Official Title of the study:

DOUBLE BLINDED RANDOMIZED PLACEBO CONTROLLED CLINICAL STUDY FOR THE EVALUATION OF THE EFFICACY OF ONE ACTIVE INGREDIENT INCORPORATED INTO A FOOD SUPPLEMENT

NCT number (not yet assigned)

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Study Protocol, Statistical Analysis Plan and Results of the Study

CIP n	P016C23_v02	**Study beginning date:**	21/06/2023	**Study conclusion/ suspension date:**	08/03/2024
Title	Double blinded randomized placebo controlled clinical study for the evaluation of the efficacy of one active ingredient incorporated into a food supplement				

Study Protocol

The primary aim of this *in vivo* clinical study is to evaluate the depigmenting effect of an active ingredient incorporated into a food supplement through the evaluation of the melanin variation parameter, assessed with the equipment Antera 3D (Miravex, Ireland), after 28 consecutive days of food supplement's once-daily intake, in comparison with the baseline and a placebo.

The secondary objective of this study is to evaluate *in vivo* the depigmenting effect of the same active ingredient incorporated into a food supplement through the evaluation of the melanin variation parameter, assessed with the equipment Antera 3D (Miravex, Ireland), after 56 and 84 consecutive days of food supplement's once-daily intake.

Other goals of this clinical study are to evaluate *in vivo* the effect of the same active ingredient incorporated into a food supplement, after 28, 56 and 84 consecutive days of a once-daily intake, in comparison with the baseline and a placebo (1) on skin whitening/brightening through the evaluation of the L* parameter, assessed with the equipment Antera 3D (Miravex, Ireland), (2) on the skin colour homogeneity through the measurement of the ITA° (Individual Typology Angle) parameter, assessed with the equipment Colorimeter® CL400, (3) on the skin tone evenness through the measurement of the standard deviation of the skin colour, assessed with the equipment Colorimeter® CL400, (4) on the skin density of the dermis, measured with the equipment Episcan 20 MHz (Longport Inc, United States), (5) on skin hydration assessed through capacitance measurements with the equipment Corneometer® CM825, (6) on the skin firmness, skin overall elasticity and skin total deformation measured with the equipment Cutometer® SEM 575 (Courage+Khazaka electronic GmbH, Germany), and (7) on skin radiance through clinical evaluation performed by an expert.

It is also objective of this clinical study to evaluate (8) subjects' tolerance, acceptability, perception of efficacy and future use/purchase intention towards the food supplements by filling out a subjective evaluation questionnaire after 28, 56 and 84 consecutive days of food supplement's once-daily intake.

To perform this study, 110 female healthy subjects, with ages between 25 and 60 years old, presenting mild to moderate fine lines and wrinkles, mild to moderate skin spots and phototype III to V, according to the Fitzpatrick phototyping scale, are enrolled. The subjects are randomly divided in two groups, the test group, taking the active ingredient incorporated into a food supplement and the placebo group, taking the food supplement without the active ingredient.

A 2 weeks washout period is performed, if subject has taken any kind of supplements (Vitamins, antioxidants, nutraceuticals) before the beginning of the study. In this period subjects cannot use any kind of supplements.

Each subject shall take 1 capsule of the food supplement per day, with lunch, for 84 consecutive days.

Statistical Analysis Plan

A descriptive statistical analysis of the skin depigmenting, skin whitening/brightening, skin density, skin hydration, skin firmness, skin overall elasticity, skin total deformation and skin radiance results are performed for each group at each evaluation time-point, including the calculation of mean, standard deviation, and graphic representations.

Normality tests (Shapiro-Wilk test) are performed in order to assure normal distribution of the objective data obtained.

If normal distribution of the data is verified:

- paired-sample t-tests are applied to compare the results obtained for each parameter before (t0) versus after 28 (t1), 56 (t2) and 84 (t3) days of each food supplement's intake;
- independent t-tests are applied to compare the differences to baseline obtained with the investigational product versus the ones obtained with the comparator product at each time-point of evaluation (t1, t2 and t3).

For non-normal distributions non-parametric tests are used:

- Wilcoxon-tests are applied to compare the results obtained for each parameter before (t0) versus after 28 (t1), 56 (t2) and 84 (t3) days of each food supplement's intake;
- Mann-Whitney tests are applied to compare the differences to baseline obtained with the investigational product versus the ones obtained with the comparator product at each time-point of evaluation (t1, t2 and t3).

The significance value is established at 0.05 and at a power of 0.95.

Descriptive statistics of the subjective evaluation is performed at each time-point of evaluation with the calculation of number and percentage of subjects responding to each answer option and the results presented in graphic representations.

Results

The most relevant results that can be concluded from this study are:

	Investigational product	**Comparator product placebo**
Skin depigmenting	The product showed a skin depigmenting effect after 84 days of products' intake, in comparison to the baseline. After 28 and 56 days of products' intake, no improvement was observed.	No improvement in the skin depigmenting was observed after 28, 56 and 84 days of products' intake, in comparison to the baseline.
	The investigational product has no effect on the skin depigmenting, in comparison to the placebo.	
	– 28.00%, 52.08% and 61.22% of the subjects agreed to strongly agreed that their skin is with less hyperpigmentation spots 28, 56 and 84 days after the daily intake of the investigational product, respectively. – 26.53%, 55.10% and 53.07% of the subjects agreed to strongly agreed that their skin is with less hyperpigmentation spots 28, 56 and 84 days after the daily intake of the placebo, respectively.	
Skin whitening/ brightening	The product demonstrated a skin whitening/ lightening effect after 56 and 84 days of products' intake, in comparison to the baseline. After 28 days of products' intake, no improvement was observed.	The product demonstrated a skin whitening/ lightening effect after 56 and 84 days of products' intake. After 28 days of products' intake, no improvement was observed.
	The investigational product has a skin whitening/brightening effect, in comparison to the placebo, after 84 days of products' intake.	
	– 32.00%, 60.42% and 55.10% of the subjects agreed to strongly agreed that their skin had a luminous effect 28, 56 and 84 days after the daily intake of the investigational product, respectively. – 32.65%, 51.02% and 57.14% of the subjects agreed to strongly agreed that their skin had a luminous effect 28, 56 and 84 days after the daily intake of the placebo, respectively.	
Skin colour	The product revealed a skin tone lightening effect after 28, 56 and 84 consecutive days of products' intake, in comparison to the baseline.	The product revealed a skin tone lightening effect after 56 days of products' intake, in comparison to the baseline. After 28 and 84 days of products' intake, no improvement was observed.
	The investigational product has an almost statistically significant improvement in the skin tone lightening, in comparison to the placebo, after 84 days of products' intake, supporting the skin whitening/lightening effect observed previously.	
Skin tone evenness	No improvement in the skin tone evenness was observed after 28, 56 and 84 days of products' intake.	No improvement in the skin tone evenness was observed after 28, 56 and 84 days of products' intake.
	The investigational product has no effect on the skin tone evenness, in comparison to the placebo.	
	– 32.00%, 60.42% and 67.34% of the subjects agreed to strongly agreed that their skin is with more homogeneous skin tone 28, 56 and 84 days after the daily intake of the investigational product, respectively. – 32.65%, 59.18% and 59.18% of the subjects agreed to strongly agreed that their skin is with more homogeneous skin tone 28, 56 and 84 days after the daily intake of the placebo, respectively.	
Skin density	No improvement in the density of the dermis was observed after 28, 56 and 84 days of products'	No improvement in the density of the dermis was observed after 28, 56 and 84 days of products'

	intake, in comparison to the baseline. Instead, a significant decrease was observed.	intake, in comparison to the baseline. Instead, a significant decrease was observed.
	The investigational product has no effect on the density of the dermis, in comparison to the placebo.	
	– 14.00%, 33.33% and 32.65% of the subjects agreed to strongly agreed that their skin is denser 28, 56 and 84 days after the daily intake of the investigational product, respectively. – 22.45%, 36.73% and 38.77% of the subjects agreed to strongly agreed that their skin is denser 28, 56 and 84 days after the daily intake of the placebo, respectively.	
Skin hydration	No improvement in the skin hydration was observed after 28, 56 and 84 days of products' intake, in comparison to the baseline.	No improvement in the skin hydration was observed after 28, 56 and 84 days of products' intake, in comparison to the baseline. Instead, a decrease was observed after 84 days of products' intake.
	The investigational product has no effect on the skin hydration, in comparison to the placebo.	
	– 50.00%, 60.42% and 61.23% and 36.73% of the subjects agreed to strongly agreed that their skin is more hydrated 28, 56 and 84 days after the daily intake of the investigational product, respectively. – 36.73%, 51.02% and 69.39% of the subjects agreed to strongly agreed that their skin is more hydrated 28, 56 and 84 days after the daily intake of the Placebo, respectively.	
Skin total deformation	No improvement in skin total deformation was observed after 28, 56 and 84 days of products' intake, in comparison to the baseline. Instead, an increase was observed after 84 days of products' intake.	No improvement in skin total deformation was observed after 28, 56 and 84 days of products' intake, in comparison to the baseline. Instead, an increase was observed after 84 days of products' intake.
	The investigational product has no effect on the skin total deformation, in comparison to the placebo.	
Skin overall elasticity	No improvement in skin overall elasticity was observed after 28, 56 and 84 days of products' intake, in comparison to the baseline.	The product showed to increase the skin overall elasticity after 84 days of products' intake, in comparison to the baseline. After 28 and 56 consecutive days of products' intake, no improvement was observed.
	The investigational product has no effect on skin overall elasticity, in comparison to the placebo.	
Skin firmness	No improvement in skin firmness was observed after 28, 56 and 84 days of products' intake, in comparison to the baseline.	The product showed to increase the skin firmness after 84 days of products' intake, in comparison to the baseline. After 28 and 56 consecutive days of products' intake, no improvement was observed.
	The investigational product has no effect on skin firmness, in comparison to the placebo.).	
	– 18.00%, 37.50% and 42.86% of the subjects agreed to strongly agreed that their skin is firmer 28, 56 and 84 days after the daily intake of the investigational product, respectively. – 28.57%, 40.81% and 42.85% of the subjects agreed to strongly agreed that their skin is firmer 28, 56 and 84 days after the daily intake of the placebo, respectively.	
Skin radiance	The product demonstrated a skin radiance effect after 28, 56 and 84 days of products' intake, in comparison to the baseline.	The product demonstrated a skin radiance effect after 28, 56 and 84 days of products' intake, in comparison to the baseline.

	The investigational product has no effect on the radiance condition of the subject's skin, in comparison to the placebo.

Additionally, the following results were obtained from the subjects' subjective evaluation:

- In a general way, 73.47% and 63.26% of the subjects evaluated the investigational product and placebo as good to very good, respectively, after 84 days of daily intake;
- 92% and 91.84% of the subjects agreed to strongly agreed that it was practical to include the investigational product and placebo in their daily routine, respectively;
- 75.51% and 67.35% of the subjects agreed to strongly agreed that their skin is with better appearance 84 days after the daily intake of the investigational product and placebo, respectively;
- 89.80% and 75.51% of the subjects agreed to strongly agreed that they would like to continue taking the supplement and placebo, respectively;
- 89.80% and 77.56% of the subjects agreed to strongly agreed that they would recommend the supplement and placebo to a friend, respectively.

The product was in general well tolerated by the subjects with no subjects reporting any serious adverse events. However, five subjects reported mild discomforts, which were resolved during the study.

The benefits of the investigation superposed the risks, as the efficacy degree of the products was assessed and no adverse events were observed.

The principal hypothesis of this clinical study is that the melanin variation parameters will decrease at least 7.02% after 28 days of the intake of the investigational product, compared to the placebo. This hypothesis was not verified with this clinical investigation.

In conclusion, the investigational product showed to have a skin whitening/brightening effect, in comparison to the placebo, after 84 days of products' intake, while no effects were observed for the skin depigmenting, skin tone evenness, skin density, skin hydration, skin total deformation, skin overall elasticity, skin firmness and skin radiance, in comparison to the placebo, after 28, 56 and 84 consecutive days of products' intake.