ONCE UPON A TIME...
THERE WAS A PLANT.

Giada Maramaldi
PROLOGUE

Understanding of life

«Herbs and plants are medical jewels gracing in the woods, fields and lanes which few eyes see and few minds understand.»

Linnaeus (1707-1778)

«Almost all aspects of life are engineered at the molecular level, and without understanding molecules we can only have a very sketchy understanding of life itself.»

Francis Crick
(Nobel Prize for Medicine in 1962)
A QUICK LOOK TO OUR PATH

PROLOGUE
Understanding of life
More than one metabolism
The many reported biological activities of quercetin
Quercetin as a secondary metabolite

The new Phytosome® solution: Quercevita™
Quercevita™ on cells

Quercevita® on human volunteers: a first skin aid

Quercevita™ improves the Skin Antioxidant Power and decreases ROS production

EPILOGUE
Quercevita™ in our and your formulations
The fire sermon

Quercevita™ on cells

Quercevita™ in our and your formulations
MORE THAN ONE METABOLISM

COMPOUNDS OF PRIMARY METABOLISM
Primary metabolism refers to compounds absolutely necessary for life such as:
- Energy sources
- Genetic material
- Proteins
- Components of cells membranes

COMPOUNDS OF SECONDARY METABOLISM
Secondary metabolism refers to molecules that are NOT required for the short term functioning of an organism.
- Toxic to the animals that eat the plant
- Pigments in flowers to attract pollinators
MORE THAN ONE METABOLISM: MANY COMPOUNDS!

ALKALOIDS
- caffeine
- morphine
- nicotine

COUMARINS
- coumarin
- aesculin

TERPENES
- menthol
- glycyrrhizin

AND FLAVONOIDS!
- quercetin
FLAVONOIDS AS SECONDARY METABOLITES

Anthocyanins and flavonoids are a very large group of compounds which primarily act as pigments.

Their biological effects are mainly due to their antioxidant activity, but flavonoids have a far more significant role in topically applied products (many act as co-factors for all kind of enzymes).

![Quercetin](quercetin.png)

Quercetin is probably the most frequently occurring botanical pigment!
THE MANY REPORTED BIOLOGICAL ACTIVITIES OF QUERCETIN

Quercetin is a powerful antioxidant (20 times more potent than ascorbic acid, 50 times more potent than tocopherol). This is made possible thanks to the presence of a large number of phenolic hydroxy groups that enable free radicals to leapfrog over the aromatic system.

Quercetin is a powerful enzyme manipulator, it inhibits 5 lipoxygenase (Thorne et al).

It is a selective inhibitor of pro-inflammatory metabolites.

HOWEVER

Quercetin is poorly bioavailable.
THE NEW PHYTOSOME® SOLUTION

Phospholipids are obtained from Sunflower:
- Great skin affinity
- No GMO free issues or declarations needed

QUERCEVITA®
THE NEW PHYTOSOME® SOLUTION

STARTING HERBAL MATERIAL

- *Sophora japonica* L.
- Common name: Japanese pagoda tree
- Botanical family: *Fabaceae*
- Cultivated/Wild: Cultivated
- Part of the plant utilized: Flower

quercetin; CAS [117-39-5]; C15H10O7; M.W. 302.24
# QUERCEVITA™ ON CELLS
## IN VITRO EFFICACY ON BASOPHIL CELLS

<table>
<thead>
<tr>
<th>Study name:</th>
<th>Degranulation test on basophil cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental model</td>
<td>RBL (rat basophil cells) incubated and expressing the IgE human receptor. β-hexaminidase is measured as an index of basophil degranulation.</td>
</tr>
<tr>
<td>Measured parameters</td>
<td>β-hexaminidase expressed as optical density at 405 nm.</td>
</tr>
<tr>
<td>Results</td>
<td>Effective in inhibiting basophil degranulation in a dose-dependent way, reaching a maximal inhibition of <strong>83.6%</strong> at 10 μg/ml.</td>
</tr>
<tr>
<td>Indications</td>
<td>Soothing, lenitive, antiallergic</td>
</tr>
<tr>
<td>Treatment</td>
<td>Quercevita® was tested at four different concentrations (1.25, 2.5, 5 and 10 μg/ml) and compared to a positive control (HA), to a negative control (spontaneous degranulation) and to the maximal stimulation (Tritox X).</td>
</tr>
</tbody>
</table>
Aim of the test is to evaluate the ability of Quercevita™ to inhibit basophil degranulation.

**Basophil cells** are the **terminal cells** of the **allergic reaction** in vitro.

**Basophil degranulation** is induced by **specific allergens** interacting with the IgE receptor located on the granulocyte surface.

Basophil degranulation was compared to a positive and to a blank control.

**β-hexaminidase** (with histamine and leukotrienes) is a marker of degranulation.
Basophil degranulation is induced by specific allergens interacting with the IgE receptor located on the granulocyte surface. Basophil degranulation was compared to a positive control.

Basophil degranulation was inhibited by **83.6%** at a 10μg/ml conc. (0.01% in weight)
ONCE UPON A TIME…
THERE WAS A WISE PERSON.
In this discourse, the Buddha preaches about achieving liberation from suffering through detachment from the five senses and mind. He describes the sense bases and resultant mental phenomena as "burning" with passion, aversion, delusion and suffering. Meditation and disenchant liberate from the burning senses.

Quercevita® has the potential to liberate from the fire of skin challenges.
# QUERCEVITA™
## CLINICAL EFFICACY AS SOOTHING AND ANTI-IRRITANT

<table>
<thead>
<tr>
<th>Study name:</th>
<th>Lenitive and anti-itch efficacy following to acute irritation induced by various skin challenges</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental model</td>
<td>Following to basal measurements, the volunteers’ back is divided into 4 areas challenged by different stimuli.</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>30 healthy volunteers aged 18-65 years.</td>
</tr>
</tbody>
</table>
| Measured parameters | **Histamine**: wheal size and itch score (VAS)  
**UV**: erythema index by Mexameter;  
**SLS** and **glycolic acid**: skin hydration (TEWL), irritation index (Mexameter) |
| Results | Wheal size by histamine challenge decreased by **13.25%** at 30’ (p<0.001); erythema index by UV challenge reduced by **10.05%** (p<0.01); erythema index induced by SLS challenge decreased by **17.01%** (p<0.0001) at 4h; TEWL induced by SLS decreased by **38.10%** (p<0.0001) at 4h, TEWL induced by glycolic acid decreased by **45.69%** (p<0.0001). |
| Indications | Soothing, lenitive, antiallergic |
| Treatment | Quercevita™ was tested at **1%** compared to a placebo formulation and to a positive reference formulation (containing Desclorophenyramine maleate) |

ABICH Study No. IE760/14-01
QUERCEVITA™
CLINICAL EFFICACY AS SOOTHING AND ANTI-IRRITANT

The volunteers’ back is divided in 4 areas:
1: Histamine
2: UV
3: SLS
4: Glycolic Acid

Each area is challenged by a different stimulus and the topical efficacy evaluated by different parameters and instruments.
# QUERCEVITA™

**CLINICAL EFFICACY AS SOOTHING AND ANTI-IRRITANT**

<table>
<thead>
<tr>
<th><strong>Histamine 1% (prick test)</strong></th>
<th>Eval: wheal size; itching score (VAS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl NEG (untreated)</td>
<td>Placebo</td>
</tr>
<tr>
<td>Quercevita™</td>
<td>Polaramin</td>
</tr>
</tbody>
</table>

Evaluation at T0, T10’, T30’

<table>
<thead>
<tr>
<th><strong>UV</strong></th>
<th>Eval: Erythema index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl NEG (untreated)</td>
<td>Placebo</td>
</tr>
<tr>
<td>Quercevita™</td>
<td>Polaramin</td>
</tr>
</tbody>
</table>

Eval. at T0 e T4h from product application

<table>
<thead>
<tr>
<th><strong>SLS</strong></th>
<th>Eval: TEWL; Erythema index; Hydration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl NEG (untreated)</td>
<td>Placebo</td>
</tr>
<tr>
<td>Quercevita™</td>
<td>Polaramin</td>
</tr>
</tbody>
</table>

Evaluation at T0, T2h, T4h, T24h/TEWL

<table>
<thead>
<tr>
<th><strong>Glycolyc Acid</strong></th>
<th>Eval: TEWL; Erythema index; Hydration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ctrl NEG (untreated)</td>
<td>Placebo</td>
</tr>
<tr>
<td>Quercevita™</td>
<td>Polaramin</td>
</tr>
</tbody>
</table>

Evaluation at T0, T2h, T4h, T24h/TEWL
The size of the wheal generated by the prick test after 30’ decreased by 13.25% (overperforming the positive benchmark!). At the same control neither the placebo nor the treated area revealed any statistically significant decrease.
The (subjective) itch intensity decreased in line with the positive benchmark according to a subjective VAS (Visual Analogue Score) ranging 1-10 reaching the same low value. (p<0.0001 for all).
The erythema index after 4 hours had decreased by over 10% in the Quercevita™ treated area (p<0.01). Scale ranges 0-999.
The erythema index measured by Mexameter significantly decreased both in the Quercevita™ (by 17.01%, p<0.0001) and the positive control area.

The mean hydration value overperformed the benchmark at all tested times (data not shown, +24% vs +19%, p<0.0001)
The TEWL induced by SLS has decreased in 4 hours’ time, in the area treated by Quercevita™ by **38.10%** (overperforming the positive benchmark!).
The Erythema Index induced by Glycolic acid challenged as decreased in 4 hours’ time, in the area treated by Quercevita™ by 16.83% (p<0.0001). On the other side, mean hydration increased by 16.48% (p<0.01), overperforming the positive benchmark. Additionally, TEWL decreased by 45.69% (p<0.001).
# QUERCEVITA™
## IN VITRO EFFICACY AS FREE RADICAL SCAVENGER

<table>
<thead>
<tr>
<th>Study name:</th>
<th>Inhibition of ROS production on keratinocytes following to UVA induced oxidative stress</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental model</td>
<td>Inhibition of ROS in response to UVA exposure by fluorescence marker (DCFH converted to fluorescent DCF) directly proportional to the ROS quantity inside the cytoplasm / tested in triplicate</td>
</tr>
<tr>
<td>Measured parameters</td>
<td>Fluorescin intensity as a measure of ROS production. ROS interact with dichlorofluorescein to dichlorofluorescin, a highly fluorescent molecule.</td>
</tr>
<tr>
<td>Results</td>
<td>Quercevita™ at the lowest dosage performed as the positive benchmark ascorbic acid but weight % was 7.5% lower. The best result, overperforming ascorbic acid, was achieved at the 0.05 mg/ml dosage and with the highest energy challenge, when ROS production was reduced by 39.87%.</td>
</tr>
<tr>
<td>Indications</td>
<td>Antioxidant, free radical scavenger</td>
</tr>
<tr>
<td>Treatment</td>
<td>The tested emulsion contained 1% Quercevita™</td>
</tr>
</tbody>
</table>
Quercevita™ emulsion 1% was effective in reducing UVA induced ROS production at all tested dosages with a dose response evidence at the lower ones. The highest energetic challenge has not reduced the efficacy of Quercevita™. At the lowest dosages performances were in line with ascorbic acid (but weight % was 7.5 times lower!)
QUERCEVITA™
TESTED FORMULATIONS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqua (water)</td>
<td>53.68%</td>
</tr>
<tr>
<td>Glycerin</td>
<td>3.00%</td>
</tr>
<tr>
<td>Disodium EDTA</td>
<td>0.10%</td>
</tr>
<tr>
<td>Imidazolylindin urea</td>
<td>0.30%</td>
</tr>
<tr>
<td>Cetyl alcohol / C12-20 Acid PEG-7 Ester</td>
<td>14.00%</td>
</tr>
<tr>
<td>PEG-90 Stearate / Glyceryl stearate</td>
<td>4.50%</td>
</tr>
<tr>
<td>Triglycerides Caprylic / Capric</td>
<td>4.00%</td>
</tr>
<tr>
<td>Polydecene</td>
<td>0.02%</td>
</tr>
<tr>
<td>Polysorbate-60</td>
<td>0.50%</td>
</tr>
<tr>
<td>Ascorbyl palmitate</td>
<td>0.50%</td>
</tr>
<tr>
<td>Quercevita®</td>
<td>1.00%</td>
</tr>
<tr>
<td>Phenoxyethanol</td>
<td>0.60%</td>
</tr>
<tr>
<td>Dimethicone</td>
<td>1.00%</td>
</tr>
</tbody>
</table>

The tested formulation, compared to the placebo, is a quite simple system which has nonetheless shown a high efficacy level.
Study name: Determination of Skin Antioxidant Potential (SAP)

Experimental model: Evaluation of the Skin Antioxidant Potential by labelling the skin with an ESR active free radical; an active substance will increase SAP: Quercevita™ has been compared to a placebo and to a tocopherol standard (1%).

Numerosity: At least 3 different skin biopsy for each formulation at each time (tot. 36)

Measured parameters: SAP (Skin Antioxidant Potential) using Electrone Spin Resonance. SAP value is expressed in % calculated on the placebo treated skin on a time-dependent penetration profile. AUC results integrate the antioxidant potential and the time parameter.

Results: Quercevita™ at 0.5% in an inert formulation has increased the SAP by 125% compared to Tocopherol standard 1% (measured by AUC).

Indications: Antioxidant, free radical scavenger, skin protection enhancer.
The SAP (Skin Antioxidant Power), expressed in %, is a quantitative determination of the antioxidant activity inside the epidermal and dermal skin layers.

- Each skin has its own intrinsic antioxidant capacity.
- The skin is labelled by an ESR active test radical permeating the skin from the saturated filter paper underneath.
- When an active substance is applied to the stratum corneum, it will diffuse un the under layers and increase the SAP.
- When the test radical meets with the test substance, a specific signal is detected.
- From there, the SAP is evaluated at different times.
QUERCEVITA™

IN VITRO EFFICACY AS FREE RADICAL SCAVENGER IN SKIN

The SAP (Skin Antioxidant Power), expressed in %, is a quantitative determination of the antioxidant activity inside the epidermal and dermal skin layers.

AUC of penetration-time profiles is higher, for Quercevita®, by 125% (at half the concentration) compared to the Tocopherol standard.
EPILOGUE

Quercevita™ in our and your formulas

Flavonoids comprise a large group of compounds of vegetal origin which, in many cases, are responsible for the biological activity of botanical extracts.

The application of flavonoids in personal care products is less developed, although some major applications comprise anti-ageing, anti-cellulite and soothing.

The benefit of a bioavailable purified flavonoid as Quercevita™ has a significant potential in cosmetic formulas as a skin first aid agent and has shown clinically a remarkable soothing activity on various challenges and parameters.

Quercevita™ was shown effective in inhibiting basophil degranulation thus qualifying as a lenitive and soothing active ingredient.

Quercevita™ was shown effective in increasing the skin antioxidant intrinsic potential (SAP) overperforming the tocopherol standard by over the double at half the concentration.

Potential applications comprise sun bare, baby care, products for aesthetic medicine treatments, sensitive skin.
ON THE MARKET

Why is Quercevita™ innovative?

Quercevita™ is the first Phytosome® to be used in personal care formulas with **sunflower** phospholipids.

Sunflower phospholipids used in Quercevita™ are IP (Identity preserved) and not GMO.

Products with Quercetin launched 2003-2013 worldwide

January 2003 - December 2013: ONLY 145 products launched, less than 15/year on average, worldwide!

Source: GNP Mintel
ON THE MARKET

Why is Quercevita™ innovative?

Quercetin containing products are spread according to the following distribution:

Products with Quercetin launched 2003-2013 worldwide

- 63%: Vitamins and dietary supplements
- 22%: Cardiovascular
- 12%: Antioxidant
- 12%: Antihistaminic
- 10%: Decongestant
- 2%: Dermatology

Let’s grow these classes.
## ON THE MARKET

### Why use Quercevita™?

Forecast Consumption of Substantiated Botanical Actives in Personal Care in Europe by Function, 2013 to 2018

<table>
<thead>
<tr>
<th>Function</th>
<th>EUR Million</th>
<th>2013</th>
<th>2018</th>
<th>CAGR, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-aging</td>
<td>78.0</td>
<td>96.7</td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td>Hair care</td>
<td>17.6</td>
<td>21.9</td>
<td>4.5</td>
<td></td>
</tr>
<tr>
<td><strong>Anti-inflammatory</strong></td>
<td><strong>14.3</strong></td>
<td><strong>17.4</strong></td>
<td><strong>4.0</strong></td>
<td></td>
</tr>
<tr>
<td>Skin lightening/whitening</td>
<td>8.5</td>
<td>10.3</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>Sun protection</td>
<td>5.9</td>
<td>7.0</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>Slimming</td>
<td>3.9</td>
<td>4.7</td>
<td>4.3</td>
<td></td>
</tr>
<tr>
<td>Anti-acne</td>
<td>2.0</td>
<td>2.5</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>105.0</strong></td>
<td><strong>160.5</strong></td>
<td><strong>4.3</strong></td>
<td></td>
</tr>
</tbody>
</table>

SOOTHING is the second largest category of skin care products worldwide!

*Source: Kline*
ON THE MARKET
Potential applications

- Sun care
- Jellyfish
- Post-aesthetic treatments
- Baby care
- Nettle
- Mosquitos