Quillaja saponaria saponin-rich extract shows anti-inflammatory activity, protecting and repairing against UV-induced skin damage

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Abstract

The saponin-rich extract from Quillaja saponaria's tree has been used in the cosmetic industry as a natural surfactant to create natural derived emulsions. We have tested the extract for its anti-inflammatory properties both in vitro and clinically. Experiments in PMA induced keratinocytes showed the extract (either at 9% or 15% saponin content) being able to reduce PMA stimulated pro-inflammatory markers CXCL5, CCL3, IL23A, IL17C, IL6ST. The reduction of the interleukins (but not of the chemokines) was dose dependent on the saponin concentration. To further confirm the anti-inflammatory action of the extract and explore its clinical significance, a clinical study was run. Twenty healthy volunteers were UV irradiated and changes in skin redness and TEWL were measured. The Quillaja extract (15% saponin) at 0.5% or 1% in a gel formulation was tested as prevention (before irradiation) or as treatment (after irradiation). When redness was measured, both Quillaja formulations at 0.5% and 1% were significantly effective ($p<0.05$) as prevention (reduction of 10% and 12% respectively) and even more effective as treatment (reduction of 20% and 25% respectively). When TEWL was evaluated, the highest dose Quillaja formulation (1%) as a pre-treatment was significantly effective in reducing TEWL (-27.5%, $p<0.05$) and both formulations significantly more effective as a post-treatment (-37%, -39.2% for 0.5% and 1% Quillaja respectively, $p<0.01$). Our in vitro and clinical data show the ability of Quillaja extract to reduce the damaging effects of pro-inflammatory inducers. The extract can be considered a powerful and natural adjuvant in topical formulations designed for before and after sun exposure.

Keywords: Quillaja; skin; inflammation; TEWL; UV

Introduction

The quest to develop scientific evidence associated with the use of natural ingredients has been one of the focuses of the cosmetic industry in the last several years, fueled by an increasing number of consumers looking for naturality but also for science and proven efficacy. One of the most successful natural ingredients in the food and pharma industry has been the extract derived from the Quillaja saponaria tree. The tree is endemic to Chile and grows in dry environments 2,000 meters above sea level. The extract has a specific saponin content [1,2] (**Figure 1**) and has been used mostly as a stabilizer of colloidal structures in food and cosmetics [3,4] and as a very successful immune adjuvant for vaccines, due to its immunomodulatory properties in pharmaceutics [1,5,6]. Also, the extract has shown antimicrobial and antiviral properties [7,8] and its use has been proposed as a sebum regulator in skin and scalp dermatological conditions [9]. In the cosmetic industry, Quillaja saponaria derived saponins

have been mostly used as natural surfactants, specifically to create natural derived nano-emulsions [10] and/or as detergents to substitute synthetic surfactants [11]. In the present paper we wanted to explore the biological properties of the extract and investigate its potential application as an anti-inflammatory agent to reduce skin irritation and damage.

Materials and Methods

In vitro

Two different extracts from Quillaja saponaria, derived from the tree biomass, and with a different content of saponins (9% and 15%), were incubated with normal human epidermal keratinocytes (NHEK) at a non-cytotoxic concentration of 0.00037%, to test Quillaja extracts' ability to reduce a pro-inflammatory response induced by Phorbol Myristate Acetate (PMA), an activator of the inflammatory response (12). NHEK were seeded in 24 well plates and cultured for 48 hours. The medium was then replaced with fresh medium containing or not Quillaja extracts, and cells incubated for an additional 24 hours. PMA (0.1 μg/ml) was then added with fresh medium in combination or not with Quillaja extracts. Cells were further incubated for an additional 8 hours. In parallel, a non-PMA stimulated control was performed. At the end of the incubation the cells were washed and frozen. To detect the mRNA expression of specific markers, RNA was extracted and run on an Affymetrix chip. The data was expressed as Arbitrary Units (a.u.). At least ≤ 0.5 decrease fold change and a ≥ 2 increase fold change in mRNA expression was considered significant.

Clinical Study

We tested the highest saponin content Quillaja extract (15% saponins) formulated in a gel at 0.5% or 1%, to protect but also to repair the skin of healthy volunteers irradiated with a solar spectrum (UVA+UVB) at a fixed dose of 1.5 MED (protective protocol) or 1 MED (repair protocol). Twenty healthy caucasian women (average age 47 years old) were enrolled by a board-certified dermatologist to participate in the study. Skin redness and Trans Epidermal Water Loss (TEWL) were evaluated instrumentally as end points when formulations were applied either before or after UV irradiation. Two different skin areas were treated with the Quillaja formulations (2mg product/cm^2 skin), one area was treated with a Placebo formula (2mg product/cm^2 skin) (no Quillaja), and another area remained untreated. Skin redness was evaluated using a Mexameter 18 while TEWL was measured using a Tewameter® TM 300 (both instruments are manufactured by Courage+Khazaka, electronic GmbH, Germany).

Statistical Analysis

Data was submitted to two-way paired Student's t-test. The inter-group statistical analysis was carried out on percentage variations obtained in the Quillaja treated vs placebo areas at each experimental time. p-value < 0.05 was considered statistically significant.

Results

In vitro data in NHEK showed a significant increase of chemokines and cytokines induced by PMA (CXCL5, CCL3, IL23A, IL17C, IL6ST, see full description in figure legend) (**Figure 2**). This effect was reduced by the presence of Quillaja extracts containing saponins at different concentrations (9% and 15%) (**Figure 2**). Interestingly, the effect was dose dependent on the saponin concentration for the

Interleukins reduction (but not for the chemokines) (**Figure 2**). These results suggest an anti-inflammatory action associated with the use of saponin-rich Quillaja extracts.

In the clinical study, when UV-induced skin redness was evaluated, both Quillaja formulations given as a pre-treatment were significantly effective in reducing redness (-10%, -12% for 0.5% and 1% Quillaja respectively, $p<0.05$ vs Placebo, Student's t test) (**Figure 3**) and even more effective as a post-treatment (-20%, 25% after 2 hours treatment from irradiation with 0.5% and 1% Quillaja respectively, $p<0.05$ vs Placebo, Student's t test) (**Figure 4**). When UV-induced TEWL was evaluated, the highest dose Quillaja formulation (1%) as a pre-treatment was significantly effective in reducing TEWL (-27.5%, $p<0.01$ vs Placebo, Student's t test) (**Figure 5**) and both formulations significantly more effective as a post-treatment (-37%, -39.2% after 24 hours treatment from irradiation with 0.5% and 1% Quillaja respectively, $p<0.01$ vs Placebo, Student's t test) (**Figure 6**).

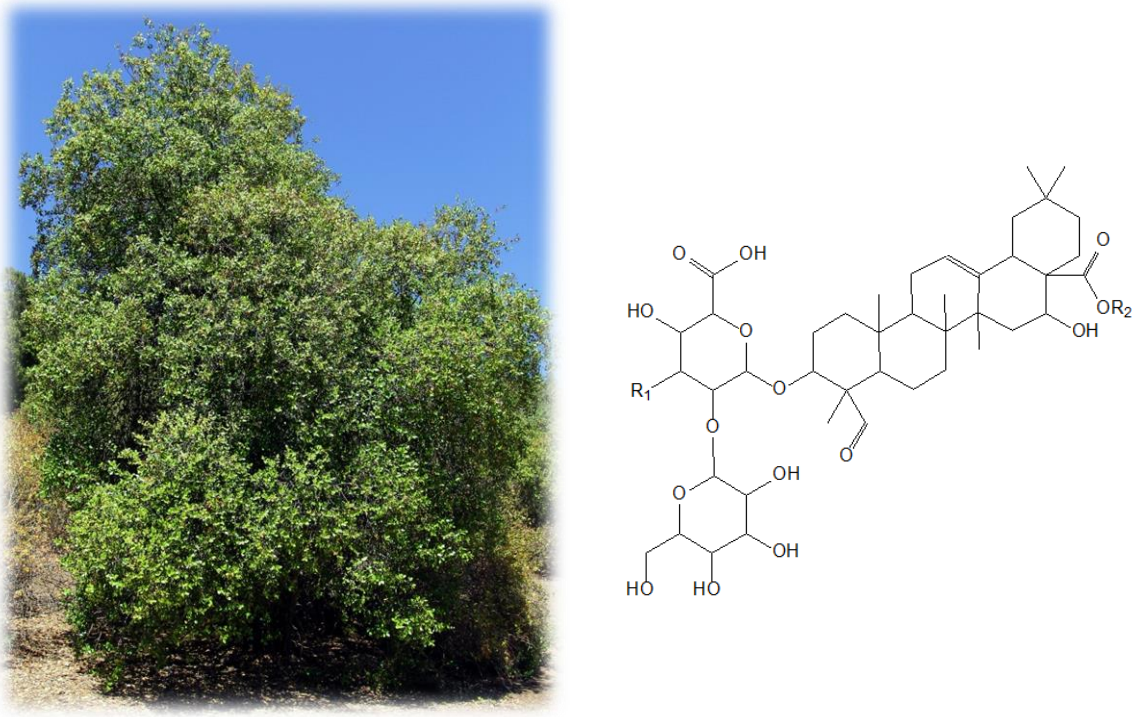

Figure 1. Quillaja saponaria tree and its saponins.

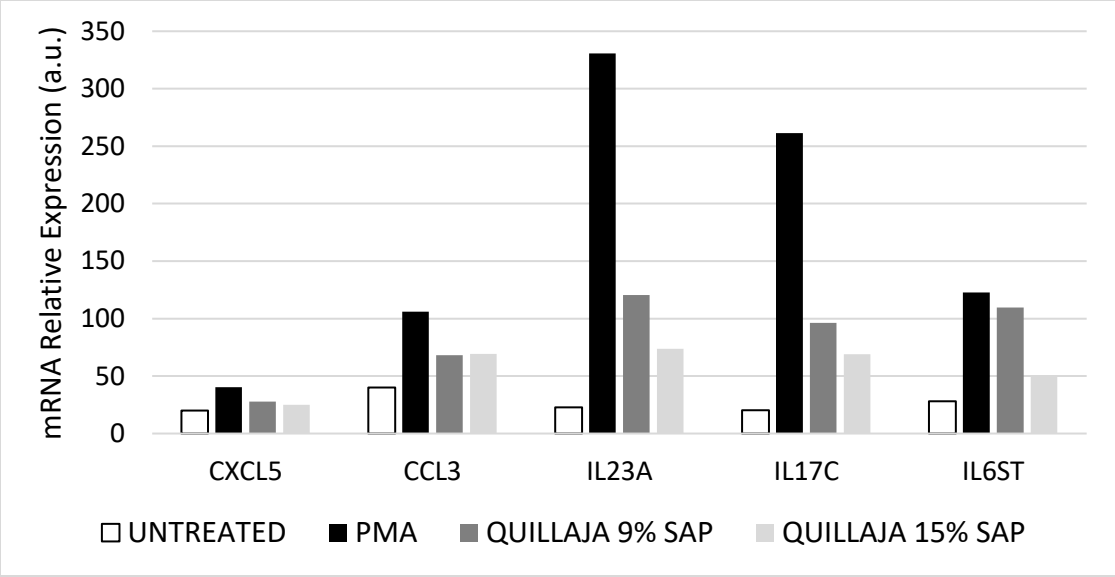

Figure 2. Quillaja Extract containing either 9% or 15% saponins (SAP), when used at a concentration of 0.00037%, reduced mRNA transcription for pro-inflammatory cytokines and chemokines in the presence of Phorbol Myristate Acetate (PMA) at 0.1 μg/ml. CXCL5: CXC motif chemokine 5, CCL3: chemokine C-C motif ligand 3, IL23A: interleukin 23 subunit alpha, IL17C: interleukin 17C, IL6ST: interleukin 6 signal transducer; a.u.: arbitrary units.

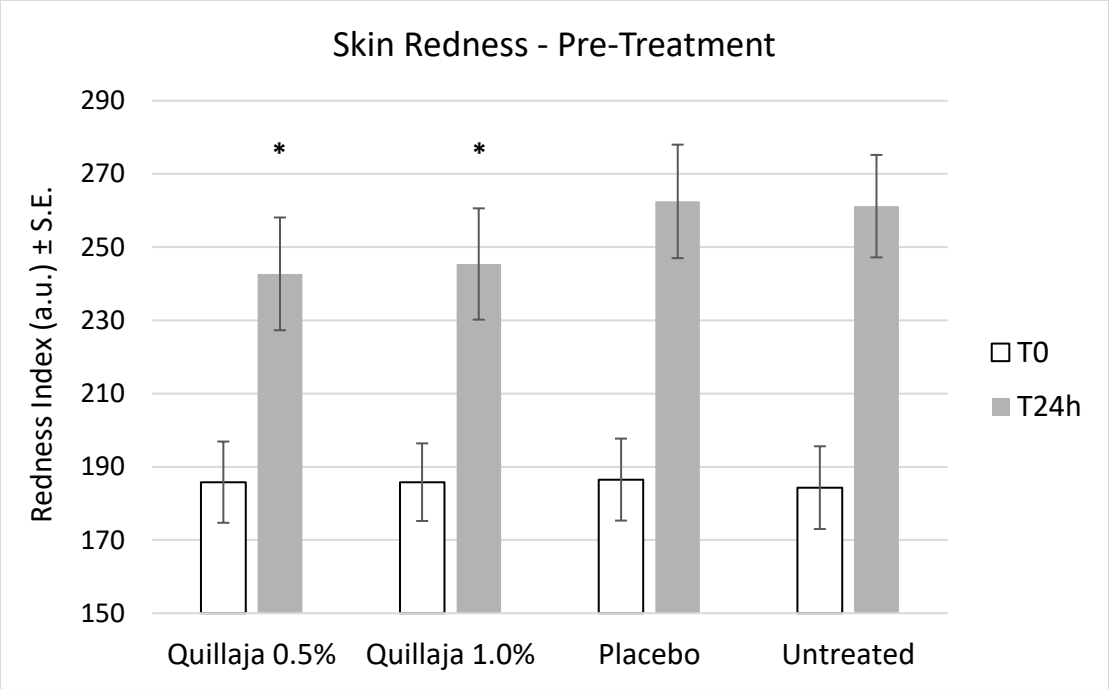

Figure 3. In a clinical study, Quillaja extract containing 15% saponins, used in a formulation at 0.5% and at 1.0%, significantly reduced UV-induced skin redness after 24 hours compared to a Placebo formulation and to Untreated. a.u.: arbitrary units, S.E.: standard error, *p<0.05 vs Placebo.

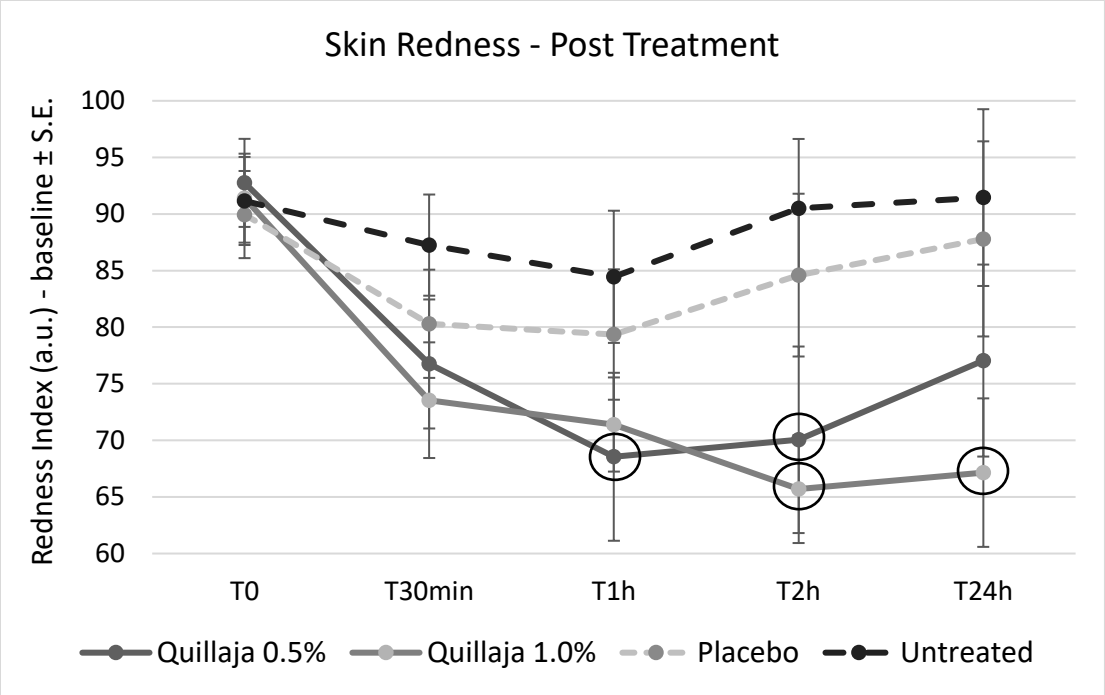

Figure 4. Quillaja extract containing 15% saponins, used in formulation at 0.5% and at 1.0%, significantly reduced UV-induced skin redness overtime compared to a Placebo formulation and to Untreated. a.u.: arbitrary units, S.E.: standard error, in circle: $p<0.05$ vs Placebo.

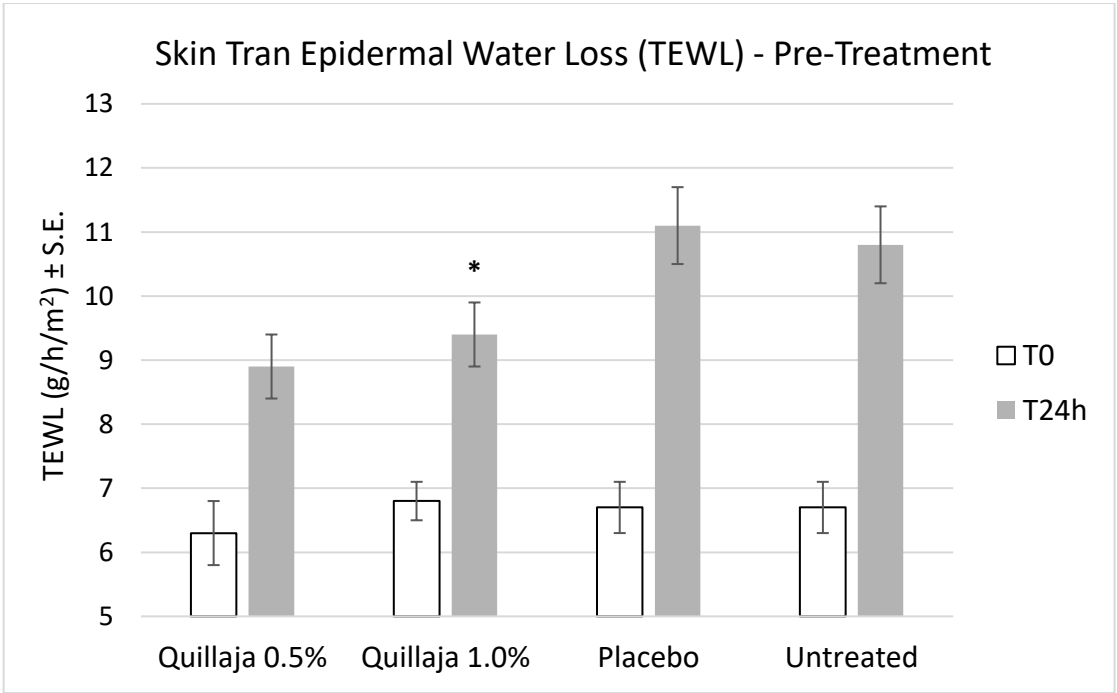

Figure 5. Quillaja extract containing 15% saponins, used in a formulation at 0.5% and at 1.0%, significantly reduced UV-induced TEWL after 24 hours compared to a Placebo formulation and to Untreated. a.u.: arbitrary units, S.E.: standard error, *$p<0.05$ vs Placebo.

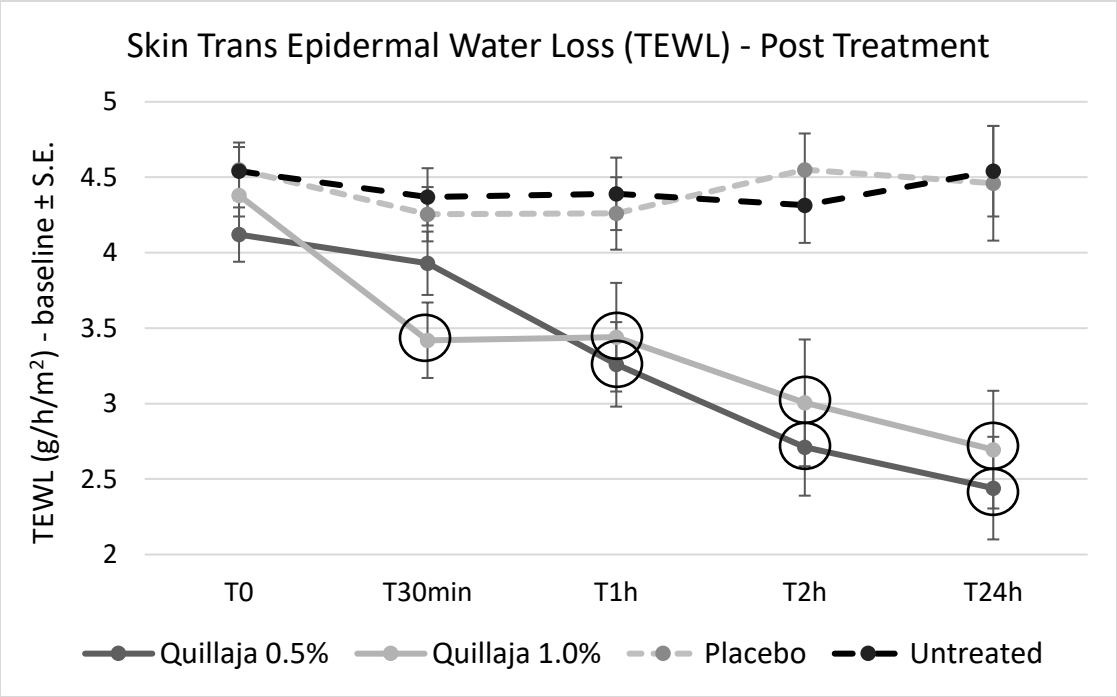

Figure 6. Quillaja extract containing 15% saponins, used in formulation at 0.5% and at 1.0%, significantly reduced UV-induced skin TEWL overtime compared to a Placebo formulation and to Untreated. a.u.: arbitrary units, S.E.: standard error, in circle: $p<0.01$ vs Placebo.

Discussion

In this work we have shown the capacity of saponin-rich Quillaja extract to reduce inflammation markers in normal human epidermal keratinocytes (NHEK), and to prevent/treat UV-induced skin redness and Trans Epidermal Water Loss (TEWL) in a clinical study, when introduced in a cosmetic formulation against a placebo formulation. The observed regulation by Quillaja extract on pro-inflammatory markers in NHEK has never been described and warrant further investigation. It would be interesting to understand the mechanism associated with this effect. PMA is known to activate NFkB through a PKC pathway in epidermidis and keratinocytes [12]. NFkB is a transcription factor responsible for the activation of genes associated with inflammation. It is possible that Quillaja extract interferes with NFkB activation or block downstream pathways. More experiments would be needed to demonstrate this mechanism. Also, it is important to determine the role of saponins as the active ingredient. It is in fact possible a role of the polyphenols present in the extract to contribute to the anti-inflammatory action. Previous literature has identified saponins and quillaic acid (their aglycone) as molecules involved with inflammation reduction [13]. Although, it is also possible the contribution of phenolic components in the extract to the overall effect [14]. In our experiment, the observation of a dose dependent activity on interleukins (ILs) reduction by the extract more concentrated in saponins (15%) suggests a major role of the saponins in the reduction of the inflammatory markers. Interestingly, the dose dependency was not seen when chemokines were reduced suggesting a specific mechanism for ILs.

Based on the results from the in vitro tests, we tested the hypothesis that Quillaja extract would protect and/or repair the skin from the damaging effect of UV light. It is known, in fact, that UV light induces skin inflammation/redness, damages the stratum corneum and disrupts the skin barrier [15]. In the clinical test conducted on healthy volunteers, we tested the extract at the highest saponin concentration (Quillaja 15% saponins) and formulated at 0.5% and 1% in a water-based gel to minimize the base effect. The base (without the extract) was used as the Placebo. Data were compared between the 4 groups that included untreated. The formulations containing the Quillaja extracts were statistically and significantly effective when compared to Placebo (see results), with the highest concentration (1%) more effective to reduce UV-induced skin redness (both as pre- and post-treatment), and maintaining a long-lasting effect compared to the lowest concentration (0.5%) (See **Figure 4**). When tested for TEWL, Quillaja extract formulations were equally effective (both as pre- and post-treatment). It appears that the extract has a more potent effect on TEWL reduction, i.e. improving the skin barrier, being both concentrations very effective. It will have to be analyzed if the efficacy is associated with reduction of UV-induced oxidative stress or a more downstream mechanism related to modulation of the inflammatory response, with a possible repairing effect.

Conclusions

We have demonstrated, both in vitro and clinically, the capacity of saponin-rich Quillaja extract to reduce the damaging effect of pro-inflammatory inducers (PMA and UV) in a dose dependent manner. We believe that by helping to reduce UV induced skin damage, Quillaja Extract can be considered a powerful and natural adjuvant in topical formulations designed for before and after sun exposure.

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