From Tradition

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The antiageing efficacy of donkey milk in combination with pomegranate extract and UV protection

A traditional ingredient in a modern formulation

KEYWORDS: Donkey milk, pomegranate, UV filter, anti-wrinkle, whitening, hydration.

Abstract Donkey milk, allegedly used by Cleopatra, is a complex, traditional cosmetic ingredient composed of hydrating substances. In this work, donkey milk was mixed with pomegranate extract and an optimised blend of UV filters in a cosmetic O/W emulsion. The antiageing efficacy of this synergistic blend was assessed by imaging, biomechanical and electrometric methods on 32 volunteers, after 28 days of application. The wrinkle count decreased by 32.9% and the wrinkle length was reduced by 9.6%. Skin hydration increased by 11.4%, while skin firmness and elasticity increased by 9.6% and 16.1%, respectively. Furthermore, skin colour homogeneity was enhanced. Thus, this product was proven to have antiageing effects, both by preventing photoageing and by diminishing existing signs of ageing.

INTRODUCTION

Skin ageing is influenced by both intrinsic and extrinsic factors that promote the accumulation of physicochemical alterations in the cutaneous structure and function (1, 2). These alterations reveal themselves as clinical changes, including e.g. wrinkling, dyschromias and elastosis (3). Ultraviolet (UV) radiation exposure, particularly UVA and UVB, is the major extrinsic ageing factor responsible for premature skin ageing (4, 5).

Donkey milk is highly hydrating due to its protein-rich content, namely casein, α-lactoalbumin and β-lactoglobulin (6, 7) and it contains traces of lactic acid, lactate and calcium, claimed to have positive effects on aged skin (8, 9).

An O/W emulsion for topical application was developed to address signs of skin ageing (skin firmness, elasticity and wrinkle reduction, among others) by a combination of different cosmetic ingredients: donkey milk, pomegranate extract and UV filters (SPF 20). The UV filters were selected in order to provide a broad-spectrum (UVA and UVB) protection when blended in the final product. The synergistic efficacy of these raw materials was assessed *in vivo*.

METHODS

In vivo efficacy evaluation studies were performed on a selected population of 32 women likely to use the product over a period of 28 days (measurements performed on D0 and D28).

The main inclusion criteria: women with ages between 45-65 years old, phototype (Fitzpatrick) I to IV, all skin types, with wrinkles around periocular area, corresponding to the target market of the product. The number of subjects defined in protocol was sufficient to assess the efficacy of the product. All the subjects were selected after being given informed consent. The study respected the principles of the Helsinki Declaration and the subsequent amendments.

After a defined period of acclimatization (at least 20 min) in a fully controlled room (21°C ± 1°C; 50% Relative Humidity ± 5%) one of the hemifaces was randomly selected. All the measurements were obtained on that selected hemiface and a standardized procedure was used to guarantee that the measurements were performed exactly on the same place of the hemiface.

Standardized photographic images of the front face and both hemifaces were obtained with visible, cross polarized and UV light before and after the treatment (D0 and D28) with the system VISIA®-CA (Canfield Scientific, USA), in order to quantify the evolution and appearance of spots, i.e. brown areas, UV areas, normal visible areas and real spots. For each parameter, a feature count inside the area and an area in % of pixels were calculated.

3D images of the skin topography in the periocular area were obtained by a digital fringe projection using DLP® micro mirror displays. A fringe standard was projected on the skin and detected by the CCD camera of the optical system. The 3D effect was calculated by the deflection in

the fringes, which represent the skin profile qualitatively and quantitatively. A Phase Shifting Rapid *In vivo* Measurement of Skin system was used (PRIMOS® 3D 40x30 mm evaluation area, Gfm, Germany). At each experimental time, the wrinkle parameters and standard roughness were calculated in the fully aligned image (Ra = arithmetic mean of the skin surface, Rz = mean of the 5 highest peaks and the 5 lowest valleys in the image area). The defined wrinkle parameters were: wrinkle count and wrinkle average length. To assess the efficacy of the product, all the subjects included in the study were taken into account.

A skin biomechanical evaluation was performed using a Cutometer® dual MPA 580 with a 2 mm probe to assess the firming effect and elasticity recovery. This system measures the elasticity of the upper skin layers by applying a negative pressure, which deforms the skin mechanically. The total skin displacement R0 (Uf) (mm), was obtained to calculate the firmness of the skin. As the skin becomes more firm, the R0 value decreases (it measures the maximum amplitude achieved by the suction). This parameter represents the passive behaviour of the skin to force and is a measurement of firmness. A lower result is related with a higher firming effect. The R2 (Ua/Uf) (%), the elasticity parameter, was also obtained. This parameter represents the ratio between the ability of returning to the original position and the maximum amplitude. It is a standard parameter of elasticity. The closer the value is to 1 (100 %) the more elastic the curve.

The hydration content was obtained by an electrometric system using a Corneometer® CM825 connected to a Cutometer® dual MPA 580. Measurements were performed in the periocular area. Trans-epidermal water loss (TEWL) was used as an assessment of the skin barrier by using a Tewameter® TM 300 on a randomly selected facial area.

The methodology used to calculate the mean values was based on clinical trials methodology. Briefly, all calculations were made by taking into account the difference of each individual subject at the beginning and in the end of the study (mean of the differences as a percentage), in order to eliminate the interindividual variability.

This reflects the average of the differences.

All statistical analysis was performed using parametric statistical tests (student t-test) for paired comparisons after confirming the normality of the distribution, in order to reduce the variability. IBM SPSS 23® software was used to perform all the calculations. A 95% confidence level was adopted for all the statistical calculations.

All the accessory calculations were performed using MS Excel 2016®. The % change expressed in all the tables and graphics represent the mean values of each individual change.

RESULTS

As shown in Figure 1, the topical application of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20 for 28 days resulted in a statistically significant decrease in both the wrinkle count and the wrinkle length in the periocular area ($p < 0.0001$). By comparing the relative transformation on completion of the study in relation to D0, this product produced a marked alteration of the cutaneous topography: there was a 32.9% reduction in wrinkle count and a 9.6% reduction in wrinkle length. Figure 2 illustrates the visible results associated with the data in Figure 1.

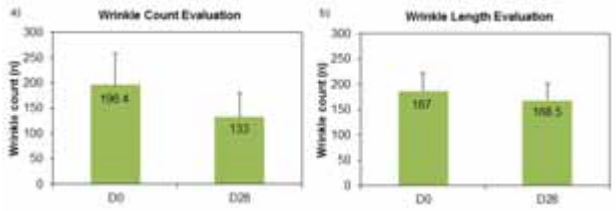

Figure 1. The influence of topical application of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20 on (a) wrinkle count (D0 to D28) and (b) wrinkle length evolution (D0 to D28) (mean ± SD values, n = 32).

The application of the same product did not result in a statistically significant change in skin roughness over the duration of the study. By comparing the relative transformation on completion of the study in relation to D0, there was a 12.0% and 14.7% non-significant increase in the Ra and Rz variables, respectively (data not shown). Nevertheless, this suggests a maintenance of the skin smoothness in the measured areas.

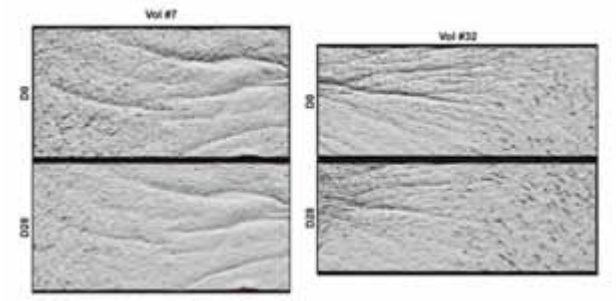

Figure 2. Photographs of examples of the evolution of wrinkle reduction (D0 to D28) in two subjects, showing the anti-wrinkle effect of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20.

A 3.5% and 6.8% reduction in the number of brown spots and the number of UV spots respectively, were obtained after 28 days of product application (Table 1). There was also a reduction of the area of UV spots of 6.8% (Table 1). Altogether, these results indicate that the application of the tested product was associated with an increase in the homogeneity of the skin colour. Figure 3 shows an example of the increase in skin colour homogeneity.

Figure 3. Photographs of one example of the increase in skin colour homogeneity (D0 to D28) by topical application of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20.

Skin hydration was observed to be increased by 11.4%, alongside an increase in TEWL of 21.4%. Skin firmness and

elasticity increased by 9.6% and 16.1%, respectively. These results are shown in Table 1.

	Time	Mean ± SD	p-value
Skin hydration (AU)	D0	68.9 ± 9.7	< 0.0001
	D28	76.1 ± 8.1	
TEWL (g/h.m²)	D0	7.9 ± 2.7	0.0069
	D28	9.1 ± 2.8	
Skin firmness	D0	0.261 ± 0.126	0.017
	D28	0.204 ± 0.039	
Skin elasticity	D0	0.460 ± 0.107	0.011
	D28	0.521 ± 0.130	
UV spots counts	D0	256.4 ± 77.7	0.018
	D28	243.2 ± 82.8	
UV spots area	D0	29.5±13.7	0.003
	D28	26.7 ±12.2	
Brown spots counts	D0	310.4 ± 41.7	0.002
	D28	299.7 ± 44.5	

Table 1. Evolution of skin hydration, TEWL, skin firmness and skin elasticity with the topical application of an O/W emulsion with donkey milk, pomegranate extract and a SPF of 20 (D0 to D28) (mean ± SD values, n = 32).

DISCUSSION AND CONCLUSION

According to the *in vivo* results, the topical application of donkey milk, pomegranate extract and UV filters in an O/W emulsion vehicle showed promising results in addressing skin ageing. It was assumed that this occurred due to a synergistic effect between the ingredients.
Skin homogeneity was calculated by counting the number and area of different types of spots in the face. In brown spots this increase homogeneity was observed in 25 of the 32 subjects. The effect is statistically significant. Again, this is a cosmetic product and it's not supposed to reduce all the brown spots. Nevertheless, the increase in the overall brown dark aspect was the effect, and in fact the subjects have a more homogeneous skin color.
It is suggested that donkey milk, due to its high whey-protein content, was responsible for the significant increase in skin hydration (6,7). The increase in skin hydration was consistent with an increase in TEWL. When the ingredients provide some water to the skin due to their hygroscopic properties, the TEWL value can increase instead of decreasing. This seems to be the effect in this formulation, which suggests an increase in the water content of the upper layers of the skin.
Concerning skin firmness, reported in Table 1, the variable R_0 was reduced meaning that the skin was more firm.
The findings obtained in this work are consistent with previous studies, which revealed the efficacy of pomegranate extract in diminishing the signs of skin ageing due to its properties, not only as an antioxidant (10), but also for its role in reversing hyperpigmentation by inhibiting tyrosinase activity (11). Donkey milk may have also contributed, in synergy with the pomegranate extract, to the increase in skin colour homogeneity due to the tyrosinase-inhibiting capacity of α-lactoalbumin and β-lactoglobulin (12). The use of pomegranate extracts in skincare has also been associated with the inhibition of photoageing induced by UV radiation (13).
Thus, together with UV filters, whose key role in preventing extrinsic ageing has been extensively reviewed (14-17), it is suggested that this formulation exerts its effects both by preventing photoageing and by diminishing extant signs of ageing in photoaged skin.

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