MARRUBIUM VULGARE STEMS G™

IRB STEMS
THE WIDEST RANGE OF
PURE PLANT STEM CELLS
AT THE HIGHEST AVAILABLE
CONCENTRATION WITHOUT
PRESERVATIVES

COSMETICS
COSMETICS

MARRUBIUM VULGARE STEMS G™

HTN™ biotechnology, exclusively owned by IRB, is the unique process able to physiologically orientate the production of specific substances involved in defensive responses of the plant against environmental physical and biological stress such as phenylpropanoids. Through the in vitro culture of plant stem cells in protected and highly controlled conditions IRB achieves the production of these substances with considerable biological properties.

MARRUBIUM VULGARE, also known as White Horehound or Marrubio, is an edible herbaceous plant of the Lamiaceae family, spontaneously growing in temperate areas of Europe, America and Asia. Horehound medicinal properties were esteemed by the Romans and the Arabs. It is often used in the popular medicine as a remedy for respiratory conditions and to improve the digestive function. The plant has also anti-inflammatory properties and is used externally for skin problems.

THE DETOXIFICATION SYSTEM

The skin is the largest organ of the human body and one of its major functions is to defend the body from many different environmental stresses such as UV, chemical irritants, heavy metals and other pollutants, known as xenobiotics. This continuous exposure will result in skin irritation followed by decreased barrier function and penetration of the substances in the body.

To manage this challenge, our skin has developed a complex detoxification system made of molecules and enzymes that can neutralize the external substances. This important protective function is performed by two enzymatic systems known as the Phase I and Phase II enzymes.

Phase I enzymes are involved in oxidation, reduction and hydrolysis of the foreign substances and are, in some cases, responsible for their bio-activation, hence increasing their potential harmfulness.

Phase II enzymes modify the xenobiotics to achieve complete detoxification by conjugating the toxic molecules with hydrophilic residues and making them easier to excrete. Among the Phase II enzymes there are also many antioxidant enzymes like SOD, catalase, heme oxygenase and thioreduxin reductase.

The genetic activation of Phase II enzymes is under control of the nuclear transcription factor Nrf2 which is normally located in the cell cytoplasm and bound to the repressor Keap1. Following increase of the oxidative stress or xenobiotic levels, the Nrf2 separates from Keap1 and enters into the cell nucleus where it activates the transcription of the detoxification, antioxidant and cytoprotective proteins (Phase II enzymes).

Nrf2 levels in the nucleus can be increased by plant molecules (eg. sulforaphane) so that the detoxification system can be alerted and pre-activated. These substances are known as second-generation antioxidants to be distinguished form the traditional first-generation antioxidants with mainly radical scavenging properties (eg. vitamin C).
THE WHEN:
THE FIRST AND UNIQUE THIRD GENERATION ANTIOXIDANT:
ENSURING MULTI-PHASE PROTECTION THROUGH RADICAL
SCAVENGING AND MAXIMISATION OF SKIN SELF DEFENSIVE
SYSTEMS

THE WHY:
HTN™ TECHNOLOGY
Thanks to the HTN™ technology Marrubium vulgare stems G™ is rich
of phenylpropanoids, active substances involved in defensive
responses of the plant, and particularly FORSYTHOSIDE B and
VERBASCOSIDE.

<table>
<thead>
<tr>
<th>Properties</th>
<th>1st generation antioxidant</th>
<th>2nd generation antioxidant</th>
</tr>
</thead>
<tbody>
<tr>
<td>FORSYTHOSIDE B</td>
<td>antioxidant</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>activator of Nrf2</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>inducer of Phase II enzymes</td>
<td>✔</td>
</tr>
<tr>
<td>VERBASCOSIDE</td>
<td>antioxidant</td>
<td>✔</td>
</tr>
<tr>
<td></td>
<td>inducer of Phase II enzymes</td>
<td>✔</td>
</tr>
</tbody>
</table>

Marrubium vulgare stems G™ is therefore the first and unique third
generation antioxidant able to ensure to the skin a multi-phase
protection against a wide range of oxidative and environmental
stresses:
› immediate protection through strong radical scavenging
› long-term protection through maximisation of skin self defensive
systems
ANTIOXIDANT ACTIVITY

The TEAC (Trolox Equivalent Antioxidant Capacity) method is commonly used to evaluate the antioxidant capacity of a substance compared with that of Trolox, a water-soluble derivative of vitamin E.

Marrubium vulgare stems G™ has a strong antioxidant activity, 2 times more efficient than common natural antioxidant benchmarks such as resveratrol and vitamin C. (*) This test has been performed on the powder form of Marrubium vulgaris cell cultures in order to avoid experimental interferences.

ACTIVATION OF Nrf2

Human keratinocytes (HaCat) have been incubated for 24 hours with forsythoside B and verbascoside at increasing concentrations (0.006-0.015%) and the nuclear level of transcription factor Nrf2 has been assessed by Western blotting.

Forsythoside B and verbascoside dose dependently increase the levels of Nrf2 protein thus activating the physiological detoxification system of the human keratinocytes and hence the synthesis of Phase II enzymes.
INDUCTION OF PHASE II ENZYMES

Heme oxygenase 1 (HO-1) is a Phase II enzyme normally present also in human skin where it plays an antioxidant defensive role. In fact several stimuli such as oxidative stress, cytokines and bacterial compounds can considerably induce its synthesis thus improving the skin self defensive systems.

Human keratinocytes (HaCat) have been incubated, at different times, with forsythoside B (0.015%) and verbascoside (0.012%) and the expression levels (mRNA) of heme oxygenase have been assessed by quantitative PCR analysis.

The mRNA level of the protective enzyme heme oxygenase was strongly increased by forsythoside B and verbascoside in a time dependent manner. Especially forsythoside B increased the expression level up to 8000%, if compared with untreated control. This stimulating activity also resulted to be dose dependent for both substances (data not shown).

Consequent to the gene expression induction, western blot analysis confirmed that the protein level of heme oxygenase in HaCat was also increased.

Forsythoside B and verbascoside significantly induce the expression and the synthesis of the Phase II enzyme heme oxygenase 1 in human keratinocytes thus improving their physiological self defensive system.
STIMULATING EFFECT ON PHASE II ENZYMES IN 3D HUMAN EPIDERMIS

Transcriptional effects of Marrubium vulgare stems G™ were evaluated on reconstructed human epidermis in order to assess effects on Phase II enzymes.

After 24 hours of incubation with the ingredient* (0.125%) the mRNA expression level of typical Phase II enzymes was evaluated by quantitative PCR.

(*) This test has been performed on the powder form of Marrubium vulgaris cell cultures in order to avoid experimental interferences.

Marrubium vulgare stems G™ increases the expression levels of the enzymes thioredoxin reductase 2 (TXNRD2) and glutathione S-transferase I (GSTP1). These Phase II enzymes are directly involved in the response to xenobiotics mediated by Nrf2 activation.

Marrubium vulgare stems G™ is able to potentiate the enzymes belonging to the endogenous cytoprotective system by activation of the Nrf2 transcription pathway.

TXNRD2 is a dimeric NADPH-dependent enzyme that catalyzes the reduction of thioredoxin and is a key enzyme in the regulation of the intracellular redox environment.

GSTP1 is a member of glutathione S-transferases, a family of enzymes that play an important role in detoxification by catalyzing the conjugation of many xenobiotics with reduced glutathione to facilitate their excretion.
BIBLIOGRAPHY

THE HOW:

COSMETIC USES
- detoxifying formulations
- anti-stress day/night creams
- anti-pollution creams
- photo-aging prevention

POTENTIAL CLAIMS
- Marrubium vulgare stems G is the first and unique third generation antioxidant
  - ensures multi-phase protection: immediate antioxidant activity and long-term protection
  - maximises skin self defensive systems
  - enhances the capacity of the skin to resist to oxidative stress (also UV induced)
  - protects the skin against environmental stresses

RECOMMENDED CONCENTRATION:
- 1 - 3%

FORMULATION GUIDELINES:
- compatible with O/W emulsions, serum, ...
- pH ≤ 6
- introducing during the cooling phase (<50°C)

PRODUCT SPECIFICATIONS

<table>
<thead>
<tr>
<th>Product code</th>
<th>IRB MVSGX</th>
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</thead>
<tbody>
<tr>
<td>Product name</td>
<td>MARRUBIUM VULGARE STEMS G</td>
</tr>
<tr>
<td>Product composition</td>
<td>Marrubium vulgare cell cultures 20%, glycerin 80%, xanthan gum 0.3%</td>
</tr>
<tr>
<td>Appearance</td>
<td>Amber to light brown coloured liquid with characteristic odour</td>
</tr>
<tr>
<td>Total aflatoxins</td>
<td>absent</td>
</tr>
<tr>
<td>GMO</td>
<td>absent</td>
</tr>
<tr>
<td>Pesticides</td>
<td>absent</td>
</tr>
<tr>
<td>Microbiological Specifications</td>
<td>Total microbial count bacteria: ≤ 100 CFU/g, fungi ≤ 100 CFU/g</td>
</tr>
<tr>
<td>Packaging</td>
<td>1 Kg</td>
</tr>
<tr>
<td>Storage</td>
<td>Store the product in the original, well closed container, in a cool, dry area and protected from light</td>
</tr>
<tr>
<td>Shelf life</td>
<td>12 months</td>
</tr>
</tbody>
</table>

PRODUCT COMPOSITION

- Marrubium vulgare cell cultures 20%
- glycerin 80%
- xanthan gum 0.3%

APPEARANCE
- Amber to light brown coloured liquid with characteristic odour

TOTAL AFLATOXINS
- Absent

GMO
- Absent

PESTICIDES
- Absent

MICROBIOLOGICAL SPECIFICATIONS
- Total microbial count bacteria: ≤ 100 CFU/g
- Fungi ≤ 100 CFU/g

PACKAGING
- 1 Kg

STORAGE
- Store the product in the original, well closed container, in a cool, dry area and protected from light

SHELF LIFE
- 12 months
This information and further technical advice are based on our present knowledge and experience and we reserve the right to make any changes according to further developments and acquisition of new data. The only guaranteed analytical specifications are those appearing in the certificate of analysis sent with each delivery. Performance of the product described herein should be verified by testing carried out under the conditions specified. The results obtained may vary and cannot be guaranteed. The End-User is responsible for the quality of the final product, and for the safety and technical suitability of all products, including the End-User’s own formulation. IRB will not assume any expressed or implied liability in connection with any use of this information.