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Original Article

SPLIT-FACE PLACEBO CONTROLLED EVALUATION OF THE *IN VIVO* ANTI-AGEING EFFICACY OF LINEMINUS™ CREAM (*POLYGONUM MINUS* EXTRACT) IN HEALTHY ASIAN SKIN TYPE FEMALE SUBJECTS

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ABSTRACT

Objective: Although changes in pigmentation distribution are known to be a major characteristic of cutaneous ageing in Asian skin, wrinkling was also shown to be a main feature of skin ageing in Asians. The role of reactive oxygen species and the decrease in anti-oxidant enzymes activity with age was recently confirmed for both intrinsic and extrinsic ageing, making oxidative stress of primary importance in the overall ageing process.

Polygonum minus is found in local Malaysian herbs known as "kesum". Previous studies have demonstrated the anti-oxidant properties of kesum as a food ingredient or medicinal plant. However, little is known about the *in vivo* anti-oxidant and resulting anti-wrinkle efficacy of *Polygonum minus* in a cosmetic product. Lineminus™ is an extract developed from *Polygonum minus* leaves (with 3 pending patents: Malaysia PI2012005685, Malaysia PI20125248, and Malaysia PI2012003882/ PCT/MY2013/000033).

The aim of this study was to evaluate the efficacy and safety of Lineminus™ cream versus its placebo in healthy Asian females (split-face).

Methods: Methods used were shadow casting analysis of crow's feet silicone replicas, self-assessment and dermatological evaluation. Twenty three healthy Asian females, aged between 48-60 years old, having wrinkles on the crow's feet area were enrolled.

Results: Results showed that application of Lineminus™ cream induced a significant decrease in "number of wrinkles" (-17.6% and -20.1%, $p<0.05$), "total length" (-15.9% and -25.7%, $p<0.05$) after 1 and 2 months and "mean length" after 2 months (-8.6%, $p<0.05$). When variations from the skin treated with Lineminus cream were corrected from the ones of the skin treated with Placebo Cream, there was a statistically significant difference in "number of wrinkles" after 1 month (-12.4%, $p<0.05$) and "mean length" of wrinkles after 2 months (-7.3%, $p<0.05$). Subjects significantly agreed to most questions asked to them about Lineminus™ cream's efficacy after 1 and 2 months. Finally, the tolerance of both creams was found to be very good.

Conclusion: Based on these results it is possible to conclude that Lineminus™ Cream is safe and has an anti-wrinkle effect and that Lineminus™ *(Polygonum minus* extract) has beneficial cosmetic anti-wrinkle properties. These results also show that in addition to *Polygonum minus*'s well-known anti-oxidant efficacy, the plant extract also demonstrates a significant clinical anti-wrinkle effect with clear reduction of crow's feet wrinkles.

Keywords: Cream, skin, lineminus, wrinkles, placebo, Polygonum minus, kesum, replicas, testing.

INTRODUCTION

Although changes in pigmentation distribution (mottled pigmentation, pigmentation spots etc.) are known to be a major characteristic of cutaneous ageing in Asian skin [1], wrinkling was also shown to be a main feature of skin ageing in Asians [1, 2] as well as Caucasians [3]. Skin ageing is the result of two different and cumulated processes: intrinsic and extrinsic ageing (also known as photo-ageing) [3-6]. While intrinsic ageing is natural and mainly due to the passage of time (influence of genetic factors, oxidative stress, cellular senescence etc.) and its consequences, photo-ageing is mainly linked with the detrimental effects of solar exposure on the skin (prominent in Asian countries compared to Western countries due to their geographical location), although pollution, diet and smoking are also contributing factors [4-7]. The role of reactive oxygen species (ROS) and the decrease in anti-oxidant enzymes activity with age has been described previously [4], but the role of ROS has recently been confirmed for both processes of ageing, not only intrinsic ageing [4], making oxidative stress of primary importance in the overall ageing process [4]. This partly confirms the previous conclusions that extrinsic ageing is superimposed on intrinsic ageing [4] and that it also accelerates many key aspects of intrinsic ageing. The role of anti-oxidants is to neutralize free radicals by supplying another electron, delivering an electron pair to an oxygen molecule and stabilizing it in the process [3]. The use of anti-oxidants to hinder the pathways of collagen breakdown is thought to inhibit photo-ageing by preventing collagenase production and its detrimental influence on collagen [3]. It appears therefore that anti-oxidant properties provided to the skin could act as anti-ageing and improve its clinical features such as wrinkles.

Polygonum minus (also known as *Persicaria minor*) is found in local Malaysian herbs and it is popular with its local name "kesum" [8-11]. Previous studies have demonstrated the anti-oxidant properties of kesum as a food ingredient or medicinal plant [8-11], with sometimes comparing it to other herbs known for their anti-oxidant properties [8, 10]. Similar studies were also performed on a wide range of other plants from Malaysia, India etc. to assess their anti-oxidant properties [12, 13]. However, little is known about the *in vivo* anti-oxidant efficacy and resulting anti-wrinkle efficacy of *Polygonum minus* contained in a cosmetic product following topical application on the face. Lineminus™ is an extract developed from *Polygonum minus* leaves (with 3 pending patents: Malaysia PI2012005685, Malaysia PI20125248, and Malaysia PI2012003882 / PCT/MY2013/000033). Lineminus™ cream contains Lineminus™

as its main active ingredient with other ingredients to complete the formulation of the cream.

The present study was performed in order to evaluate the efficacy and safety of Lineminus cream as an anti-wrinkle cream in a panel of healthy Asian females.

MATERIALS AND METHODS

Material and product preparation

The extract was obtained from a commercial batch of Lineminus™. The standardized aqueous extract was prepared by a water extraction of *Polygonum minus* leaves using the high pressure water extraction technology (Pending Patent Malaysia PI2012003882 / PCT/MY2013/000033) comprising the steps of a) subjecting the dried leaves to hot water extraction by percolation; b) filtering; c) followed by concentration and by condensation; d) drying without any carrier; and e) size reduction, obtaining the dry extract powder. The dry extract powder was standardized for content of Quercetin-3-Glucuronide and Quercitrin.

The Lineminus™ Cream gel was produced under GMP manufacturing facility with ingredients such as: Isohexadecane, Polyacrylamide & C13-14 Isoparaffin & Laureth-7, Butylene Glycol, Cyclopentasiloxane & Cyclotetrasiloxane & Dimethiconol, Cyclotetrasiloxane & Cyclopentasiloxane, Lineminus Powder Extract.

Aim of the study

The objective of this study was to evaluate the *in vivo* anti-wrinkle efficacy and safety of Lineminus cream versus its placebo cream, within the same group of healthy Asian skin type female subjects (split-face design). Objective measurements of the skin, dermatological evaluation as well as complementary subjective evaluation by the subjects were used to demonstrate the efficacy and safety of Lineminus cream and to compare it to its placebo cream.

Study design

This was a single centric study where all subjects were aware of the expected effects of the tested products. However, they were not informed which of the two products was the placebo. This was a split-face comparative study (Lineminus cream versus placebo) where each subject was used as her own control. The study was performed in SPINCONTROL ASIA Co., Ltd., Bangkok, Thailand from November 21, 2012 until January 23, 2013. This non-interventional study was performed according to the most recent recommendations given by the World Medical Association (Helsinki Statement 1964, amended in Seoul, Korea, 2008).

Inclusion criteria

Twenty three healthy Asian skin type female subjects, aged between 48 and 60 years old, having wrinkles on both crow's feet areas with a grade ≥ 3 according to the "Skin Ageing Atlas" (corresponding to moderate to severe wrinkles) [14], without any skin hypersensitivity or known allergy to cosmetic products, were enrolled in this study. All the subjects gave their informed written consent before beginning the study.

Exclusion criteria

Subjects were not allowed to take part in the study if they were pregnant or breastfeeding a child at the time of enrollment or if they had stopped breastfeeding within the past 3 months. Subjects with existing cutaneous or general disease and subjects following any chronic or intermittent medicinal treatment were not included in the study. Subjects having made injections of anti-wrinkle products or having undergone techniques with aesthetic aim in the past year as well as having applied products with an anti-wrinkle action (Retinoic acid, retinol, retinaldehyde, A.H.A etc.) in the 2 weeks preceding the start of the study were also not included.

Study restrictions

At the baseline visit, subjects were asked to respect a list of restrictions throughout the 2-month study in order to confirm their inclusion. These restrictions were related to the non-use of other cosmetic products or aesthetic procedures as well as medication that could interfere with the study and impact on the results. This also included sun and UV light exposure which should not have been intentional and prolonged.

Study procedures

Wrinkle evaluation was performed using skin rubber replicas (Silflo, Flexico Developments Ltd., UK) of the crow's feet area followed by shadow casting analysis (Fig. 1) with the Quantirides® system (Fig. 2) and associated software (Courage & Khazaka, Germany). The replica, which represents a negative imprint of the skin, is illuminated uniformly with a defined white light mounted at a specific angle (35°). The shadows visible on the replica created by the oblique light are captured with a high resolution camera, which is mounted vertically to the replica. The principle of the shadow casting analysis comes from the geometrical relationship between the depth, width and length of one wrinkle and the surface of the shadow it generates when illuminated by an oblique lighting.

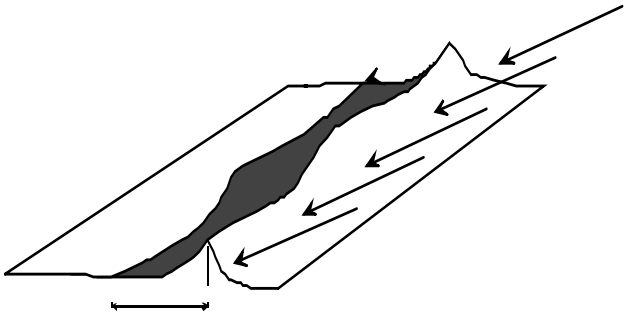

Fig. 1: Principle of the shadow casting method

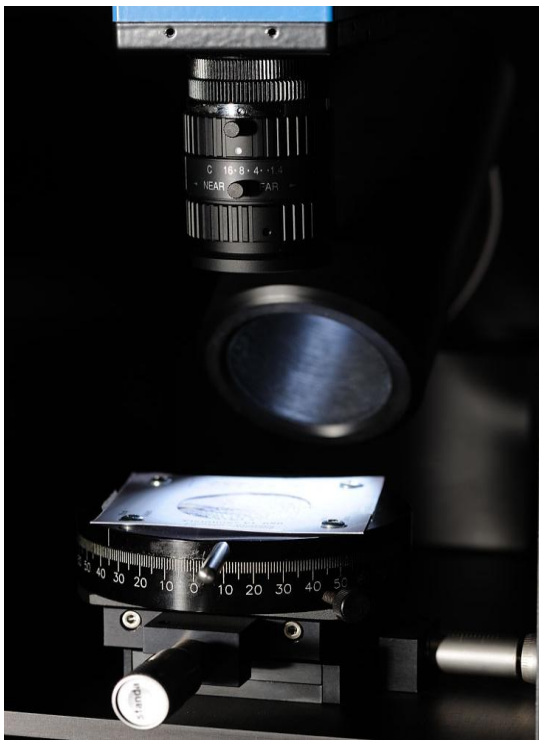

Fig. 2: Quantirides® system

The crow's feet area was previously found to be the major site of wrinkles in Asian skin [15] and skin replicas analysis, which is commonly used in anti-wrinkle studies, was previously taken as the method to study changes in skin micro-relief and wrinkles with increasing age [16]. It seemed therefore relevant to assess wrinkles of the crow's feet area for this study. Parameters such as number, length and depth of wrinkles were analyzed. This method was associated with subjective evaluation from the subjects who were

asked to answer self-assessment questionnaires following products application. A set of 13 questions was asked to subjects after 1 and 2 months of products application for each treated half-face. Seven questions were related to the evaluation of product efficacy (anti-wrinkle, anti-ageing, improvement of eye contour appearance) while the remaining six questions focused on product cosmetic acceptability (ease to spread, appreciation of the fragrance etc.). For each question, subjects had the option to choose one of the four possible answers: totally agree, rather agree, rather disagree and totally disagree.

Finally, dermatological evaluation of skin tolerance was performed by a dermatologist in order to ensure that there was no change in the subjects' skin quality following application of the test products. This evaluation was based on visual evaluation (objective attributes such as erythema, edema and scaling) as well as an interview of the subjects to report functional signs (subjective attributes such as stinging, itching, sensation of warmth etc.). When a sign was observed by the dermatologist or reported by the subject, it was graded by the dermatologist using a 5-point scoring system, from 0 = absent/no evident reaction to 4 = extreme reaction. An overall tolerance conclusion (for each subject as well as for the whole panel) was made by the dermatologist at the end of the study, based on the evaluations made throughout the study as well as spontaneous reporting of undesirable events by the subjects. This overall conclusion by the dermatologist was made using a 4-point scoring system, from 1 = poor tolerance to 4 = very good tolerance. All measurements and evaluations were performed at baseline (except self-assessment), after 1 and after 2 months of products application. Ambient conditions were monitored for each visit (acceptable range of 20-24°C and 40-60% relative humidity) to ensure that there would be no effect of these factors on measurements and evaluations.

Primary and secondary outcome measurements and evaluation

The primary techniques used were skin rubber replicas followed by analysis with shadow casting method and self-assessment using questionnaires. The second outcome evaluation was dermatological control of skin tolerance which was based on objective (evaluation by the dermatologist) and subjective evaluation (feedback from subjects).

Adverse events

The reporting of any adverse event by the subjects themselves, the dermatologist or the technicians taking part in the study was done using an adverse event report form. The type of sign, its intensity, duration, evolution and decision made by the investigator regarding the subject's participation in the study were collected and fed back to the study sponsor. Finally, the investigator defined the relationship between the observed sign and the tested product (unlikely, possible, certain or un-assessable). Subject follow-up was also implemented depending on the type and intensity of sign observed as well as whether the subject had to be withdrawn from the study following appearance of the undesirable event.

Statistical analysis

Data from subjects who successfully completed the whole study with attendance to all three visits was exploited for the analysis. Data from any subject withdrawn from the study was removed from the final results, except if this withdrawal was linked with an adverse event, in which case data from this subject was kept into results from dermatological evaluation.

Comparison in time for each product was done using the Shapiro-Wilk test (significance threshold: 1%) to check the normality of the distributions, followed by the Student t-test (normality of distributions checked) or by the Wilcoxon test (normality of the distributions rejected) to analyze the evolution of the measured parameters during the study for each product. The significance threshold of both tests was 5%.

Comparison between the two products at T0 and at Tn-T0 was done using Shapiro-Wilk test (threshold: 1%). Statistical comparison of the two products at T0 and on the differences (Tn-T0), for each of the measured parameters, was performed with the Student test (normality of distributions checked) or the Wilcoxon test (normality of the distributions rejected). The significance threshold was set to 5%.

The analysis of self-assessment questionnaires involved establishing frequency tables that took into account the number of responses and calculated the frequency of the different possible answers (given as a percentage) to each qualitative question. For each question, results were shown in tabular form (number of individuals and frequency). Answers to self-assessment questionnaires were combined into two percentages: a percentage representing favorable opinion (combining "Totally agree" and "Rather agree" answers) and a percentage representing unfavorable opinion (combining "Totally disagree" and "Rather disagree"). The statistical difference in frequencies (percentages) between favorable and unfavorable opinions was analyzed using the Chi-squared test (significance level set to 5%).

Results from dermatological evaluation were compiled into grades, from 1 = poor tolerance to 4 = very good tolerance. This data was therefore not analyzed using any statistical analysis.

RESULTS

Study population

One subject was absent and another subject withdrew herself from the study at the last visit (T+2 months) because of personal reasons, therefore these were not related to the tested products. Crow's feet replica from this latter subject could not be exploited for the analysis with shadow casting method. Therefore twenty one subjects successfully completed the study. The recruited panel at baseline was aged between 48 and 60 years old (mean age: 54.0 years old).

Products safety evaluation

None of the subjects from this study, dermatologist or technicians reported any adverse event during the 2-month study, for both treated half-faces.

No functional or clinical sign was observed for none of the subjects on each half-face, at none of the study kinetics namely baseline, after 1 and after 2 months of products application (Table 1).

Therefore the dermatologist who made the evaluations concluded that the skin tolerance of subjects participating in this study was very good for LINEMINUS CREAM as well as for the PLACEBO CREAM after twice daily application on one half-face during 2 months (Table 2).

Table 1: Mean results (±SD) of dermatological scoring (functional and clinical signs) on the subjects' face for each test product at baseline, after 1 and 2 months of products usage (4-level system with 0 = Absent/ No evidence reaction, 1 = Slight, 2 = Moderate, 3 = Severe and 4 = Extreme reaction)

	Functional signs				**Clinical signs**			
	LINEMINUS CREAM		**PLACEBO CREAM**		**LINEMINUS CREAM**		**PLACEBO CREAM**	
Kinetics	**Mean**	*S.D.*	**Mean**	*S.D.*	**Mean**	*S.D.*	**Mean**	*S.D.*
Baseline	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*
T+1 month	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*
T+2 months	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*
Variation after 1	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*

month								
Variation after 2 months	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*	**0.0**	*0.0*

Table 2: Mean results (±SD) of skin tolerance determined after 2 months of products usage for each test product, based on the individual scores of skin tolerance given by the dermatologist (1 = Poor tolerance, 2 = Medium tolerance, 3 = Good tolerance and 4 = Very good tolerance)

Product	Overall skin tolerance after 2 months (**Mean ±** *S.D.)*
LINEMINUS CREAM	4.0 ± *0.0*
PLACEBO CREAM	4.0 ± *0.0*

Products efficacy evaluation

Results from replicas analysis with the shadow casting method showed that application of LINEMINUS CREAM (Table 3) induced a decrease in all measured parameters after 1 and 2 months of application when compared to baseline values, except for the mean depth of wrinkles after 1 month of treatment. These decreases reached statistical significance for the parameters "number of wrinkles" after 1 month (-17.6%, *p<0.05*) and 2 months (-20.1%, *p<0.05*), "total length" after 1 month (-15.9%, *p<0.05*) and 2 months (-25.7%, *p<0.05*) and "mean length" after 2 months only (-8.6%, *p<0.05*). These results show that the anti-wrinkle efficacy of LINEMINUS CREAM progressively increased between 1 and 2 months of treatment, as seen by higher percentages of reduction of wrinkle number and length. It should be noted that the slight increase in the "mean depth" parameter is believed to be due to the fact that the product acted primarily on the fine lines. In this case, at the end of the 2-month treatment only bigger wrinkles remained, and this contributed more to the mean parameters such as mean depth.

Results from replicas analysis with the shadow casting method showed that application of the PLACEBO CREAM (Table 4) induced a reduction in all assessed parameters after 1 and 2 months of product application, however, the only variation which reached statistical significance was "number of wrinkles" after 2 months only (-15.8%, *p<0.05*). The slight decrease in all assessed parameters is believed to be linked with the moisturizing efficacy of the placebo formula which helped improving fine lines through an effect on the skin micro-relief.

Table 3: Mean results of parameters from silicone replicas analysis with shadow casting for the whole panel at baseline, after 1 and 2 months of LINEMINUS CREAM usage and variations (% and p value) compared to baseline after 1 and 2 months

	LINEMINUS CREAM				
Kinetic	**Number of wrinkles**	**Total wrinkle area (mm^2)**	**Total length (mm)**	**Mean length (mm)**	**Mean depth (µm)**
Baseline	88.0	21.1	87.2	1.0	113.2
T+1 month	72.6	17.2	73.3	1.0	119.4
T+2 months	70.4	16.9	64.7	0.9	112.8
Variation after 1 month	-17.6% (*p<0.01)*	-18.1% (*ns)*	-15.9% (*p<0.05*)	-1.6% (*ns*)	+5.5% (*ns*)
Variation after 2 months	-20.1% (*p<0.05)*	-19.6% (*ns)*	-25.7% (*p<0.01*)	-8.6% (*p<0.01*)	-0.3% (*ns*)

Table 4: Mean results of parameters from silicone replicas analysis with shadow casting for the whole panel at baseline, after 1 and 2 months of PLACEBO CREAM usage and variations (% and p value) compared to baseline after 1 and 2 months

	PLACEBO CREAM				
Kinetic	**Number of wrinkles**	**Total wrinkle area (mm^2)**	**Total length (mm)**	**Mean length (mm)**	**Mean depth (µm)**
Baseline	80.6	19.3	78.6	0.9	114.5
T+1 month	76.5	16.3	75.9	0.9	109.3
T+2 months	67.9	15.7	67.1	0.9	114.0
Variation after 1 month	-5.2% (*ns)*	-15.5% (*ns)*	-3.4% (*ns)*	+0.8% (*ns*)	-4.6% (*ns)*
Variation after 2 months	-15.8% (*p<0.05*)	-18.8% (*ns)*	-14.6% (*ns)*	-1.4% (*ns)*	-0.5% (*ns)*

Table 5: Mean variations (expressed in percentage and with p value) of parameters from silicone replicas for LINEMINUS CREAM corrected from the variations observed on the skin treated with PLACEBO CREAM for 1 and 2 months

Variations	**Number of wrinkles**	**Total wrinkle area (mm^2)**	**Total length (mm)**	**Mean length (mm)**	**Mean depth (µm)**
After 1 month	-12.4% (*p<0.05*)	-2.6% (*ns*)	-12.4% (*ns*)	-2.4% (*ns*)	+10.0% (*p<0.05*)
After 2 months	-4.3% (*ns*)	-0.7% (*ns*)	-11.1% (*ns*)	-7.3% (*p<0.05*)	+0.1% (*ns*)

When variations of wrinkle parameters from shadow casting method on the skin treated with LINEMINUS CREAM were corrected from the variations observed on the skin treated with PLACEBO CREAM (Table 5), results show that there was a decrease in all measured parameters after 1 and 2 months of product usage, except for the mean depth of wrinkles. As mentioned previously, the increase in mean depth is believed to be linked with the primary efficacy of the LINEMINUS CREAM on fine lines before deeper wrinkles. The decrease in two measured parameters reached statistical significance, namely "number of wrinkles" after 1 month (-12.4%, *p<0.05*) and "mean length" of wrinkles after 2 months of product application (-7.3%, *p<0.05*). Since these results represent

the efficacy of LINEMINUS CREAM corrected from the efficacy of PLACEBO CREAM, they evidence the anti-wrinkle effect of *Polygonum minus* (main ingredient of LINEMINUS CREAM) on crow's feet fine lines and wrinkles.

Figure 3 shows photographs of skin replicas of the crow's feet area taken before and after application (1 and 2 months) of LINEMINUS CREAM for one subject who displayed improvements of crow's feet wrinkles similar to the average improvements (A, B and C). Parts highlighted in green on the bottom photographs (D, E and F) represent the shadow created by the Quantirides equipment on the replicas.

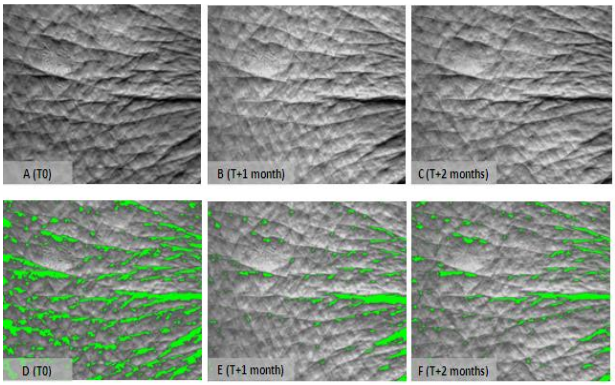

Fig. 3: Photos of skin replicas before (A, top left photograph) and after treatment with LINEMINUS CREAM for 1 (B, top middle photograph) and 2 months(C, top right photograph) (D, E and F represent the photographs with analyzed shadow)

Results from self-assessment using questionnaire showed that 8 out of the 13 items asked to subjects were significantly agreed by the panel after 1 month of application of LINEMINUS CREAM and 11 out of 13 items were significantly agreed after 2 months of product application (Table 6).

The highest percentages of agreement obtained after 1 month were related to the moisturizing effect (87% of agreement) and smoothing effect (83% of agreement) of LINEMINUS CREAM on the eye contour. These percentages increased after 2 months of product usage (90% of agreement obtained for both items), and subjects also noticed an improvement of the fine lines on their eye contour (90% of agreement). Regarding cosmetic acceptability, subjects found that the product was easy to spread and that it did not leave any sticky or oily feeling on the skin (91% of agreement obtained for the 3 items). These properties were also confirmed (significance threshold reached) after 2 months of product usage as well as an easy absorption of the product into the skin (90% of agreement).

Results from self-assessment using questionnaire showed that 8 out of the 13 items asked to subjects were significantly agreed by the panel after 1 month of application of PLACEBO CREAM and 11 out of 13 items were significantly agreed after 2 months of product application (Table 7).

Table 6: Mean results (percentage of positive opinions) from self-assessment of product efficacy and cosmetic acceptability after 1 and 2 months of application of LINEMINUS CREAM (p (Chi-2) value to confirm statistical significance, n=23 after 1 month and 21 after 2 months)

	AFTER 1 MONTH		*AFTER 2 MONTHS*	
EFFICACY AND COSMETIC ACCEPTABILITY OF LINEMINUS CREAM	***%Positive opinion***	***p(Chi-2)***	***%Positive opinion***	***p(Chi-2)***
With this product, eye contours seem more moisturized	87%	3.93E-04	90%	2.08E-04
With this product, fine lines around the eye contours seem diminished	70%	6.06E-02	90%	2.08E-04
With this product, deep wrinkles around the eye contours seem diminished	52%	8.35E-01	76%	1.64E-02
With this product, eye contours seem smoother	83%	1.76E-03	90%	2.08E-04
With this product, eye contours seem tighter	74%	2.18E-02	86%	1.06E-03
With this product, eye contours seem younger	52%	8.35E-01	67%	1.27E-01
With this product, your skin around the eye contour had a youthful appearance	61%	2.97E-01	76%	1.64E-02
This product is easy to spread	91%	7.44E-05	100%	4.59E-06
This product absorbs easily to the skin	74%	2.18E-02	90%	2.08E-04
This product has a pleasant fragrance	61%	2.97E-01	67%	1.27E-01
This product does not leave sticky feeling on the skin	91%	7.44E-05	86%	1.06E-03
This product does not leave oily residue on the skin	91%	7.44E-05	95%	3.38E-05
The product gives a pleasant feeling of freshness at application	74%	2.18E-02	71%	4.95E-02

Table 7: Mean results (percentage of positive opinions) from self-assessment of product efficacy and cosmetic acceptability after 1 and 2 months of application of PLACEBO CREAM (p (Chi-2) value to confirm statistical significance, n=23 after 1 month and 21 after 2 months)

	AFTER 1 MONTH		*AFTER 2 MONTHS*	
EFFICACY AND COSMETIC ACCEPTABILITY OF PLACEBO CREAM	***%Positive opinion***	***p(Chi-2)***	***%Positive opinion***	***p(Chi-2)***
With this product, eye contours seem more moisturized	87%	3.93E-04	90%	2.08E-04
With this product, fine lines around the eye contours seem diminished	70%	6.06E-02	90%	2.08E-04
With this product, deep wrinkles around the eye contours seem diminished	52%	8.35E-01	67%	1.27E-01
With this product, eye contours seem smoother	87%	3.93E-04	90%	2.08E-04
With this product, eye contours seem tighter	74%	2.18E-02	86%	1.06E-03
With this product, eye contours seem younger	48%	8.35E-01	62%	2.75E-01
With this product, your skin around the eye contour had a youthful appearance	43%	5.32E-01	57%	5.13E-01
This product is easy to spread	91%	7.44E-05	95%	3.38E-05
This product absorbs easily to the skin	74%	2.18E-02	95%	3.38E-05
This product has a pleasant fragrance	65%	1.44E-01	67%	1.27E-01
This product does not leave sticky feeling on the skin	96%	1.19E-05	86%	1.06E-03
This product does not leave oily residue on the skin	96%	1.19E-05	95%	3.38E-05
The product gives a pleasant feeling of freshness at application	74%	2.18E-02	76%	1.64E-02

The highest percentages of agreement obtained after 1 month were related to the moisturizing effect (87% of agreement) and smoothing effect (87% of agreement) of PLACEBO CREAM on the eye contour. These percentages increased after 2 months of product usage (90% of agreement for both items), and subjects also noticed

an improvement of the fine lines on their eye contour (90% of agreement). Regarding cosmetic acceptability, subjects found that the product was easy to spread and that it did not leave any sticky or oily feeling on the skin (91% of agreement obtained for the first item and 96% for the following two). These properties were also confirmed (significance threshold reached) after 2 months of product usage as well as an easy absorption of the product into the skin (95% of agreement).

Products performance comparison

Comparison of results from shadow casting analysis of crow's feet silicone replicas (Table 8) made at baseline (T0) for both sides of the face did not show any significant difference for none of the measured parameters. This means that the crow's feet area of all subjects at baseline was not found to be significantly different between each side of the face, making the comparison between products after treatment possible and without any correction required.

There was a statistically significant difference between LINEMINUS CREAM and PLACEBO CREAM in favor of LINEMINUS CREAM in terms of decrease of the number of wrinkles after 1 month of product application (-15.5 ± 22.4 versus -4.2 ± 22.1, *p=4.54E-02*). There was also a statistically significant difference between LINEMINUS CREAM and PLACEBO CREAM in favor of LINEMINUS CREAM in terms of decrease of the mean depth of wrinkles after 2 months of product application (-0.1 ± 0.1 versus 0.0 ± 0.1, *p=3.52E-02*).

Subjects were not able to notice more visible anti-ageing effects from the LINEMINUS CREAM compared to the PLACEBO CREAM after 1 month of usage (Table 9). However, they significantly agreed that their skin treated with LINEMINUS CREAM had a more youthful appearance on the eye contour (76% of agreement, *p=1.64E-02*) and that deep wrinkles around the eye contours seemed diminished when treated with LINEMINUS CREAM (76% of agreement, *p=1.64E-02*)) for 2 months (Table 10). They did not significantly agree with these statements after 2 months of application of PLACEBO CREAM, which confirms LINEMINUS CREAM's long term visible effect on crow's feet wrinkles.

DISCUSSION

It was previously shown that phenolic compounds were the main contributor of antioxidant activity in plants [10] and that *Polygonum minus* had the highest total phenolic content and reducing power, when compared to other herbs and spices such as ginger, turmeric, curry leaves etc. [8, 10]. *Polygonum minus* was also suggested to be a potential source of natural antioxidants with similar characteristics to the synthetic antioxidant butylated hydroxyanisole (BHT) [8]. Lineminus™ is an extract developed from *Polygonum minus* leaves with 3 pending patents. The interest of this study was to assess, for the first time, the effects of *Polygonum minus*, contained in a cosmetic product, in Asian subjects.

Table 8: Mean values (±SD) of parameters from silicone replicas for the skin treated with LINEMINUS CREAM and the skin treated with PLACEBO CREAM for 1 and 2 months. P value to confirm statistical difference between the two products

		Number of wrinkles		**Total wrinkle area**		**Total length**		**Mean length**		**Mean depth**	
		Mean ± *S.D.*	p-value	**Mean** ± *S.D.*	p-value	**Mean** ± S.D.	p-value	**Mean** ± S.D.	p-value	**Mean** ± S.D.	p-value
T0	LINEMINUS CREAM	88.0 ± *37.4*	3.11E-01	21.1 ± *12.2*	1.94E-01	87.2 ± *42.1*	3.28E-01	1.0 ± *0.2*	3.03E-01	113.2 ± *14.0*	6.81E-01
	PLACEBO CREAM	80.6 ± *36.8*		19.3 ± *13.1*		78.6 ± *43.3*		0.9 ± *0.2*		114.5 ± *17.6*	
T+1 month	LINEMINUS CREAM	72.6 ± *34.9*	5.63E-01	17.2 ± *10.5*	6.72E-01	73.3 ± *43.2*	7.63E-01	1.0 ± *0.2*	6.85E-01	119.4 ± *22.5*	4.03E-02
	PLACEBO CREAM	76.5 ± *38.2*		16.3 ± *9.5*		75.9 ± *45.3*		0.9 ± *0.2*		109.3 ± *16.7*	
T+2 months	LINEMINUS CREAM	70.4 ± *34.2*	7.53E-01	16.9 ± *11.2*	6.53E-01	64.7 ± *36.1*	7.80E-01	0.9 ± *0.2*	4.42E-01	112.8 ± *15.8*	7.31E-01
	PLACEBO CREAM	67.9 ± *34.6*		15.7 ± *10.8*		67.1 ± *43.4*		0.9 ± *0.2*		114.0 ± *17.9*	
Variation after 1 month	LINEMINUS CREAM	-15.5 ± *22.4*	4.54E-02	-3.8 ± *8.7*	1.53E-01	-13.8 ± *31.1*	2.23E-01	0.0 ± *0.2*	6.31E-01	6.2 ± *21.7*	4.68E-02
	PLACEBO CREAM	-4.2 ± *22.1*		-3.0 ± *10.9*		-2.7 ± *29.5*		0.0 ± *0.1*		-5.2 ± *17.1*	
Variation after 2 months	LINEMINUS CREAM	-17.7 ± *34.3*	3.75E-01	-4.1 ± *10.8*	8.12E-01	-22.4 ± *37.1*	1.21E-01	-0.1 ± *0.1*	3.52E-02	-0.4 ± *12.3*	9.73E-01
	PLACEBO CREAM	-12.7 ± *29.3*		-3.6 ± *10.3*		-11.5 ± *29.7*		0.0 ± *0.1*		-0.5 ± *18.1*	

Table 9: Mean results (percentage of positive opinions) from self-assessment of product efficacy and cosmetic acceptability after 1 month of LINEMINUS CREAM and PLACEBO CREAM usage (p (Chi-2) value to confirm statistical significance)

	LINEMINUS CREAM		***PLACEBO CREAM***	
EFFICACY AND COSMETIC ACCEPTABILITY OF THE PRODUCT	***%Positive opinion***	***p(Chi-2)***	***%Positive opinion***	***p(Chi-2)***
With this product, eye contours seem more moisturized	87%	3.93E-04	87%	3.93E-04
With this product, fine lines around the eye contours seem diminished	70%	6.06E-02	70%	6.06E-02
With this product, deep wrinkles around the eye contours seem diminished	52%	8.35E-01	52%	8.35E-01
With this product, eye contours seem smoother	83%	1.76E-03	87%	3.93E-04
With this product, eye contours seem tighter	74%	2.18E-02	74%	2.18E-02
With this product, eye contours seem younger	52%	8.35E-01	48%	8.35E-01
With this product, your skin around the eye contour had a youthful appearance	61%	2.97E-01	43%	5.32E-01

This product is easy to spread	91%	7.44E-05	91%	7.44E-05
This product absorbs easily to the skin	74%	2.18E-02	74%	2.18E-02
This product has a pleasant fragrance	61%	2.97E-01	65%	1.44E-01
This product does not leave sticky feeling on the skin	91%	7.44E-05	96%	1.19E-05
This product does not leave oily residue on the skin	91%	7.44E-05	96%	1.19E-05
The product gives a pleasant feeling of freshness at application	74%	2.18E-02	74%	2.18E-02

Table 10: Mean results (percentage of positive opinion) from self-assessment of product efficacy and cosmetic acceptability after 2 months of LINEMINUS CREAM and PLACEBO CREAM usage (p (Chi-2) value to confirm statistical significance)

	LINEMINUS CREAM		*PLACEBO CREAM*	
EFFICACY AND COSMETIC ACCEPTABILITY OF THE PRODUCT	***%Positive opinion***	***p(Chi-2)***	***%Positive opinion***	***p(Chi-2)***
With this product, eye contours seem more moisturized	90%	2.08E-04	90%	2.08E-04
With this product, fine lines around the eye contours seem diminished	90%	2.08E-04	90%	2.08E-04
With this product, deep wrinkles around the eye contours seem diminished	76%	1.64E-02	67%	1.27E-01
With this product, eye contours seem smoother	90%	2.08E-04	90%	2.08E-04
With this product, eye contours seem tighter	86%	1.06E-03	86%	1.06E-03
With this product, eye contours seem younger	67%	1.27E-01	62%	2.75E-01
With this product, your skin around the eye contour had a youthful appearance	76%	1.64E-02	57%	5.13E-01
This product is easy to spread	100%	4.59E-06	95%	3.38E-05
This product absorbs easily to the skin	90%	2.08E-04	95%	3.38E-05
This product has a pleasant fragrance	67%	1.27E-01	67%	1.27E-01
This product does not leave sticky feeling on the skin	86%	1.06E-03	86%	1.06E-03
This product does not leave oily residue on the skin	95%	3.38E-05	95%	3.38E-05
The product gives a pleasant feeling of freshness at application	71%	4.95E-02	76%	1.64E-02

CONCLUSION

Based on the results from this clinical study it is possible to conclude that Lineminus™ Cream, containing extracts from plants which are common in Asia, is safe and has an anti-wrinkle effect. The results also show that Lineminus™ (*Polygonum minus* extract) has beneficial cosmetic anti-wrinkle properties which were not only confirmed by objective evaluation (analysis of silicone replicas) but they were also observed by the subjects after 2 months of daily application. These results also show that in addition to *Polygonum minus*'s well-known anti-oxidant efficacy, the plant extract demonstrates as well a significant clinical anti-wrinkle effect with clear reduction of crow's feet wrinkles.

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