A CRANBERRY EXTRACT WITH THE HIGHEST PERCENTAGE OF A-TYPE PROANTHOCYANIDINS (PACs-A) ON THE WORLD MARKET.

UTI SUPPORT AND ANTIVIRAL ACTIVITY
Cranberries are rich in phenols, which show potent antioxidant activity, prevent the adhesion of bacteria to host cells in the urinary tract infections (UTI) due to *Escherichia coli*, prevent the formation of gastric ulcers, and fight virus infections.
UroPathogenic Escherichia Coli (UPEC) infections

- a) UPEC colonization of the periurethral and vaginal areas with colonization of the urethra;
- b) ascending into the bladder;
- c) adherence to the surface and interaction with the bladder epithelium defense system;
- d) biofilm formation;
- e) invasion and replication by forming bladder intracellular bacterial communities (IBCs);
- f) kidney colonization and host tissue damage with increased risk for bacteremia/septicemia
Cranberry extracts contain proanthocyanidins (PACs), which are divided into two types:

**Type-B PACs** are present in foods rich in tannins such as grapes and chocolate. These PACs exert no effects on UTI.

**Type-A PACs** possess a second ether bond between the ring A of the lower unit and the carbon in 2 of the upper unit (O7 → C2). PACs-A were found to inhibit P-fimbrial adhesion *in vitro* and to play a significant role in UTI prevention.

Cranberry extracts containing 72 mg PAC produce an active and significant bacterial anti-adhesion in human urine.
There are several methods used to quantify PACs in cranberry. The classical Bates-Smith method (incorrectly referred to as UV, despite the reading is made at 540 nm) and the European Pharmacopoeia method depolymerize the PACs and express their content on cyanidin chloride basis. However, due to the complexity of the structures of PACs and the links of type A, the results can often be inaccurate and not reproducible.

The DMAC (dimethylaminocinnamaldehyde) method is more accurate than other methods and has been successfully used to quantify cranberry PACs. However, DMAC does not distinguish PAC A from PAC B, therefore further chemical analysis with HPLC-mass spectrometry is necessary.

Comparison of analytical methods for the quantification of PAC in an extract standardized to 5% PAC equivalents of PAC-A2 (Extrasynthese).
Take home message #1

1) BL-DMAC is the best method for quantification
2) BL-DMAC does not distinguish PAC-A and PAC-B
3) PAC-A content correlates with anti-adhesion activity

*The more accurate is the PAC-A quantification, the more effective the cranberry extract will be.*
Biosfered uses in house **HPLC coupled to Ion Trap Mass Spectrometry** to evaluate PACs quality and content.

**Quantification of PAC-A and PAC-B**

Mass spectrum of PAC A2
We have performed a **comparative analysis** between Oximacro and some cranberry extracts present in the international market.

Oximacro possesses the highest **Total PAC content***

*calculated with the DMAC method and validated by external analytical labs.
Chemical composition of Oximacro®

A. Eluogram from Sephadex-LH20 column
B. Fraction 1: anthocyanins and rutin.
C. Fraction 2: quercetin and isorhamnetin.
D. Fractions 3 and 4: isomers of PAC-A dimers.
E. Fractions 3 and 4: isomers of PAC-A trimers.
We have performed a **comparative analysis** between Oximacro® and some European and US end products present on the market as a support for UTI by using the BL-DMAC method for total PAC content and HPLC-ESI-MS/MS chromatography for authentication of PACs-A and PACs-B.

<table>
<thead>
<tr>
<th>Product name</th>
<th>Company</th>
<th>Exp.</th>
<th>PACs conc. (mg/g)</th>
<th>% PAC A</th>
<th>% PAC B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oximacro®</td>
<td>Biosfered</td>
<td>Jul-18</td>
<td>360</td>
<td>85%</td>
<td>15%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>306 mg/g</td>
<td>54 mg/g</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Origin</th>
<th>European Products</th>
<th>US Products</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Total PACs (mg/g)</td>
<td>26</td>
<td>25</td>
</tr>
<tr>
<td>% PAC-A</td>
<td>73%</td>
<td>80%</td>
</tr>
<tr>
<td>% PAC-B</td>
<td>27%</td>
<td>20%</td>
</tr>
</tbody>
</table>

We have analyzed >40 products and the results will soon be published.
Take home message #2

Oximacro® offers the possibility to use a cranberry extract with the highest content of standardized and authenticated PACs-A, unprecedented on the world market.

\[ \sim 120 \text{ mg of Oximacro} \rightarrow 36 \text{ mg PAC-A} \]
Antioxidant activity of Oximacro® with respect to Trolox in vitro.
No matter the test, Oximacro® shows a higher* antioxidant power.

*The lowest the value the highest the activity
Take home message #3

Oximacro® shows a strong antioxidant activity.

Oximacro® shows a high stability also at high temperatures (55°C).

- Oximacro® PACs content was assayed by the BL-DMAC method, while the dimer and trimer PAC-A and PAC-B percentages were determined by HPLC-ESI-MS/MS.
- A balanced group of female (N=60) volunteers ranging from 19 to over 51 years and males (10) over 51 years was divided in two groups:
  - The experimental group received 1 capsule containing Oximacro® (36 mg PAC-A) twice a day (morning and evening) for 7 days and the placebo group was given the same number of capsules with no PACs.
  - After 7 days of Oximacro® administration, a significant difference was found between placebo and Oximacro® groups for both females (Mann-Whitney = 875; P <0.001) and males (Mann-Whitney = 24; P =0.016).
Effectiveness of Oximacro®


Leukocyte esterase (LE) is a urine test for the presence of white blood cells and other abnormalities associated with infection. The combination of the LE test with the urinary nitrite test provides an excellent screen for establishing the presence of a UTI.
Effectiveness of *Escherichia coli* (UPEC) was the dominant pathogen (about 85%), followed by *Klebsiella pneumoniae* (about 7%), *Proteus mirabilis* (about 5%) and *Enterococcus faecalis*, *E. cloacae*, *Streptococcus bovis* and *Providencia stuartii* (for the remaining percentage).
Effectiveness of Oximacro®


Uroculture shows a complete recovery
Same effects were obtained with administration of Monuril®, trometamol salt of fosfomycin.
Dimers, trimers or polymeric PACs?

Dimeric and trimeric PACs-A from Cranberry are found in the plasma and prevent adherence of P-fimbriated *E. coli* isolates from the urinary tract to cellular surfaces containing α-Gal(1 4)β-Gal receptor sequences similar to those on Uroepithelial cells.

Polymeric PACs remain mostly in the gut where they bind to Type-P fimbriae without affecting strains proliferation and/or transmigration to bladder by type-1 pili.

However, polymeric PACs cannot reach the bladder epithelium; thus, they cannot reduce *E. coli* replication by formation of bladder intracellular bacterial communities (IBCs) where quiescent intracellular reservoirs (QIRs) form and reside in the underlying urothelium.
Take home message #4

The high content of dimeric and trimeric PACs-A of Oximacro® allows a deeper interaction with UPEC in the bladder urothelium.

Oximacro® used at 72 mg PAC-A is highly effective as a support for urinary tract health.

Our studies demonstrate that the use of dosages based on PAC-A rather than total PACs is strongly suggested.
Because of its recognized anti-adhesive activity against bacterial pathogens, Oximacro® may represent a natural source of new antiviral microbicides.
Antiviral activity of Oximacro® was tested in vitro on HSV-1 and HSV-2 replication. Pretreatment of Vero cells with Oximacro® 1 h before infection produced a significant concentration-dependent inhibition of both clinical isolates of HSV-1 and HSV-2.

Inhibition of Herpes Simplex Type 1 and Type 2 infections by Oximacro®, a cranberry extract with a high content of A-type Proanthocyanidins (PACs-A). Terlizzi et al., 2016 Antiviral Research, 132:154-164
Oximacro® prevents adsorption of HSV-1 and HSV-2 to target cells. Prechilled Vero cells were treated with various concentrations of Oximacro®, or heparin at 4°C for 30 min and then infection was carried out with precooled HSV-1 or HSV-2 at a MOI of 0.002 for 3 h at 4°C in the presence of compounds as indicated in the figure below. Oximacro® impairs the attachment of HSV in a concentration-dependent manner and to a similar degree as observed in the virus yield.
In order to evaluate whether the whole cranberry or the PAC-A exerted a biological activity on Herpes virus we analyzed the anti-HSV activity of the five Oximacro®-derived purified fractions.

We found that only identified fractions 3 and 4, corresponding to dimers and trimers of PAC-A are responsible for the inhibitory activity of the whole extract.
Interaction between the A-type PACs present in Oximacro® and the HSV envelope glycoproteins.

Oximacro® binds the ectodomain of HSV glycoproteins (gD and gB)* in a concentration- and time-dependent manner, thus inhibiting their functions in virus attachment and entry.

*Results obtained with Western Blot Analysis and by Confocal Laser Scanning Microscopy localization
Inhibition of Influenza-A and Influenza-B infections by Oximacro®, a cranberry extract with a high content of A-type Proanthocyanidins (PACs-A). Terlizzi et al., 2017 in press

**Antiviral activity of Oximacro®**

Oximacro® inhibits IV replication *in vitro*

Oximacro® has virucidal activity on Influenza viral particles

*In silico* modeling of PAC-A dimer binding to extravirionic side of IV-HA protein, involved and required in the first phases of IV replication cycle.

Proanthocyanidins A2 of Oximacro® are able to interact with HA protein of IV, determining a inhibition of its function.
Take home message #5

Oximacro® is a valid support for viral (Herpes and Influenza) suppression because of its unique high content of PACs-A
Alcohol-free fluid extract (36 mg PACs-A/ml/gr)
Take home message #6

Oximacro® is also available as a fluid extract with a calibrated amount of PAC-A
Company and product registration

New Dietary Ingredient Notification report number 918
Applications

- pills
- capsules
- tablets
- emulsions
- suspensions
- liquid preparations
Vitafoods Europe

The global nutraceutical event

9-11 May 2017 Palexpo, Geneva

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Thank you