

Expression of differential genes involved in the maintenance of water balance in human skin by *Piptadenia colubrina* extract

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Summary

Background Hydration and integrity of the *stratum corneum* (SC) is an important determinant of skin appearance, metabolism, mechanical properties, and barrier function. The presence of aquaglyceroporins and envelope proteins are crucial to provide greater corneocyte cohesion to keep water and other moisturizers in the skin. **Aims** In this study, we evaluated the ability of *Piptadenia colubrina*, a plant native of South American rain forests, in the expression of genes involved in skin capacitance and SC integrity.

Methods The expression of genes for aquaporin-3 (AQP3), loricrin, involucrin (INV), and filaggrin (FLG) was measured by real-time PCR, using an *in vitro* model of human keratinocytes incubated with concentrations of 2.5, 5, 10, and 20 mg/mL of a hydroglycolic extract of *P. colubrina* (HEPC). The amount of AQP3 protein was also tested by immunohistochemistry in human skin explants. Clinical trials were conducted to evaluate the effects of a gel-cream containing HEPC on the glycerol index and skin capacitance.

Results Hydroglycolic extract of *P. colubrina* increased both the expression and immunoreactivity of AQP3 in cultured keratinocytes and human skin explants. The gene induction to envelope proteins FLG and INV was also observed after cell incubation with HEPC. Skin capacitance was significantly improved in human volunteers under treatment with HEPC-containing cream.

Conclusions The extract of *P. colubrina* promotes cellular hydration and induces gene expression of envelope proteins providing greater corneocyte cohesion to keep water and other moisturizers in the skin and an appropriate epidermal adhesion. The *in vitro* findings were clinically confirmed and encourage the clinical use of this compound in skin care products.

Keywords: *Piptadenia colubrina*, aquaporin-3, cornified envelope, filaggrin, involucrin, glycerol

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Introduction

The outermost region of the skin establishes and maintains an effective barrier that controls water exchange and protects against dehydration,¹ which is

crucial for physiological homeostasis.^{2,3} The epidermis also serves as a protective barrier that excludes harmful organisms such as viruses, bacteria, fungi, and other antigenic particles.⁴

Stratum corneum (SC or cornified layer) consists of the outer physical barrier originated from epidermal differentiation in which keratinocytes present flattened and dead cells.⁵ During this process, different proteins belonging to a cluster of genes in chromosome 1q21-termed epidermal differentiation complex (EDC) are expressed.^{6,7} Several of these genes encode for proteins involved in the formation of the cornified envelope, including loricrin (LOR), involucrin (INV), small proline-rich proteins (SPRRs), S100 proteins family, filaggrin (FLG), profilaggrin, and trichohyalin.⁷

Recent studies have shown that variation in EDC genes results in the pathogenesis of at least three common skin disorders, ichthyosis vulgaris (IC), atopic dermatitis (AD) and psoriasis. Deficiencies in the profilaggrin system (FLG), such as mutation R501X and 2282del4, have been identified as a cause of IC as a semi-dominant inheritance and a major risk factor for the development of IC.⁸ A review by McGrath⁹ in 2008 demonstrated that a mutation for loss of function in FLG is also a major risk factor for AD, and is associated with AD persisting into adulthood and with increased severity of atopic asthma and other health diseases.

Other EDC genes are associated with different disorders. Mutation in the LOR gene causes a variant form of Vohwinkel syndrome,¹⁰ a rare genetic disorder characterized by disturbed cornification. A low LOR expression was found in Italian families in individuals affected with psoriasis.¹¹ The expression of some S100 proteins (A2, A7, A8, A9, and A15) is increased in psoriasis and inflammation of the skin.¹² Increased expression of SPRR1 and SPRR2 was determined in orthokeratotic and parakeratotic hyperkeratosis (e.g., in psoriasis vulgaris and lichen planus).¹³

The water content of SC has a major influence on the appearance and physical properties of the skin. Different factors could control SC water content, such as the external humidity, lipid and protein composition, and concentration of water-retaining osmolytes or natural moisturizing factors (NMFs). Several different compounds such as free amino acids, ions, and other small solutes function as NMFs.¹⁴ Reduced hydration of SC has been found in several skin diseases, including AD, eczema, psoriasis, senile xerosis, and hereditary ichthyosis.¹⁵

Another important factor that plays a key role in skin hydration and integrity of the envelope is the presence of aquaglyceroporins.¹⁶ The aquaglyceroporins

(aquaporin-3 [AQP3], AQP7, AQP9, and AQP10) are a subset of the aquaporins (AQP0–AQP12), a family of membrane-transport proteins that assemble in membranes as tetramers and act primarily as water-selective pores, facilitating osmotically-driven water transport across the cell plasma membrane.^{17,18} Therefore, the aquaglyceroporins are permeable to water as well as to small solutes, such as glycerol and urea. AQP3 is expressed in the basal layer of keratinocytes in normal skin,^{19,20} where it functions primarily to allow glycerol to move into more superficial layers of the epidermis and SC. Studies using mice deficient in AQP3 demonstrated that they have dry skin with reduced SC hydration, decreased elasticity, and impaired biosynthesis, suggesting the importance of AQP3 in skin physiology and providing a rational scientific basis for the long-standing practice of including glycerol in cosmetic and medical skin preparations.^{14,21}

The use of active substances from natural sources, particularly plants, is potentially efficient in preventing physiological disorders, particularly those involving skin esthetics. Among these substances, polysaccharides of diverse origins and structural characteristics have been studied as biological-response modifiers.²² In this study, a native leguminous tree from South American rain forests *Piptadenia colubrina* Bth. (botanical synonym *Anadenanthera colubrina* [Vell.] Brenan), popularly known as Angico-branco,²³ was used as a source of polysaccharides, and its involvement in skin hydration and integrity was investigated. The expression of the genes for AQP3, LOR, INV, and FLG was measured by real-time PCR, using an *in vitro* model of human keratinocytes incubated with a hydroglycolic extract of *P. colubrina* (HEPC). The amount of AQP3 protein was also confirmed by immunohistochemistry in human skin explants. In addition, clinical trials were conducted to evaluate the effects of a gel-cream containing HEPC on the glycerol index and skin capacitance (corneometric index).

Material and methods

Plant material

A hydroglycolic extract of *P. colubrina* bark (Aquasense) was manufactured and provided by Chemyunion Química Ltda (Sorocaba, SP, Brazil). The INCI name of this compound is Water (and) Butylene Glycol (and) *P. colubrina* Peel Extract. Crude plants obtained from Brazilian suppliers were sliced into small pieces and extracted with an aqueous glycol solution. The extract was assayed by HPLC and presented total polysaccharide

contents ranging from 0.05% to 0.25% p/p. HEPC is recognized through patent applications PI-0702480-0, deposited in National Institute of Industrial Property (INPI) in 07/10/2007, and PCT/IB2008/050750, deposited in Patent Cooperation Treaty (PCT) in 02/29/2008.

Cell culture and treatment protocol

Normal human keratinocytes were obtained from commercial suppliers (Cell Applications, Inc., San Diego, CA, USA) and subcultured at 37 °C in 5% CO₂ in a humidified incubator. At 80–90% confluence, cells were seeded into six-well culture plates (1 × 10⁵ cells/well) and incubated with concentrations of 2.5, 5, 10, and 20 mg/mL of HEPC. Possible interference of hydroglycolic vehicle was evaluated and demonstrated no changes in cell response compared with untreated control cultures. Selection of these concentrations was based on previous results of cytotoxicity assays (data not shown). Cells were exposed for various times (0, 2, 4, 6, 8, and 24 h) as described by Sugiyama *et al.*²⁴

Real-time reverse transcription-polymerase chain reaction

For these analyses, RNA from cell culture was extracted with Trizol reagent (Invitrogen, São Paulo, Brazil). For RT-PCR, the RNA (1 µg) was treated with DNaseI (Fermentas, Ontario, Canada) and submitted to first strand cDNA synthesis with SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Gene expression (cDNA) was performed by Real-Time PCR with *Platinum* SYBR Green PCR Master Mix (Applied Biosystems, São Paulo, Brazil) in an ABI 7300 Real-Time PCR System (Applied Biosystems). The specific primers used for amplification were: aquaporin-3 5'-TCATTATGCTGAGGATTT-3' (forward), 5'-GGCCTCCCATCTCCTTCA-3' (reverse); FLG 5'-GTTACAATTCCAATCCTGTTGTTTTTC-3' (forward), 5'-CGTTGCATAATACTTGGATGATC-3' (reverse); INV 5'-GTGGGGGAGAGAGGGAATTA-3' (forward), 5'-CTCACCTGAGGTTGGGATTG-3' (reverse); LOR 5'-CATGATGCTACCCGAGGTTT-3' (forward), 5'-ACTGGGGTTGGGAGGTAGTT-3' (reverse); P53 (tumor suppressor gene *p53*) 5'-CTCACTCCAGCCACCTGAA-3' (forward), 5'-AAATGGAAGTCTGGGTGCT-3' (reverse); and hypoxanthine phosphoribosyltransferase (HPRT) 5'-CACTTGGATATGATCAATGGCTTCT-3' (forward), 5'-AAGGGAGGCTGTGCTATGA-3' (reverse) (Sinapse Biotecnologia, São Paulo, Brazil). HPRT was used as the reference gene. Amplification conditions were 50 °C for 2 min and 94 °C for 10 min, followed by 40 cycles of 94 °C for

15 s and 60 °C for 1 min. The dissociation curve was performed as follows: 95 °C for 15 s, 60 °C for 30 s, and 95 °C for 15 s. The relative mRNA levels were calculated according to Pfaffl.²⁵ We decided to consider induction of expression when the expression increased twofold or more, and repression when the expression decreased at least 50%.

Immunohistochemistry for AQP3

Skin biopsies obtained from patients undergoing plastic surgery were incubated with HEPC at the concentration of 10 mg/mL for 24 h. Skin explant sections (20 mm thick) were cut in a cryostat (Leica, Heidelberg, Germany) and collected on glass slides. After fixation in formaldehyde, samples were incubated with H₂O₂ to block endogenous peroxidases. Sections were incubated for 1 h in phosphate-buffered saline containing rabbit anti-AQP3 antiserum diluted 1:600 (Chemicon International, Millipore, Billerica, MA, USA) and then with anti-rabbit secondary antibody (1:200, Histostain-SP, Zymed; Invitrogen). The immunoreactive product was amplified by using a streptavidin-peroxidase conjugated and stained with diaminobenzidine. Finally, sections were counterstained with hematoxylin–eosin and documented by digital imaging (Leica).

Clinical studies

Thirty healthy female subjects with normal to dry skin, aged 30–55 years, and with skin types I–IV according to Fitzpatrick²⁶ were enrolled in this study. The randomized forearms of each volunteer were treated for 14 days with a gel-cream containing 5% of HEPC (GC-HEPC) or a placebo (INCI name: Water (and) Polyacrilamide (and) C13–14 Isoparaffin (and) Laureth-7 (and) Lanolin Alcohol (and) Mineral Oil (and) Disodium EDTA (and) Potassium Sorbate (and) Phenoxyethanol). Placebo was applied to the corresponding area of the forearm opposite to that receiving HEPC. According to a standard procedure, before each test, the subjects rested for 30 min in an air-conditioned area with a constant 22 ± 1 °C room temperature and 50 ± 5% relative humidity. The clinical studies were carried out with approval from the Ethics Committee of the Leonor Mendes de Barros Hospital, São Paulo, Brazil.

Determination of glycerol index in the *stratum corneum*

The SC of 15 volunteers was sequentially (five times) extracted from forearms by D-Squame (CuDerm Corp., Dallas, TX, USA) strippings, as described by Choi *et al.*,²⁷

on day 1 (D1), D7, and D14 after 120 min following the application of a GC-HEPC or a placebo. The glycerol content from the strippings was pooled and removed with an aqueous solution of 10% Cocamide DEA (90%) (Oxiteno, São Paulo, Brazil). Aliquots of each sample solution were incubated in triplicate with Free Glycerol Reagent (Sigma, St. Louis, MO, USA) for 15 min, and the optical density was measured at 540 nm. Glycerol index was expressed as mg/mL, calculated by reference to a standard curve constructed with known amounts of Glycerol Standard (Sigma).

Evaluation of skin capacitance by corneometric assessment

Capacitance measurements (corneometric index) were made from the remaining 15 volunteers. Three successive readings were taken and averaged prior to application of the products (baseline – T0) and on D1, D7, and D14 after 30, 60, and 120 min (T30, T60, and T120) following application of a GC-HEPC or the placebo. Corneometric index was measured using a CM 825 Corneometer™ (Courage-Khazaka Electronics GMB H, Cologne, Germany).

Statistical analysis

Statistical significance for real-time PCR was assessed by Student's *t*-test. For the glycerol index, a parametric method, the one-way analysis of variance (ANOVA) followed by the Tukey test, was used to compare data among all groups. For the clinical trials, the *t*-test hypothesis was used to evaluate treatment and time effects. Statistical significance was considered when $P < 0.05$.

Results

Expression of aquaporin-3 gene

The expression of AQP3 was studied by exposing the skin-culture cells to HEPC for different periods of time. The AQP3 mRNA expression was detected by real-time PCR. As seen in Figure 1, the expression increased after incubating for 2 h, peaked at 6 h, and decreased to the basal level after 48 h (data not shown). After the treatment time was standardized as 6 h, the expression of AQP3 was measured in different concentrations of HEPC (2.5, 5, 10, and 20 mg/mL) (Fig. 2). The concentration of 10 and 20 mg/mL induced a significant increase in AQP3 expression ($P < 0.01$). For immunohistochemical analysis, skin sections obtained

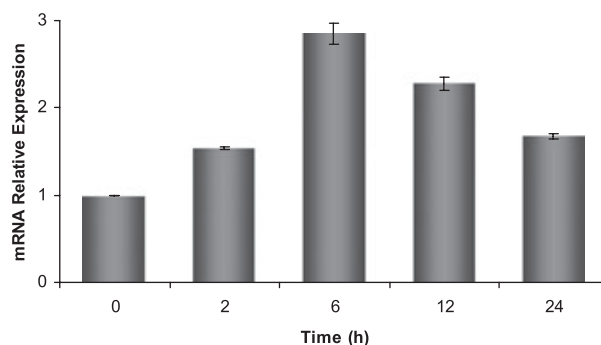


Figure 1 Aquaporin 3 (AQP3) mRNA expression in human keratinocytes cells treated with 10 mg/mL of hydroglycolic extract of *Piptadenia colubrina* (HEPC) for 0, 2, 6, 12, and 24 h. Data represent the mean ± SE of three individual experiments.

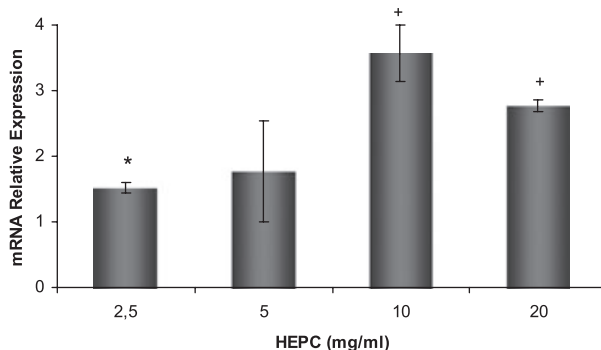


Figure 2 The effect of different concentrations of HEPC (2.5, 5, 10, and 20 mg/mL) in AQP3 mRNA expression in human keratinocytes cells demonstrated by real-time polymerase chain reaction analysis. Data represent the mean ± SE of three individual experiments. * $P < 0.05$, + $P < 0.01$ vs. control.

from plastic-surgery patients were treated with HEPC in the concentration of 10 mg/mL for 24 h and incubated with a commercial polyclonal anti-AQP3 antibody. The visualization showed an increase of expression of AQP-3 in the cell membrane of keratinocytes compared to the control group (Fig. 3), which could be attributed, in some degree, to increased trafficking of AQP3 to the membrane fraction.

Expression of p53 gene

To investigate an association between the epidermal expression of AQP3 and skin tumor formation, we measured the expression of p53 in the same samples and concentrations. Our results showed that treatment with HEPC did not modify the p53 expression in relation to control (2.5 mg/mL = 1.57 ± 0.34 ; 5 mg/mL = $0.92 \pm$

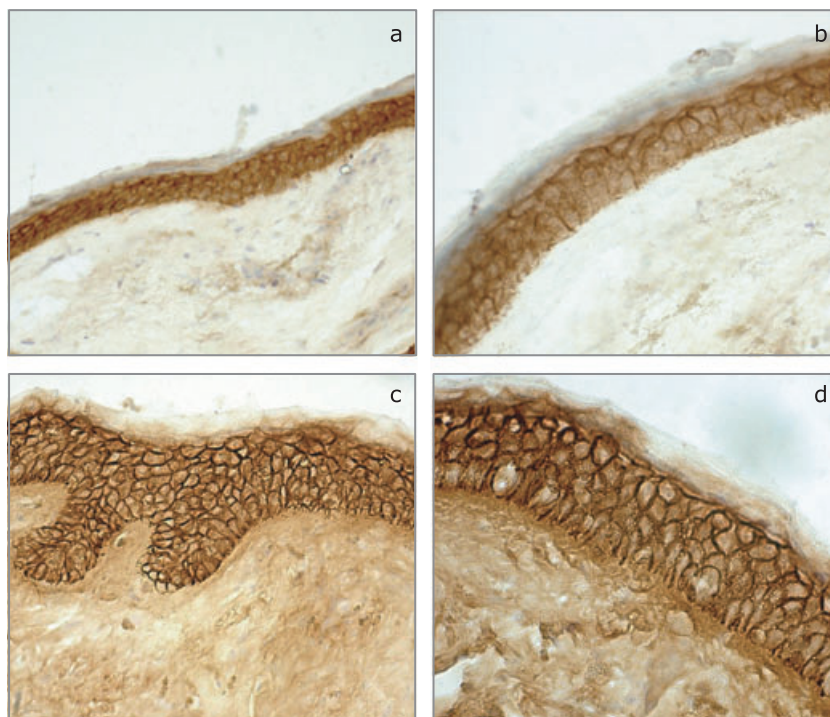


Figure 3 The effect of HEPC (10 mg/mL) on aquaporin-3 immunoreactivity in human skin explants for 24 h. Histological sections were immunostained by rabbit antiserum directed against AQP3 in untreated (a, c) and treated (b, d) explants (20 and 40× magnifications).

0.06; 10 mg/mL = 1.16 ± 0.46 and 20 mg/mL = 1.50 ± 0.46).

Expression of filaggrin and involucrin

The levels of expression of FLG and INV transcripts were determined by real-time PCR (Fig. 4) in different concentrations of HEPC (2.5, 5, 10, and 20 mg/mL). The expression of all these proteins was induced by HEPC in 6 h, and the proteins involved in formation of the cornified envelope, FLG and INV, showed peaks of expression with 10 mg/mL. The FLG gene was induced in all concentrations, whereas the INV gene was not induced in 2.5 mg/mL. When the concentration of 20 mg/mL was used, the expression of the genes decreased, indicating a possible saturation. We also investigated the expression of cell-envelope protein LOR and no significant alteration was induced by HEPC treatment (data not shown).

Quantification of glycerol in *stratum corneum*

D-Squame stripping for glycerol analyses was obtained from the SC of the volunteers' forearms on D1, D7, and D14 after 120 min following the application of GC-HEPC

or placebo. A time-dependent increase in glycerol index was found after the GC-HEPC or placebo treatment, particularly after 14 days. The glycerol concentration of GC-HEPC groups was significantly higher than those observed with all the corresponding placebos ($P < 0.001$) (Fig. 5).

Skin capacitance measurements

Corneometric index was taken prior to the first application (baseline) and at D1, D7, and D14 (T30, T60, and T120) after the treatment with GC-HEPC or placebo formulations. On D1 and D14, after 30, 60, and 120 min of GC-HEPC application, a significant improvement in the corneometric index ($P < 0.01$) was observed compared with the baseline values (T0) (Table 1). Additionally, on D1, the placebo group also showed an increase after 120 min ($P < 0.05$) (Table 1). In contrast, no significant changes over time could be observed in corneometric index on D7 after GC-HEPC application.

Discussion

Hydration of SC protects skin from dehydration and external hazards, being an important determinant of

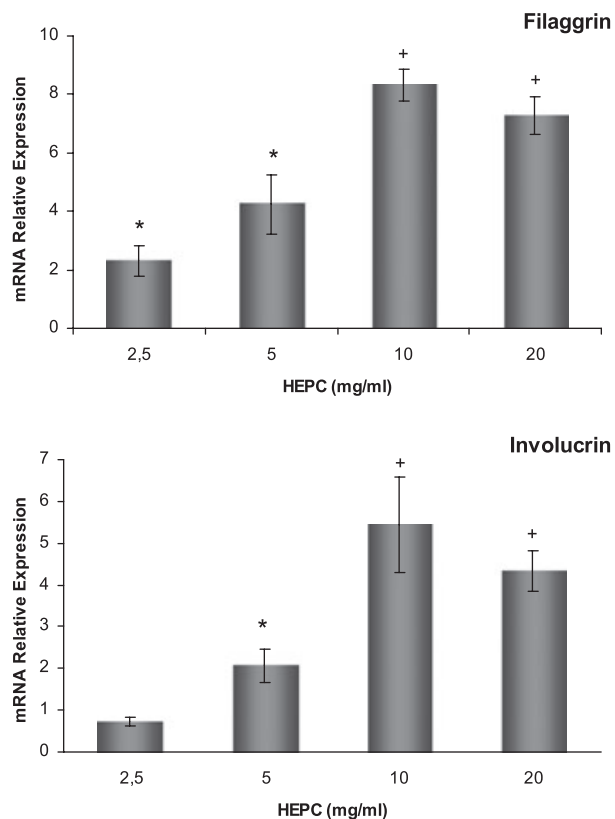


Figure 4 The effect of different concentrations of HEPC (2.5, 5, 10, and 20 mg/mL) in filaggrin and involucrin mRNA expression in human keratinocytes cells as demonstrated by real-time polymerase chain reaction analysis. Data represent the mean ± SE of three individual experiments. **P* < 0.05, +*P* < 0.01 vs. control.

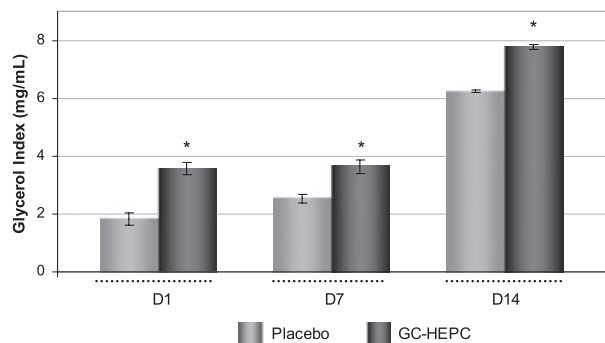


Figure 5 Glycerol quantification on *stratum corneum* of human subjects on day 1 (D1), D7, and D14 after 120 min from the application of gel-cream containing 5% of HEPC (GC-HEPC) or placebo. Data are expressed as mean ± SD. **P* < 0.001, in relation to respective placebo (ANOVA, Tukey).

skin appearance, metabolism, mechanical properties, and the barrier function.²⁸ A decrease in SC water content is found in a number of common skin diseases,

Table 1 Corneometric index performed in human subjects on day 1 (D1), D7, and D14 in baseline (T0), after 30 (T30), 60 (T60), and 120 (T120) min from the application of gel-cream containing 5% of HEPC (GC-HEPC) or placebo

	Hydrating effect (corneometric index)			
	T0	T30	T60	T120
D1				
Placebo	37.5 ± 7.5	38.5 ± 10.3	39.1 ± 9.5	41.3 ± 9.1*
GC-HEPC	38.8 ± 8.5	43.1 ± 10.8 [#]	43.3 ± 11.1 [#]	44.8 ± 9.1 [#]
D7				
Placebo	39.4 ± 5.9	40.4 ± 7.4	41.8 ± 7.2	40.5 ± 7.9
GC-HEPC	40.3 ± 6.2	40.6 ± 5.7	41.8 ± 8.1	41.1 ± 9.0
D14				
Placebo	41.3 ± 7.2	44.1 ± 7.8	45.0 ± 5.3	43.8 ± 6.2
GC-HEPC	41.0 ± 5.8	47.5 ± 5.2 [#]	47.0 ± 7.1 [#]	44.6 ± 5.2 [#]

Data are expressed as mean ± SD. **P* < 0.05, in relation to placebo T0; [#]*P* < 0.01, in relation to GC-HEPC T0 (Hypothesis *t*-test).

such as AD, eczema, psoriasis, senile xerosis, and hereditary ichthyosis.²⁹

Considering the role of AQP3 in SC hydration, which is believed to facilitate the formation of an osmotic gradient across viable layers of the skin,^{19,30} we investigated the ability of a HEPC to induce skin hydration by increasing AQP3 expression. The extract was standardized in total polysaccharides and preliminary studies were performed in human keratinocytes incubated with the concentration of 10 mg/mL of HEPC. The incubation was conducted over different time periods, and the peak of AQP-3 expression was observed after 6 h. After defining the time of exposure, we tested different concentrations of HEPC (2.5, 5, 10, and 20 mg/mL) and investigated the expression of AQP3. The increase in AQP3 expression was confirmed by the amount of mRNA and protein by RT-PCR and immunohistochemistry, respectively.

The marked increase of AQP-3 in skin sections could be explained, at least in part, to membrane translocation of AQP3 from the cytoplasmic fraction to the plasma membrane. Yasui *et al.*³¹ demonstrated an increased trafficking of AQP3 after epinephrine treatment in cell culture, which was supposed to be modulating by protein kinase C. Meanwhile, considering the significant increase of relative AQP3 gene expression (up to 3.5-fold in relation to control), an additional rise in protein immunostained in keratinocytes membrane could be attributed to HEPC treatment.

Recently, a correlation between AQP3 formation in epidermis and tumor cells was found in experiments on AQP3 null mice.³² Verkman³³ suggested that caution in use of any product that increases AQP3 expression, and

recommended animal tests by cosmetic companies in this particular case. In spite of these findings, expression of AQP3 has been increased for decades by the moisturizers that use mannitol and/or sorbitol in their compositions, with no observed relationship with cancer. The expression of AQP3 with HEPC that peaked at 6 h, returned to its basal level after 48 h of exposure (data not shown). To reinforce the safety of HEPC regarding cancer induction, we conducted experiments on p53 expression, as abnormal expression of p53 could be related to different cancer forms.^{34,35} A recent study demonstrated overexpression of p53 in actinic keratosis as well as in squamous cell carcinoma.³⁶ Our results showed that treatment with HEPC did not affect p53 expression, indicating no relationship between HEPC treatment and cancer.

The expression of other genes whose products are involved in skin hydration and integrity was also tested by real-time PCR. Keratinocytes were incubated for 6 h with a range of 2.5–20 mg/mL HEPC, and our results showed an increase in the expression of FLG (8.3-fold) and INV (5.4-fold) when incubated with 10 mg/mL HEPC.

Profilaggrin, FLG, INV, and LOR are proteins that form the epidermal cornified envelope, responsible for the association of intermediate keratin filaments and consequent enhancement of cohesion among corneocytes.³⁷ The epidermal protein profilaggrin, lately synthesized during epidermal differentiation, plays a key role in generating and maintaining SC flexibility and moisturizing.³⁸ This highly phosphorylated protein is quickly dephosphorylated and proteolyzed during the end of the transition from granular keratinocyte to the corneocyte. During the transition from the granular layer to SC, profilaggrin is converted to FLG by means of specific proteolysis and dephosphorylation.^{39–42} Monomers resulting from FLG are joined to keratin intermediate filaments and constitute the major structure responsible for cohesion.⁴³ In the SC, FLG – a cationic protein that aids the aggregation and subsequent disulfide bond among keratin filaments^{43,44} – is released from the interactions with keratin⁴⁵ and completely degraded into amino-acid constituents, such as PCA and urocanic acid. These amino acids constitute approximately 50% of NMFs and are retained within the mature SC corneocytes. NMFs are crucial for the maintenance of the epidermal moisture barrier, and they are reduced in dry skin, particularly during aging and seasonal alterations.⁴⁵ Whenever profilaggrin is found to be diminished, as in AD; or absent, as in vulgar ichthyosis, the quality of the SC is compromised because of failed NMF and consequent transepidermal water loss.^{46–48} In this

respect, a number of compounds that naturally occur in many medicinal herbs and plants, such as oleanolic acid, ursolic acid, and pentacyclic triterpenoids, have been shown to improve skin barrier function by increasing FLG expression.⁴⁹

The cornified envelope is also formed by protein precursors, such as INV and LOR, which provide a rigid bond among corneocytes during epidermal renewal. Involucrin was the first protein identified as a constituent of the epithelial cell envelope, and it is synthesized in the outer layers of stratified squamous epithelium during the final differentiation of the keratinocytes.⁵⁰ Human INV has been characterized as the favorite substrate for enzymes, such as transglutaminase, that are essential to cell-envelope formation, and is considered as the vital precursor for envelope formation and cohesion.⁵¹ Reduction of INV expression results in structural abnormalities of skin and hair.^{52,53}

The *in vitro* findings suggested increases in skin hydration and epidermal cohesion, which were clinically confirmed by the measurement of glycerol, as well as corneometric index. The glycerol content in SC showed a significant time-dependent increase after treatment of the volunteers with a GC-HEPC. Glycerol is transported into the epidermis from the blood via the AQP3 transporter located in the basal layer.^{27,29} Transgenic mice that lack this transporter show both abnormal SC hydration and reduced SC glycerol content, and the abnormal hydration can be largely reversed by exogenous glycerol administration.⁵⁴

Concurrently with the increase of glycerol content, a significant improvement in skin capacitance through corneometric assessment was observed, particularly at the onset and after 14 days of application, compared with baseline values.

The findings presented herein indicate that the extract of *P. colubrina* could promote cellular hydration and induces gene expression of FLG and INV, to provide greater corneocyte cohesion to keep water and other moisturizers in the skin. The increase of epidermal water channels (AQP-3) permits better distribution and maintenance of water, glycerol, and other skin NMFs, facilitating the repair and/or maintenance of barrier function by increasing corneocyte adhesion. Taken together, these data suggest that HEPC might be considered as an effective additive to skin care products formulation.

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