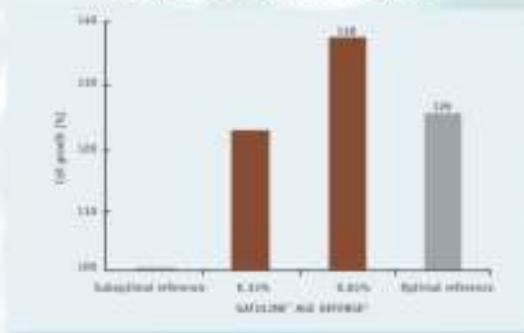
The aim of this study is to demonstrate the stimulating activity of Gatuline® Age Defense®.

The effect on proliferation was evaluated via cell numeration in a suboptimal medium.

At 0.83%, Gatuline® Age Defense® stimulates keratinocyte proliferation as efficiently as the optimal reference, rich in growth factors.

Simultaneously, cellular protein synthesis increased by about 40% (substantiated). The increase of protein synthesis is mainly correlated with the generation of new cells.

#### Proliferation of keratinocytes



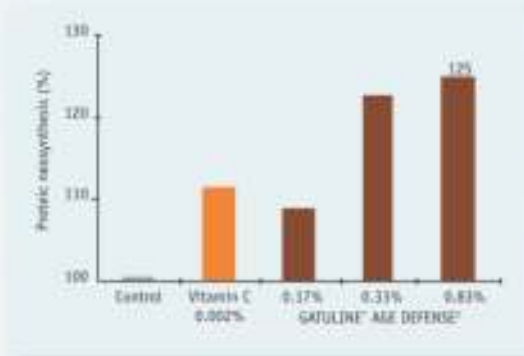
### GATULINE® AGE DEFENSE® BOOSTS HUMAN FIBROBLAST PROTEIN SYNTHESIS

The ability to stimulate protein neosynthesis was evaluated via the measurement of radiolabelled leucine incorporation.

Gatuline® Age Defense® stimulates total protein synthesis, with an efficacy superior to Vitamin C.

Fibroblasts do not undergo any cell proliferation; this result therefore affects all neosynthesized proteins, especially the insoluble extracellular proteins that constitute the extracellular matrix (substantiated).

#### Fibroblast protein synthesis



## GATULINE® AGE DEFENSE<sup>2</sup> PROTECTS ELASTIN

Anti-elastasic properties were studied with an experimental approach based on measuring the catalytic activity of fibroblast extracts.

**Gatuline® Age Defense<sup>2</sup> inhibits the elastase activity (dose-dependent).**

*In vitro* studies on cutaneous cells show that Gatuline® Age Defense<sup>2</sup> has growth factor-like biostimulating properties. It also provides anti-elastasic activity and can prevent degradation of the elastin fibers, thus preserving the mechanical properties of the skin.

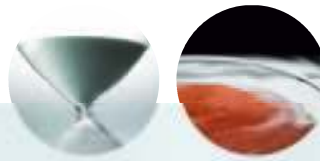
**By counterbalancing the main mechanisms involved in chronological aging, Gatuline® Age Defense<sup>2</sup> will protect the skin from the clinical aspects of aging.**

## A UNIQUE COMPOSITION

Gatuline® Age Defense<sup>2</sup> is an active ingredient derived from a selective extraction of the hydrophilic compounds of walnut. Its chemical analysis reveals a very rich composition:

- **phytic acid** or myo-inositol hexaphosphate (InsP6), a powerful chelating agent able to prevent the oxidation of biomolecules, and exhibiting anti-inflammatory properties
- **polyphenols**, such as hydrolyzable tannins (especially ellagitannins and other gallic tannins), synergistically reinforcing the properties of phytic acid by scavenging free radicals
- **oligopeptides and proteins**
- **free amino acids**, in particular aspartic acid, glutamic acid and arginin
- **sugars**
- **mineral salts** and trace elements
- **hydrosoluble vitamins** of the B group (B6, B3, and B5).





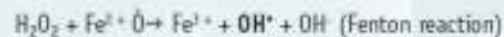
## MECHANISM OF ACTION

The composition of Gatuline® Age Defense<sup>2</sup> is indicative of its protection against cutaneous aging.

### Protection against extrinsic aging

It is generally acknowledged that the deleterious effects of both the superoxide anion ( $O_2^{\bullet -}$ ) and hydrogen peroxide ( $H_2O_2$ ) are actually due to more reactive species, from which many different experiments show that the hydroxyl radical ( $OH^{\bullet}$ ) is the most toxic candidate.

It is mainly formed via the Fenton reaction, which is initiated in the presence of catalytic quantities of transition metals like ferrous ions (FeII). This reaction is perpetuated via the Haber-Weiss cycle that regenerates FeII ions, the substrate for the Fenton reaction.



#### Haber-Weiss cycle

Particularly reactive, the hydroxyl radical  $OH^{\bullet}$  can potentially react with numerous biomolecules, and therefore damage all cellular constituents.

#### Phytic acid

Phytates are powerful iron-chelating agents that block the catalytic effect of this metal by saturating all its coordination sites: they are true Fenton reaction inhibitors.

Phytates are also said to be able to catalyze the transformation from FeII ions to ferric ions (FeIII), therefore eliminating the substrate for the Fenton reaction.

#### Polyphenols

The antioxidant properties of polyphenols are generally attributed to their scavenging properties versus the main ROS ( $O_2^{\bullet -}$ ,  $H_2O_2$ ,  $OH^{\bullet}$ ...). Polyphenols, especially gallic acid derivatives, can also interfere with the Haber-Weiss cycle by playing a part in the chelation of transition metals.

In fact, this explanation is also supported by an *in vitro* evaluation of the antioxidant potential of Gatuline® Age Defense<sup>2</sup>. It was proved to have a strong anti-free radical activity towards the hydroxyl radical and the superoxide anion, and to truly chelate the iron (substantiated).

Due to the synergistic effect between the chelating properties of phytic acid plus the scavenging properties of polyphenols, **Gatuline® Age Defense<sup>2</sup>** can prevent hydroxyl radical formation by limiting the formation of its precursive agents and also by blocking and/or eliminating FeII ions, the Fenton reaction substrate.

**By assisting the cells' antioxidant functions, Gatuline® Age Defense<sup>2</sup> regulates the ROS intracellular content.**

## Protection against chronological aging

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### *Oligopeptides*

Gatuline® Age Defense<sup>2</sup> contains oligopeptides with a molecular weight from 1000 to 5000 Daltons. These oligopeptides can penetrate through the skin barrier and act as growth factors by increasing cutaneous cell synthesis capability, as indicated by *in vitro* results on keratinocyte proliferation and on fibroblastic extracellular matrix synthesis.

### *Free amino acids*

The Krebs cycle is involved in the metabolism of glucids, lipids and proteins. It is integral to the production of energy (ATP) needed for cellular development. Gatuline® Age Defense<sup>2</sup> provides the Krebs cycle with key elements such as glutamic acid, aspartic acid and arginin (precursor agent for glutamic acid).

**With nutritive and stimulating factors, Gatuline® Age Defense<sup>2</sup> boosts the metabolism of cutaneous cells, preventing the slowing down of cell functions involved in chronological aging.**



## APPLICATIONS

Thanks to its richness, Gatuline® Age Defense<sup>2</sup> protects against all signs of aging, and is especially suited for:

### Skin care

- *anti-aging and anti-wrinkle skin care lines*
- *environmental stress protective products*
- *anti-pollution formulations*
- *mature skin care lines*
- *specific areas (eyes, lips...)*
- *face and body-firming products.*



### Hair care

- *protective preparations against environmental aggressions (UV, sand, salt, chlorine from pools...).*

### Sun care

- *pre and after-sun products*
- *sun protecting formulations.*

Gatuline® Age Defense<sup>2</sup> can be incorporated into all cosmetic products for women and men of all ages: environmental stress-protecting skin care, anti-pollution care, products for mature skin, sun care and hair care preparations.

Gatuline® Age Defense<sup>2</sup> is truly "pro-active", helping our cutaneous cells to **organize their own defenses** against all the forms of **aging (extrinsic and chronological)**. Gatuline® Age Defense<sup>2</sup> is the essence of preventive beauty care.

## SPECIFICATIONS

### *Organoleptic characteristics:*

Aspect..... clear to slightly cloudy yellow liquid

### *Physico-chemical characteristics:*

pH (pure) ..... 4.0 to 5.5

Dry matters (g/l) ..... 27 to 33

Total mineral matter (g/l) ..... 4 to 7

Total nitrogen content (g/l) ..... > 0.3

Phytic acid content (g/l) ..... > 1.0

Total phenols (expressed in gallic acid) (g/l) ..... > 0.1

Solubilities at 20°C..... soluble in water, insoluble in oils.

Preservative ..... Phenoxyethanol (0.4%), Sodium metabisulfite (0.2%)

### *Transport and storage conditions:*

To be kept at room temperature away from light. A slight and reversible turbidity may appear, without any influence on the product's activity.

### *Packing:*

Industrial standard pack..... plastic can 1 kg, 5 kg and 25 kg

Samples.....available

### *Regulatory:*

INCI name ..... **Water (and) Juglans Regia (Walnut) Seed Extract**

CAS N° ..... 7732-18-5/84012-43-1

EINECS N° ..... 231-791-2/281-688-1

Australia, Japan..... approved for cosmetic use

## CONDITIONS OF USE

The recommended use level for Gatuline® Age Defense<sup>2</sup> is 2 to 6%, depending on the type of formulation.

Gatuline® Age Defense<sup>2</sup> should be incorporated at the end of the manufacturing process, at room temperature.

Gatuline® Age Defense<sup>2</sup> is compatible with all cosmetic formulations:

- emulsions, gels, lotions and foaming products.



# FORMULAS

MASK FOR DAMAGED HAIR		MM 8453
INCI name	Trade name	%
I Cetyl alcohol (and) glyceryl stearate (and) ceteth-20 (and) steareth-20	TEFOSE <sup>®</sup> HC	5.00
Cetearyl alcohol		2.50
Cyclopentasiloxane (and) cyclodimasiloxane		2.50
Argania spinosa (argan) kernel oil		1.00
II Demineralized water		83.50
Hydroxyethylcellulose		0.30
Behentrimonium chloride		2.50
Citric acid (10% sol.)		0.10
III Cetyltrimonium chloride		1.00
IV PRESERVATIVE		0.60
Water (and) Juglans regia (walnut) seed extract	GATULINE <sup>®</sup> AGE DEFENSE <sup>®</sup>	0.50
V PERFUME		0.50
		100.00

Disperse hydroxyethylcellulose into water. Under stirring, add I heated to 75°C to II heated to 75°C. Maintain under rapid mixing (rotor stator 2500 rpm) for 3 min. Cook under normal stirring and at about 50°C, add III. Mix rapidly (rotor stator 2500 rpm) for 5 min. At about 35°C, add the components of IV and V. Complete cooling.

YOUTH AGE DEFENSE		JB 2225/D
INCI name	Trade name	%
I Polyglyceryl-6 distearate	PLUROL <sup>®</sup> STEARIQUE	5.00
Caprylic/capric triglyceride	LABRAFAC <sup>®</sup> CC	4.00
Babassu (Orbignya oleifera) oil		5.00
Cetearyl alcohol		1.00
Butyrospermum parkii (shea butter)		2.00
II Demineralized water		75.30
Glycerin		2.50
Xanthan gum		0.60
Microcrystalline cellulose (and) cellulose gum		1.00
III PRESERVATIVE		1.00
IV PERFUME		0.60
Water (and) Juglans regia (walnut) seed extract	GATULINE <sup>®</sup> AGE DEFENSE <sup>®</sup>	2.00
		100.00

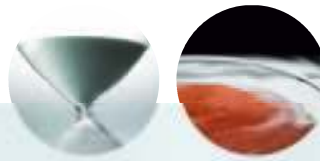
Disperse microcrystalline cellulose (and) cellulose gum into phase II water. Mix xanthan gum and glycerin and add it to phase II. Heat I and II to 75°C. Under rapid mixing (rotor stator 3000 rd/min) add I to II. Maintain rapid mixing for about 10 min. Cool under planetary stirring and at about 35°C, add phase III and IV ingredients. Complete cooling.

**ANTI-WRINKLE CARE****JB 2266/C**

	INCI name	Trade name	%
I	TRIBEHENIN PEG-20 ESTERS	EMULIUM® 22	4.00
	CAPRYLIC/CAPRIC TRIGLYCERIDE	LABRAFAC™ CC	15.00
II	DEMINERALIZED WATER		76.93
	CARBOMER		0.25
	XANTHAN GUM		0.20
III	SODIUM HYDROXIDE (10% SOL.)		0.50
IV	PERFUME		0.10
	CI 14700		0.02
V	PRESERVATIVE		1.00
	WATER (AND) JUGLANS REGIA (WALNUT) SEED EXTRACT	GATULINE® AGE DEFENSE <sup>2</sup>	2.00
			100.00

Sprinkle carbomer over water of phase II, leave to stand. Under mixing, add xanthan gum to II. Heat I and II to 75°C. Under rapid mixing (rotor stator 2000 rpm), add I to II. Maintain rapid mixing for about 5 min, then add III. Continue rapid mixing for about 2 min. Cool under planetary stirring and at about 35°C, add the ingredients of phases IV and V. Complete cooling.

This information is presented in good faith, and we believe it is correct, but no warranty as to accuracy of results, or fitness for a particular use is given, nor is freedom from patent infringement to be inferred. It is offered solely for your consideration, investigation and verification.



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GATTEFOSSÉ is an independent, multinational company headquartered in France which creates, manufactures and distributes specialty products used as ingredients by the cosmetic and pharmaceutical industries.

Present in almost 50 countries worldwide, GATTEFOSSÉ enjoys a strong know-how and position in lipochemistry, biology and extraction from natural sources.

GATTEFOSSÉ offers the cosmetic industry a variety of high performance products classified as:

- **BASES & ADDITIVES:** emulsifiers, coemulsifiers, emollients, dispersers, solubilizers, thickeners...
- **TRADITIONAL PLANT EXTRACTS**
- **SUBSTANTIATED ACTIVE INGREDIENTS** from vegetable, mineral and marine origins.

