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Cesano Maderno, 15 Ottobre  2014

# Evaluation of the anti-hair loss activity of an active ingredient named "PHYTOCOMPLEX HL" by Phototrichogram and digital images analysis

## Test report n° LM190820-14

METHOD:          **Ref. S32V**

CUSTOMER:       **NATURALIS Srl**
                         **Via Valassina 64 – 20853 Lissone  (MB) Italy**

PRODUCT:         **PHYTOCARE-HL**

                         **Ref. PROCHEMIA:  LM190820-14**

TEST START DATE:    **9/08/2014**

TEST END DATE:       **9/10/2014**

## ETHICAL AND QUALITY CRITERIA

This study was conducted in accordance with the methods of the company quality management system, according to the general principles of good laboratory practice (GLP) and good clinical practice (GCP) and according to the principles defined in the World Medical Association Declaration of Helsinki.

## AIM OF STUDY

Evaluate whether a standard hydroalcoholic lotion containing 5,0% Phytocare-HL is able to induce a stimulatory action at the level of the hair bulb with an increase in hair density per cm2 and an increase in the percentage of these in the anagen phase . The composition of the hydro-alcoholic lotion is shown below:

| n° | INGREDIENTS | % |
|----|-------------|---|
| 1 | Water | at 100% |
| 2 | Ethyl Alcohol 96% den. | 20,00 |
| 3 | PHYTOCOMPLEX HL | 5 |
| 4 | Potassium sorbate | 0,45 |
| 5 | Glycerin | 1,5 |
| 6 | Citric Acid | 0,1 |
| 7 | Ascorbic Acid | 0,15 |

# Efficacy test by Phototrichogram and digital images analysis

The phototrichogram is a non-invasive method which is based on sequential macro photographs of a selected scalp area. This method is based on the notion that anagen hairs grow at a rate of about 1 mm every 3 days, while in the same time catagen hairs will show only moderate elongation and telogen hairs will not grow at all. For the evaluation, 2 areas of about 1 cm2 each are shaved. This area is then photographed. After 3 days a second photograph is taken and the proportion of growing (anagen) hairs is evaluated. As in the classic trichogram, a significantly increased proportion of telogen hairs is indicative of acute telogen effluvium . On the contrary a reduced proportion of telogen hairs and an increased number of hair in anagen is indicative of a stimulation of hair grow.
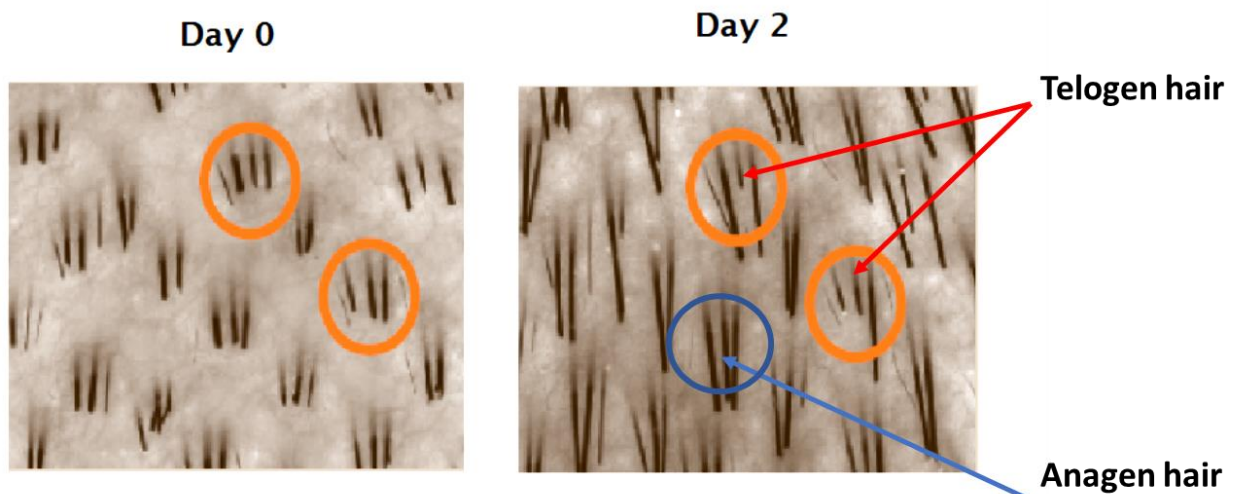


The anti-hair loss of Phytocomplex-HL was studied in a clinical study on 9 male volunteers suffering from hair thinning at the front and upper part of the scalp and with a baldness classified as type IV of Norwood scale
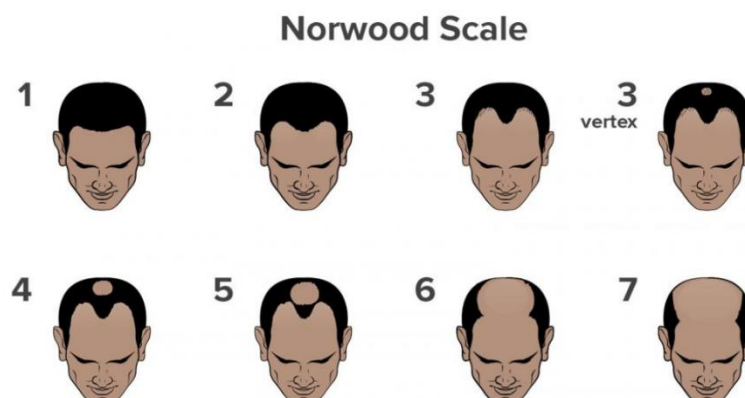
# SELECTION OF VOLUNTEERS

## Admission and recruitment criteria

At the beginning of the test, each subject read and countersigned the informed consent drawn up by the experimenters.

9 male subjects aged between 20 and 65 with baldness classified as type IV of Norwood scale

The selection was made according to the inclusion and exclusion criteria listed below.

## Inclusion criteria

- Race: Caucasian;
- Age: adults aged between 18 and 65;
- Gender: male;
- Good scalp hygiene
- No shampoo in the previous 3 hours on the test
- Absence of dermatological and scalp pathologies
- General state of health: absence of pathologies in progress or in the period immediately preceding the study;
- Phenotype (Fitzpatrick): type II-IV
- Baldness classified as type IV of Norwood scale
- Availability.

## Exclusion criteria

- Subjects being treated with any medication
- Subjects affected by scalp affections;
- Subjects with a history of intolerance to hair care products ;

## Drop – out

The following reasons were considered causes of study interruption:

- free choice of subject;
- medical reasons unrelated to the study (eg diseases etc.);
- motivations related to the study.
- No drop-out cases occurred during the study

![Prochemia Divisione Cosmetica]

| N° Vol. | Age | SIGN | WEEK 0 | | | | WEEK 8 | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | tot hair count | hair density/cm2 | Anagen hairs % | Telogen hairs % | tot hair count | hair density/cm2 | Anagen hairs % | Telogen hairs % |
| 1 | 29 | RM | 112 | 188 | 76,4 | 4,6 | 116 | 205 | 86,5 | 0,8 |
| 2 | 46 | RG | 211 | 235 | 69,5 | 5,9 | 231 | 249 | 82,8 | 1,1 |
| 3 | 39 | SS | 223 | 244 | 79,2 | 4,6 | 234 | 267 | 93,3 | 0,4 |
| 4 | 28 | MS | 159 | 174 | 75,9 | 4,8 | 171 | 195 | 89,7 | 0,5 |
| 5 | 51 | EP | 103 | 165 | 78,8 | 4,7 | 104 | 184 | 87,8 | 0,6 |
| 6 | 44 | DV | 123 | 191 | 80,2 | 3,7 | 127 | 209 | 89,9 | 0,5 |
| 7 | 32 | AZ | 175 | 209 | 82,4 | 3,6 | 181 | 227 | 91,8 | 0,4 |
| 8 | 40 | GC | 134 | 202 | 84,5 | 3,6 | 145 | 219 | 94,6 | 0,3 |
| 9 | 35 | DM | 101 | 189 | 79,3 | 3,9 | 113 | 208 | 87,5 | 0,8 |

| | WEEK 0 | | | | WEEK 8 | | | |
|---|---|---|---|---|---|---|---|---|
| | tot hair count | hair density/cm2 | Anagen hairs % | Telogen hairs % | tot hair count | hair density/cm2 | Anagen hairs % | Telogen hairs % |
| Average | 149,0 | 199,7 | 78,5 | 4,4 | 158,0 | 218,1 | 89,3 | 0,6 |
| St. dev | 44,44 | 25,42 | 4,17 | 0,74 | 48,07 | 25,39 | 3,54 | 0,25 |

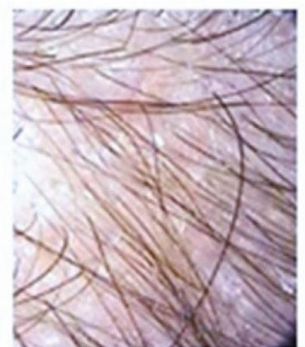| Parameter | Δ (%) | P (Anova test) |
|---|---|---|
| hair density/cm2 | +9,21 | P=0.144 |
| Anagen hairs % | +13,90 | P< 0.005 |
| Telogen hairs % | -86,40 | P< 0.005 |



T0          T=8 weeks          T0          T=8 weeks

## Statistic analysis

All data were subjected to analysis of variance (ANOVA) for repeated measurements followed by Tukey's post-test. The following levels of statistical significance were considered: n.s. (not significant): $p > 0.05$; * (significant): $p < 0.05$; ** (on average significant): $p < 0.01$; *** (highly significant): $p < 0.001$. The experimental error was expressed as a standard deviation.

## Precision of the method

The measurement uncertainty of the method is calculated on the basis of results obtained during internal repeatability tests, performed by the same operator and with the same methods on previously tested samples taking into account the individual biological variability factor.

On the basis of the data collected with the latest update, a method uncertainty value of ± 3% was calculated.
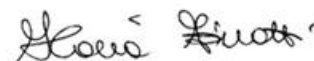
**CONCLUSIONS**

All subjects selected for the test completed the test. None of the volunteers showed burning or irritation of the scalp after 8 week of treatment with an hydro-alcoholic lotion containing 5.0% Phytocomplex HL.

Particularly the hydro-alcoholic lotion containing the Phytocare-HL at 5% object of this study, has shown to be significantly effective in increasing of both the hair density evaluated as n° hair/cm2 (+ 9,21%;  p=0.144) and the percentage of anagen hair (+13,90% ; p<0,005) and, in parallel, a reduction of % of hair in telogen (- 86,40%; p<0,005).

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