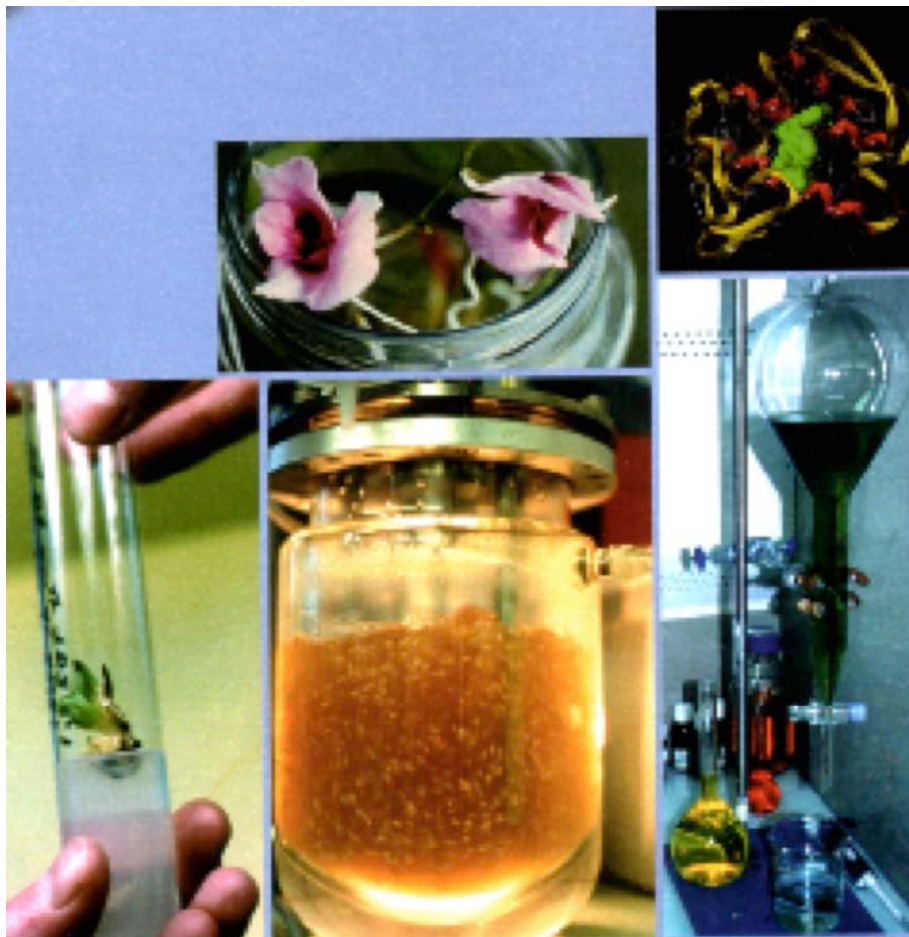


## ANTI-WRINKLES COMPLEX



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## I. SKIN

### SKIN IS A REFLECT OF BEAUTY

Despite its thinness, skin plays a key role in our physical for our physical integrity. Skin is made of three layers :

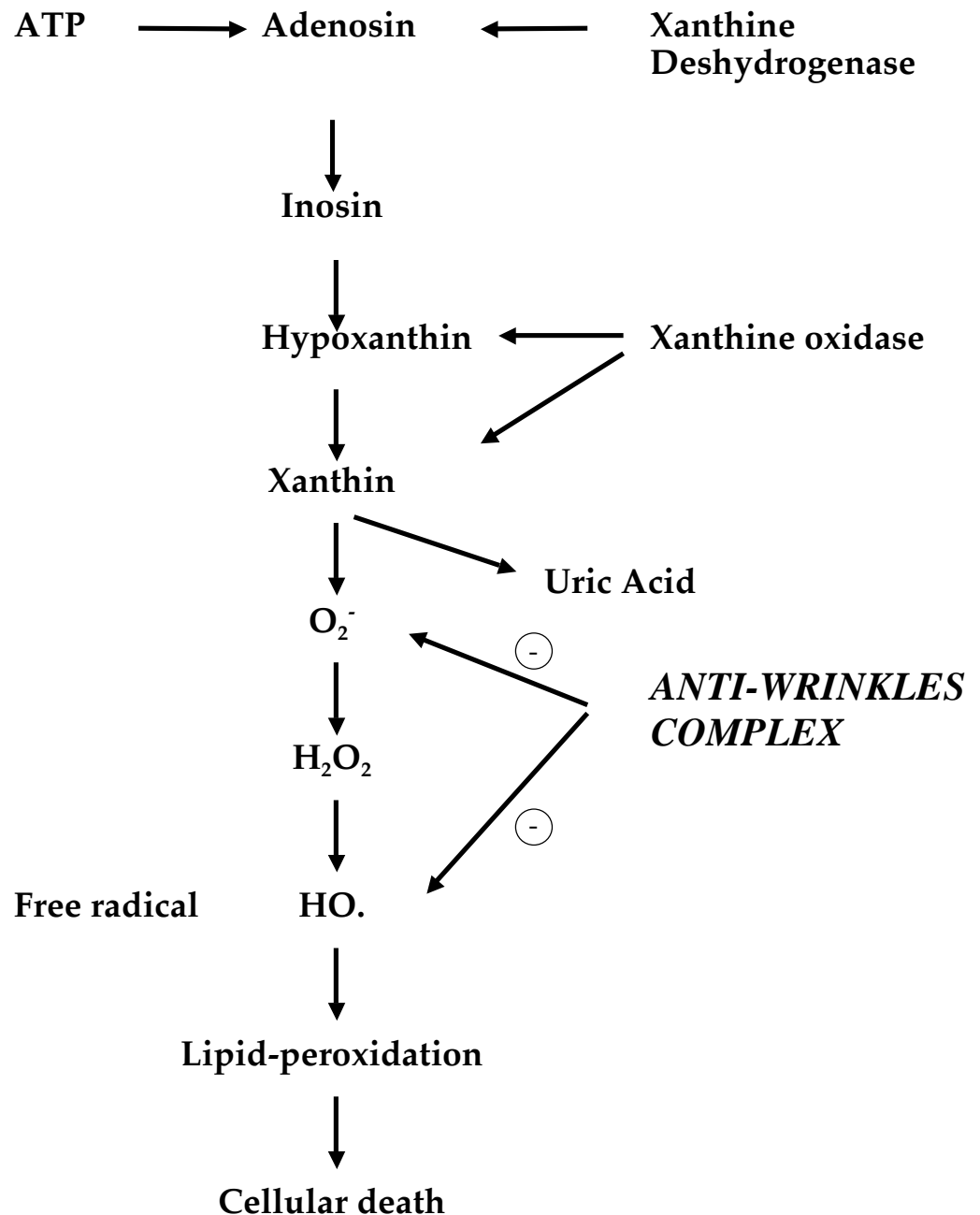
- Epidermis, the outside skin's layer,
- Dermis, made of fibroblasts secreting especially collagen elastin and hyaluronic acid,
- Hypodermis, containing fatty cells and sudoral glands.

During its lifetime, skin cells get old, thinner and start to dry, rippling and losing their flexibility and tonicity. These phenomenons are created by various factors as decrease in potential of fibroblasts division, lysis of collagen and elastin fibers. They are intensified by external factors such as ultraviolet radiation s which induce free radical formation.

Free radicals are atoms or clumps of atoms or molecules with a single electron on an external orbital creating a high instability and therefore a high reactivity. Free radicals are toxic reagents able to destabilize other molecules leading to the destruction of numerous cellular structures. For instance, a superoxide contains highly reactive oxygen targeting on :

- Proteins, subjected to polymerization and inactivation,
- Membranous lipids with the formation of other peroxides (malonaldehyde or MDA),
- DNA damages and mutations.

*All those phenomenons result in cellular death.*



The formation of free radicals is inevitable. Their production occurs continuously inside the organism and favours cutaneous aging (this production of free radicals varies according to the way of life and environment).

Then, it is essential to find substances or « actives » with antifree-radical properties. This is why, the cosmetologist is always looking for « actives » with anti-aging properties.

The second target of an anti-wrinkles « active » is the protection against dermis proteases (elastases and collagenases) and at the same time the energetic stimulation of cells in order to alleviate the decrease in various fundamental substrates synthesis.

Few products or active principles show anti-protease activity. Nevertheless the function of proteases in the aging process and then in the formation of wrinkles is very important. Collagenases, elastases and hyaluronidases have the ability to damage the substratum of skin, respectively collagen, elastin and hyaluronic acid. Consequently, on one hand, it leads to the aging phenomenon by damaging the skin and, on the other hand, by decreasing its extensibility, thus provoking wrinkles appearance.

This is why a search for anti-protease activities was realized for the anti-wrinkles complex.

### ANTI-WRINKLES COMPLEX



Anti-wrinkles plant complex offers :

- **a solution to the cutaneous aging problem** as it acts on the two major causes which are responsible for the formation of wrinkles and the withering of skin :

- Proteases which destroy collagen and elastin, the main components of the cutaneous strengthening tissues,
- The formation of free radicals.

- **a source of energy for cells**, thanks to the synergy of two plant extracts :

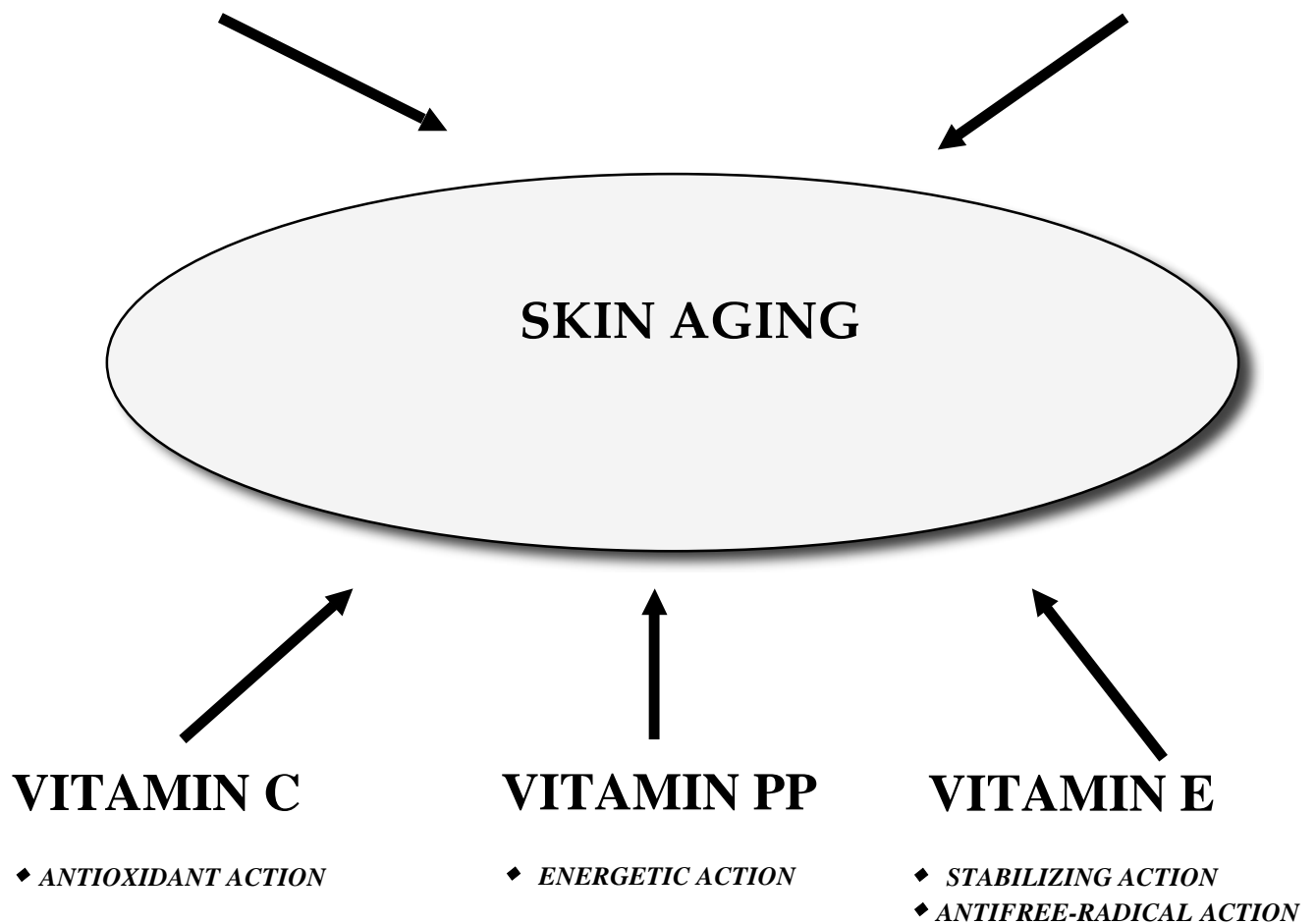
- ❑ The Cypress nut and Rosemary, main compounds of the Anti-Wrinkles Complex,  
and
  - The vitamin complexes : *vitamins C, E and PP*.

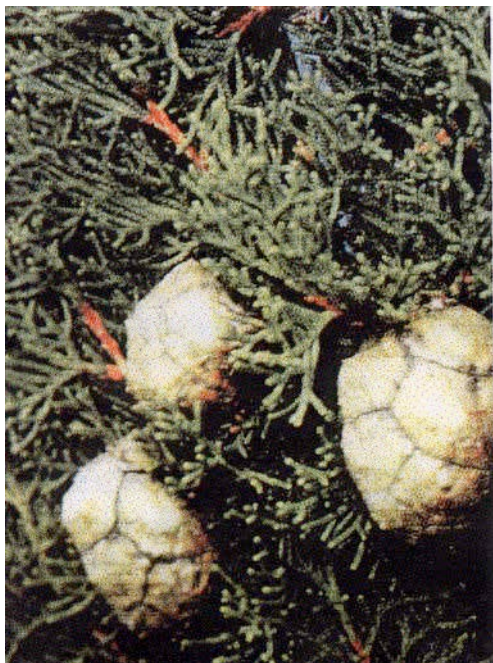
## **CYPRESS**

- *PROTECTION OF ELASTIN AND COLLAGEN FIBERS*
- ◆ *ASTRINGENT ACTION*

## **ROSEMARY**

- ◆ *TONIC AND STIMULATES FLABBY SKINS*
- ◆ *ANTIOXIDANT ACTION*



**CUPRESSALEAE Family****CYPRESS**

Latin name : *Cupressus sempervirens*

Usual name : Cypress

Part used : Cones of Cypress

**LOCATION**

In the Mediterranean area.

**CHEMICAL COMPOSITION**

IV. DITERPENIC ACIDS  
V. DIMERS AND OLIGOMERS OF  
CATECHOL AND EPICATECHOL

**PROPERTIES IN THE COMPLEX**

- ☐ PROTECTION OF ELASTIN AND  
COLLAGEN FIBERS
- ☐ ASTRINGENT ACTION



## LABIAEA Family



## ROSEMARY

Latin name : *Rosmarinus officinalis*

Usual name : *Rosemary*

Part used : Flowering plant

*0.5 to 1.5 m high shrub, with strong aromatic smell.*

## LOCATION

In Mediterranean areas (scrubs and garrigues).

## CHEMICAL COMPOSITION

- ☐ ESSENTIAL OILS (CINEOL, CAMPHOR, PINENE)
- ☐ ORGANIC ACIDS
- ☐ TERPENIC DERIVATIVES
- ☐ POLYPHENOLS

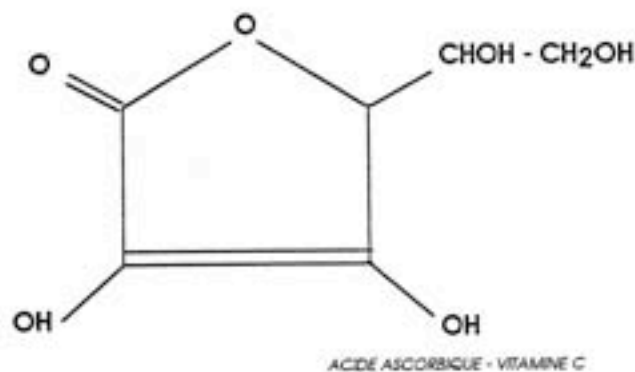
## PROPERTIES IN THE COMPLEX

- ☐ TONIC AND STIMULATING ACTION
- ☐ ANTIOXIDANT ACTION



## VITAMIN C

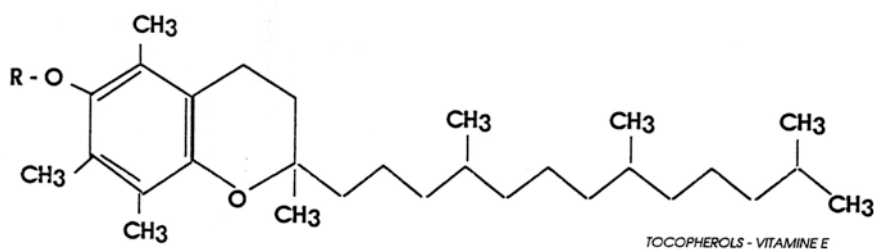
### ASCORBIC ACID



This is a hydrosoluble vitamin. Ascorbic and dehydroascorbic acid constitute the reduced and oxidized forms of a reversible oxidoreduction system. Ascorbic acid is involved in numerous biochemical pathways such as collagen synthesis. Indeed, the procollagen transformation into collagen requires the hydroxylation of two amino-acids, prolin where vitamin C is involved. Vitamin C plays an important role, and cannot be replaced. Vitamin C also interacts with free radicals by scavenging them.  
**It is then a powerful anti-oxidant.**

## VITAMIN E

### TOCOPHEROLS



This is a liposoluble vitamin. It shows anti-oxidant properties mainly at the polyunsaturated fatty acids level, which enables a stabilization of membrane lipids.

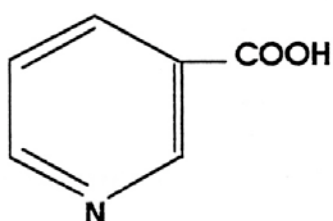
Its antifree-radical action occurs preferentially at the membrane level thanks to its lateral isoprenoid chain which is hydrophobic and liposuble. Vitamin E takes part in the formation and structure of membrane phospholipids and has a stabilizing action besides its anti-oxidant action.

The aging process is delayed. Thanks to the various properties of Vitamin E

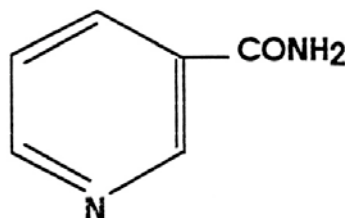
The Rosemary and Cypress nut plant complex associated with Vitamins E and C allows a perfect synergy between the various components of the complex. This close collaboration between these various molecules leads to a strengthened and optimized anti-radicalar activity.

## VITAMIN PP

## NIACINE



ACIDE NICOTINIQUE  
NIACINE - VITAMINE PP



NICOTINAMIDE  
NIACINE - VITAMINE PP

This hydrosoluble vitamin is a precursor of NAD and NADP. The term Vitamin PP includes the nicotinic acid and its amid form « nicotinamide as well as all biologically related to metabolites.

NAD is essentially located in mitochondria and contributes to the ATP formation, the source of energy of cells.

NADP is more cytoplasmic and contributes in many metabolic processes including fatty acids synthesis.

## VII. DEMONSTRATION OF THE BIOLOGICAL POTENTIAL

### ANTIFREE-RADICAL ACTIVITY

#### Test with XANTHINE OXIDASE

In order to study the anti-oxidative properties of the Anti-Wrinkles complex, an enzymatic model reaction was used. XANTHINE OXIDASE oxidizes XANTHINE to uric acid in the presence of hydrogen peroxidase with the formation of free oxygen radical. The free radical is reduced with cytochrome C (reduced form) resulting in a color change.

#### **Results :**

**A 50% inhibition of the appearance of free oxygen with a 0.5% solution of anti-wrinkles complex.**

#### Test with DPPH

The antifree-radical activity of the anti-wrinkles complex is shown using a free radical scavenger called diphenyl-pieryl-hydrazyl (DPPH) by color change at 519 nanometers.

#### **Results :**

**A 50% inhibition is observed with a 0.05% solution of anti-wrinkles complex.**

## ANTI-PROTEASE ACTIVITY

### Test with ELASTASE

The anti-protease activity of **ANTI-WRINKLES complex** has been with the elastase example. The elastine molecules produced by fibroblasts are condensed to produce flexible fibers which allows the flexibility to skin. With aging and environmental influences, there is a weakening or deterioration of elastine fibers mainly due to the action of **elastase**.

Our enzymatic test has shown an inhibition of elastase activity by the **ANTI-WRINKLES complex**. Indeed, elastase is able to cleave the synthetic substrate, N-succinyl-N-alanyl-p-nitroanilide with formation of p-nitroalane which is yellow. The reaction is monitored spectrophotometrically at 410 nanometers.

### Results :

**A 30% inhibition is observed with a 10% solution of Anti-Wrinkles Complex.**

## VIII. CONCENTRATION FOR USE

### CONCENTRATION FOR USE

- 5 to 10% in gels / creams and creams.

### CHEMICAL AND PHYSICAL CHARACTERISTICS

- pH :  $5.00 \pm 0.5$
- Dry matter :  $1.3 \pm 0.1\%$
- Colour : Opaque, brown
- Smell : characteristic



IX. EXAMPLE OF FORMULATION

## ANTI-WRINKLES GEL CREAM

0.3	<b>CARBOPOL 940</b> <i>Carbomer 940</i>
5	<b>TEFOSE 1500</b> <i>Polyglycol palmitostearate</i>
1	<b>CETIOL V</b> <i>Decyl oleate</i>
0.5	<b>PHENONIP</b> <i>Ester-p-hydroxybenzoic acid</i>
10	<b>ANTI-WRINKLES COMPLEX</b>
100 qsp	<b>DEIONIZED WATER</b>

- Mix the tefose 1500 with the cetiol at 70-80°C.
- Dissolve the carbopol in aqueous phase then add the **Anti-Wrinkles Complex** and phenonip.
- Mix the aqueous phase with the oily phase.
- Adjust pH to 5.5.

## X. TECHNICAL DATA SHEET

<b><u>Composition :</u></b>	Mix of hydroglycolic extracts of <b>Cypress Nut, Rosemary and vitamins C, E, PP.</b>
<b><u>INCI name :</u></b>	Water, Propylene Glycol, Cupressus Sempervirens Seed Extract, Rosmarinus Officinalis (Rosemary) Leaf Extract, Niacin, Tocopherol, Ascorbic Acid.
<b><u>Extract solvent :</u></b>	Propyleneglycol-water 1/1.
<b><u>Organoleptic characteristics :</u></b>	Opaque liquid, brown, characteristic smell.
<b><u>Density at 20°C :</u></b>	1.000 ± 0.05
<b><u>pH :</u></b>	5.00 ± 0.5
<b><u>Solubilities :</u></b>	Soluble in water and dilute alcohols. Insoluble in organic solvents and oils.

**Identification :**

CCM (see analysis method n°6)

<i>Rf</i>	<i>spots color</i>
0.38	blue
0.42	orange
0.46	orange
0.50	orange
0.58	orange
0.65	orange
0.70	blue
0.77	orange
0.83	orange
0.88	orange
0.97	green

**Dry matter :**

1.30 ± 0.1%

**Heavy metals :**

&lt; 10 ppm

**Microbiological characteristics :**

< 100 germs/ml  
< 10 yeasts or moulds/ml  
No enterobacteria.

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