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Article

# Vegetable moisturizing raw material from "Caatinga" Brazilian biome: safety and efficacy evaluations of O/W cosmetic emulsions containing *Kalanchoe brasiliensis* extract

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The purpose of this study was to evaluate the safety of *Kalanchoe brasiliensis* extract, followed by the development of an oil in water emulsion containing the *K. brasiliensis* leaves extract and evaluating its clinical moisturizing efficacy. The formulations containing sodium acrylates/ Beheneth-25 methacrylate Crosspolymer (and) hydrogenated polydecene (and) lauryl glucoside and 0.5% of extract were prepared. The extract was considered as non-irritating through skin irritant tests. The stability testing was carried out in different conditions for 90 days. The skin hydration was measured by capacitance measurement and transepidermal water loss using biophysical techniques. The results indicate that the formulation containing 0.5% of extract increased the hydration of the stratum corneum up to 5 h after application on the forearm. The transepidermal water loss was reduced when compared to the untreated area and placebo area. Therefore, we can conclude that the increased skin hydration and protection of barrier function can be attributed to the *K. brasiliensis* extract. This research presents a new raw material from the Brazilian Caatinga biome and shows its possible application in the development of cosmetic products.

**Keywords:** Cosmetics/products. *Kalanchoe brasiliensis*/extract/safety evaluation. *Kalanchoe brasiliensis*/ moisturizing efficacy. Stability studies. Brazil/plants.

## INTRODUCTION

Nowadays the use of natural botanical extracts in cosmetic formulation is increasing. Their increased usage as ingredients in cosmetic formulations is due to their ethnomedicinal values and the fact that they are natural in origin. The herbal extracts consist of numerous compounds that provide benefits to the skin in regard to antioxidant, anti-inflammatory, emollient and anti-aging properties (Lall, Kishore, 2014).

In this context, *Kalanchoe brasiliensis* Cambess (*K. brasiliensis*) stands out, popularly known in Brazil as 'Coirama' (Fortes *et al.,* 2008), it is a plant from the Caatinga biome, which is an exclusive biome to Brazil, and it is used for treating wounds, abscesses, boils and it is also known for its action against ulcers and gastritis (Lisboa, Ferreira, Silva, 2006). The different chemical substances present in the juice of *K. brasiliensis* leaves are polysaccharides, flavonoids, ascorbic acid and other compounds (Fortes *et al.,* 2008). The published research studies attribute the moisturizing and antioxidant activities to these compounds in the vegetal extracts (Akhtar *et al.,* 2014; Faria, Damasceno, Ferrari, 2014; Stojiljkovic, Arsic, Tadic, 2016). However, the use of *K. brasiliensis* as a possible moisturizing agent in cosmetic products has not been evaluated.

The most predominant skin care products are moisturizers (Nolan, Marmur, 2012). Most of the moisturizers

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are emulsions which consist of two immiscible phases stabilized by an emulsifier. In several cosmetic formulation preparations, derivatives of acrylates are used as the main emulsifier or co-emulsifier (Anon, 2002). These moisturizing formulations are composed of polymers, which demonstrates the increasing usage of this chemical class in products for dermatological use (Valenta, Auner, 2014). Moisturizers act through an occlusive mechanism, forming an epicutaneous lipidic film impairing the evaporation of skin moisture that prevents water loss, this is the case of oils and lipids. The humectants, i.e., glycerin, urea, and sodium pyrrolidone carboxylic acid act by attracting water from the other layers of the epidermis to the stratum corneum (Dal'Belo *et al.,* 2006).

Topically applied moisturizers are expected to deliver appropriate ingredients from semisolid dosage forms like creams or gels to the skin (Kligman, 2011). Normal skin has a natural moisturizing factor (NMF) and water contents which can be affected by factors such as excessive washing, UV radiation, age, and environmental factors such as a cold winter, and these can lead to cases of xerosis (Bonté, 2011; Wan *et al.,* 2014).

The design of topical products and measuring their interaction with the stratum corneum has increased with the advent of non-invasive measurement techniques (Kligman, 2011). To substantiate the efficacy of cosmetic products, non-invasive biophysical techniques such as measuring skin hydration along with the average characteristics of the skin surface have been introduced into cosmetology. These will help to evaluate products under real time conditions of use (Gianeti, Mercurio, Maia Campos, 2013).

This study aimed to evaluate the safety of the extract, followed by the development of an oil in water (O/W) emulsion containing *Kalanchoe brasiliensis* Cambess extract and the evaluation of its clinical moisturizing efficacy.

## MATERIAL AND METHODS

### Material

The polymers sodium acrylates/Beheneth-25 methacrylate crosspolymer (and) hydrogenated polydecene (and) lauryl glucoside (Novemer™ EC-2) and acrylates/C10-30 alkyl acrylate crosspolymer (Carbopol® Ultrez-20) were received as gifts samples from Lubrizol (São Paulo, SP, Brazil). The other components such as Disodium EDTA were purchased from DEG – Importation of Chemical Products (Sorocaba, SP, Brazil); aminomethyl propanol (AMP-95) was purchased from Via Farma (São Paulo, SP, Brazil); phenoxyethanol (and) Methylparaben (and) Ethylparaben (and) Butylparaben (and) Isobutylparaben – (Phenova), were received as gift samples from Croda do Brasil (Campinas SP, Brazil). Distilled water was used in the studies.

### Preparation of *Kalanchoe Brasiliensis* cambess extract

The leaves of *K. brasiliensis* were obtained from the local cultivation of Macaíba city. The extraction from the leaves of *K. brasiliensis* was carried out by using the standardized method (Matos, 2009). The raw material (fresh leaves without stalks) was processed in an industrial blender (Faet, mod. FWAKE, Brazil) for 5 minutes with 50% ethanol, at a ratio of 1:1 w/v (vegetable: solvent). After blending, the extract was collected and vacuum filtration was carried out to collect the hydroalcoholic extract. The organic phase was evaporated at 45 °C under vacuum using a rotary evaporator (Büchi, mod. R3, Switzerland). Subsequently, the residue obtained after evaporation was subjected to freeze-drying process by freezing the extract at -20 °C followed by lyophilization (Liotop, mod. 202, Brazil) for 48 hours to obtain the lyophilized extract of *K. brasiliensis*.

### Raw materials safety evaluation

Cutaneous compatibility of *K. brasiliensis* was assessed according to the International Contact Dermatitis Research Group Guidelines. It was evaluated by a single application to 18 volunteers of age 22 to 46 years after having given their written informed consent. The volunteers involved in the study were with phototype skin types II to IV. The volunteers were selected according to the inclusion and exclusion criteria for the study. The study was carried out with the approval of the Research Ethics Committee of the University of Cuiabá (195.680/2013). For the safety evaluation, 3 patch test devices of 1 $cm^2$ embedded with 20 µL of 0.5 and 1.0% of *K. brasiliensis* extract and water (negative control) was applied on the upper back of normal skin. The patches were removed after 48 hours of application. The skin irritation responses were evaluated at 30 minutes, 24 and 48 hours after patch removal (Felippi *et al.,* 2012; Kligman, Wooding, 1967; Wilkinson *et al.,* 1970).

### Formulation development

EDTA sodium was dissolved in part of the water and acrylates/C10-30 alkyl acrylate crosspolymer was spread on the top of EDTA sodium solution and allowed

to hydrate. After hydration of the polymer, the system was subjected to mechanical agitation (Ika, mod.RW 20 digital, Germany) at 300 rpm for 30 minutes until the polymer was completely dispersed. During stirring, Aminomethyl Propanol was added to achieve pH 6.5.

Subsequently, sodium acrylates/Beheneth-25 methacrylate crosspolymer (and) hydrogenated polydecene (and) lauryl glucoside was added and homogenized at 300 rpm for 5 minutes to obtain O/W emulsion. Freeze-dried extract of *K. brasiliensis* was added to O/W emulsion by solubilization in a part of water that would be used in the formulation (for formulations with extract) preparation. In the final step, phenoxyethanol (and) methylparaben (and) ethylparaben (and) butylparaben (and) isobutylparaben was added and agitated at 300 rpm for 1 minute. Different formulations with varying compositions were prepared and the details of formulation compositions are shown in Table I. All formulations were prepared as described above and evaluated in three different batches.

## Preliminary and accelerated stability tests

### *Centrifugation test*

The centrifugation test for the prepared formulation was performed at 24 h after preparation. The formulation was centrifuged at 3000 rpm (Fanen, mod. 206 BL, Brazil) for 30 min at room temperature (Ferrari, Rocha-Filho, 2011). After centrifugation, appearance, homogeneity and organoleptic characteristics were evaluated macroscopically (Ferrari, Rocha-Filho, 2011).

### *Thermal stress*

The prepared emulsions were kept in a thermostatic heating bath (Logen Scientific, mod. LSBMLS 2006-2, Brazil). The emulsions were exposed to temperatures from 40 to 80 °C, with the temperature increased by 5 °C and then held for 30 minutes at each temperature. After exposing the emulsions to thermal stress, emulsions were allowed to reach room temperature (25 ± 2 °C), then, organoleptic characteristics, pH values, and electrical conductivity of thermally exposed emulsions were determined (Lima *et al.,* 2008).

### *Freeze-Thaw cycles*

Samples were exposed to 6 freeze-thaw cycles. In each cycle the samples were exposed to 4 ± 2 °C for 24 h (Consul, Facilite Frost free 300 L, Brazil) followed by exposure at 45 ± 2 °C/75 ± 5% RH for 24 h (Nova Etica, mod. 520-CLDTS 150, Brazil). At the end of the 6$^{th}$ cycle organoleptic characteristics, pH and electrical conductivity were determined (Lima *et al.,* 2008).

### *pH determination*

The emulsion was diluted at a ratio of 1:10 by weight in a test tube. After dilution it was vortexed and the pH (Hanna Instruments, mod. HI 21, Brazil) was determined by inserting the electrode into the 1:10 (w/w) diluted aqueous solution of the emulsion (Lima *et al.,* 2008).

### *Electrical Conductivity Determination*

Electrical conductivity (Logen Scientific, mod. CD-300-K1, Brazil) was determined at 25 ± 2 °C by inserting the electrode into the sample (Lima *et al.,* 2008).

Samples considered stable by a preliminary test were submitted to accelerated stability, where macroscopic analyses (appearance, homogeneity and organoleptic characteristics), pH and rheological behavior of the

**TABLE I -** O/W emulsion compositions (% w/w)

| Composition | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| Dissodium EDTA | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Acrylates/C10-30 Alkyl Acrylate Crosspolymer | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Aminomethyl Propanol | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 | 0.35 |
| Sodium Acrylates/ Beheneth-25 Methacrylate Crosspolymer (and) Hydrogenated Polydecene (and) Lauryl Glucoside | 2.0 | 2.0 | 3.0 | 3.0 | 4.0 | 4.0 | 5.0 | 5.0 |
| *Kalanchoe brasiliensis* extract | 0.5 | --- | 0.5 | --- | 0.5 | --- | 0.5 | --- |
| Phenoxyethanol (and) Methylparaben (and) Ethylparaben (and) Propylparaben (and) Butylparaben (and) Isobutylparaben | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 | 0.6 |
| Water Distilled | 96.05 | 96.55 | 95.05 | 95.55 | 94.05 | 94.55 | 93.05 | 93.55 |

Notes: F = Formulation.

formulations were evaluated at 24 h after preparation. Later the samples were stored at different conditions, i.e., 4 ± 2 °C (Consul, Facilite Frost free 300 L, Brazil); 25 ± 2 °C (room temperature), and 45 ± 2 °C/75 ± 5% RH (Nova Etica, mod. 520-CLDTS 150, Brazil) for 90 days. The samples stored in these conditions were analyzed on the 30th, 60th and 90th day (Ferrari, Rocha-Filho, 2011).

### *Rheological behavior determination*

The viscosity was determined by using cone and plate viscometer (Brookfield-model DV III- USA) with Rheocalc software version 3.0.1. The rheology was determined at 25 ± 2 °C using spindle CP 52, 0.5 g of the sample was taken and the viscosity was measured by progressively increasing the rotational speed from 1-3 rpm at a rate of 0.5 rpm and vice versa for ascending and descending curves. The flow index and consistency index were calculated by the Power Law mathematical model (Ferrari, Rocha-Filho, 2011).

### *In vivo* moisturizing efficacy evaluation

This study was approved by the Research Ethics Committee of the University of Cuiabá (195.680/2013). A total of 18 volunteers of ages from 18 to 50 years old, with no previous history of skin disease were included in this study after having given their written informed consent. This study was designed as a one-sided blind, placebo-controlled study. The volunteers were instructed not to use any cosmetic products for two weeks before and on the day of the experiment, except cleaning products like soap (Maia Campos *et al.,* 2012). Prior to all measurements, volunteers were left in the room for at least 30 min in order to allow full skin adaptation to the room's temperature (21 ± 2 °C) and relative humidity (60% ± 5) (Maia Campos *et al.,* 2012).

Four sites (9 $cm^2$ each) on the volunteers' forearm skin were chosen (C1 to C4): C1 as the negative control where only measurements were taken, and the others (C2 to C4), where different formulations were applied. C2 was applied with F4 formulation (vehicle), C3 was applied with the formulation containing 0.5% of *K. brasiliensis* extract (F3), and a commercial moisturizer (Hidrafil® gel) was used as positive control in C4. The positive control was used to carry the experiment parameters. All formulations were applied to the skin surface at a dose of 2 mg/$cm^2$ with a light massage for approximately 10s (Maia Campos *et al.,* 2012). Stratum corneum moisture content was determined by non-invasive biometrical measurements using a skin capacitance meter (Corneometer® CM 825-Courage & Khazaka Electronic GmbH, Germany), whereas the transepidermal water loss (TEWL) was determined by an evaporimeter (Tewameter®TM300-Courage & Khazaka Electronic GmbH, Germany). TEWL was recorded after 30 seconds of probe equilibration on the skin and then recorded for 2 min.

The baseline measurements (control area - the region at which no formulation was applied) of skin hydration were taken at least after 30 min of acclimatization to the standard climatic conditions. The skin capacitance and TEWL were determined at 0, 1, 2, 3, 4 and 5 h after a single application of the formulations to the areas C2-C4 and to the control field without formulation (C1). For skin capacitance measurement, nine readings were taken in each field. For TEWL evaluation, one reading in each field was taken (Akhtar *et al.,* 2014; Maia Campos *et al.,* 2012).

### Statistical analysis

The statistical data analysis of the *in vivo* moisturizing efficacy of the formulations was determined by analysis of variance (ANOVA) using GraphPad Prism 5 with statistical significance of $p < 0.05$, followed by Dunnets test, for stability tests. Then, analysis of variance (ANOVA) followed by Tukey and Bonferroni were used to compare the formulations to skin capacitance meter and transepidermal water loss (TEWL) respectively for in vivo moisturizing efficacy evaluation (Ribeiro *et al.,* 2015).

## RESULTS AND DISCUSSION

### Raw material safety evaluation

After the *in vitro* cytotoxicity and phototoxicity safety tests (results not shown) of the *K. brasiliensis* extract, the preliminary skin compatibility test was evaluated. Cutaneous compatibility test mimics the first contact of the raw material with human skin, thus, predicting possible irritation and/or allergy mechanisms, indicating safety when used normally or in reasonably foreseeable conditions (Anvisa, 2012).

The cutaneous compatibility of *K. brasiliensis* study was carried out and potential risks of the extract as a cosmetic raw material for topical use was determined in a predictive way. In the skin irritation tests, none of the volunteers showed any signs of erythema, edema or papules on the skin. Therefore, the extract was considered as non-irritating. However, to obtain more robust data, it is necessary to do skin compatibility and sensitization tests by repetitive application and to test on a large number of volunteers.

## Formulation development

The sodium acrylates/ Beheneth-25 methacrylate crosspolymer (and) hydrogenated polydecene (and) lauryl glucoside, was chosen as co-emulsifier, stabilizer and rheology modifier because of its properties. This polymer was added to acrylates/C10-30 alkyl acrylate crosspolymer which further improves the viscosity, resistance to electrolytes and aesthetics in the presence of high levels of electrolytes (Ollagnier *et al.,* 2012). The inorganic electrolytes are reported to have a considerable effect on the emulsion stability (Jiang *et al.,* 2013). Accordingly, emulsions were developed with reduced oil content, stabilized by hydrophilic polymer, with or without adding the *K. brasiliensis* extract as shown in Table I.

The extraction process was previously standardized and High Performance Liquid Chromatography coupled with Diode Array Detection and Electrospray Mass Spectrometry (HPLC-DAD-MS/MS) were used on the chemical characterization of markers by the researchers (Fernandes *et al*., 2016). The authors observed the presence of a HPLC-DAD-MS/MS chromatographic profile similar to flavonoid glycosides derived from patuletin and eupafolin (Fernandes *et al*., 2016). This standardized extract was used in our research.

The *K. brasiliensis* lyophilized leaves extract powder was yellow in color and was a hygroscopic powder. The emulsions without extract were white and with extract were yellow in color.

To test the stability, the O/W emulsions prepared were subjected to preliminary stability tests (Table II). These tests highlight the earliest signs of instability in the prepared emulsions (Velasco *et al.,* 2008).

## Preliminary and accelerated stability tests

### *Preliminary stability tests*

The stability of an O/W emulsion is defined as the resistance by the dispersed water droplets against coalescence. It is dependent on a variety of factors including the presence or absence of the emulsifying agent, viscosity (influenced greatly by temperature), specific gravity, water content and the age of the emulsion (Chen, Tao, 2005). Stability tests at accelerated conditions are necessary to accelerate changes that may occur in the marketed conditions and to determine the expiry of the products.

These tests have a good probability of success (Anvisa, 2005). The formulations were subjected to a centrifugation test and remained macroscopically

**TABLE II** - Results of preliminary stability tests of emulsions with or without *Kalanchoe brasiliensis* extract

| Parameters | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 |
|---|---|---|---|---|---|---|---|---|
| | | | | **After 24 h** | | | | |
| Centrifugation | H | H | H | H | H | H | H | H |
| pH | 5.96±0.04 | 6.89±0.03 | 6.57±0.06 | 7.27±0.05 | 6.82±0.02 | 7.44±0.12 | 6.91±0.03 | 7.40±0.02 |
| Electrical conductivity (mS/cm) | 3.58±0.50 | 2.43±0.09 | 5.10±0.32 | 3.32±0.06 | 6.08±0.09 | 4.11±0.06 | 6.99±0.29 | 5.30±0.18 |
| Minimal apparent viscosity (cP) | 2715±702 | 17885±1448 | 12413±821 | 22688±148 | 17521±396 | 25161±1303 | 20472±1538 | 27745±388 |
| | | | | **After TS** | | | | |
| pH | 5.91±0.06 | 6.86±0.04 | 6.63±0.13 | 7.27±0.06 | 6.73±0.08 | 7.46±0.06 | 6.95±0.03 | 7.48±0.03 |
| Electrical conductivity (mS/cm) | 4.51±0.10 | 2.81±0.20 | 5.46±0.40 | 3.40±0.25 | 6.90±0.20* | 4.95±0.12* | 7.06±0.06 | 5.20±0.10 |
| Minimal apparent viscosity (cP) | 3399±1196* | 18214±1139* | 12997±2635 | 21199±926* | 17720±1910 | 26259±452 | 21269±1046 | 29526±1598 |
| | | | | **After FTC** | | | | |
| pH | 5.94±0.05 | 6.93±0.005 | 6.56±0.05 | 7.26±0.08 | 6.79±0.03 | 7.4±0.04 | 6.93±0.04 | 7.46±0.005 |
| Electrical conductivity (mS/cm) | 3.95±0.40* | 2.67±0.10* | 5.68±0.29* | 3.61±0.06* | 6.68±0.19* | 4.67±0.01* | 6.47±0.08* | 4.83±0.10* |
| Minimal apparent viscosity (cP) | 2796±1349* | 16771±585* | 12321±364 | 20950±732* | 16352±964 | 24139±2248 | 20123±1139 | 2803±1332 |

Notes: FTC = Freeze-Thaw Cycles; TS = Thermal Stress; H = Homogeneous; F1, F3, F5 and F7 = Sample with 0.5% *K. brasiliensis* extract; F2, F4, F5 and F8 = vehicle. * $p < 0.05$ compared to the initial time.

homogenous and no phase separation was observed after centrifugation at 3000 rpm for 30 min (Table II). The absence of phase separation is a positive factor in the stability evaluation of an emulsion; the phase separation affects all the characteristics of emulsions (Chen, Tao, 2005).

There was no significant difference ($p < 0.05$) in pH (shown in Table II) before and after freeze-thaw cycles and thermal stress tests and the emulsions were even homogenous macroscopically, which indicates that the emulsions were stable.

The common procedure to assess the phase inversions of emulsions is by measuring the electrical conductivity (Tadros, 2004). This information is important in the development of cosmetic formulations. The electrical conductivity values before and after freeze-thaw cycles were significantly different in all formulations, whereas in F5 and F6 formulations the electrical conductivity was significantly different after thermal stress (Table II). An increase in the electrical conductivity is a sign of the instability of the emulsion, but the time needed to reach instability depends on the emulsion type. It is not possible to establish a relationship between the kinetics of aging at different temperatures (Masmoudi *et al.,* 2005).

In order to understand the formulation profile, determination of minimum apparent viscosity is necessary. This method is a simple and effective method in the comparison of formulation profiles. The decrease in emulsion viscosity with time may provide information about the coalescence of emulsion (Tadros, 2004). After exposure to the above mentioned stress studies there is no significant change in all the formulations' characteristics and changes were seen in F1, F2 and F4 formulations (Table II). This can occur due to the evaporation of water from the formulations during storage, and the storage temperature often has indirect effects on emulsion viscosity (Chen, Tao, 2005).

O/W emulsions in which plant extract was incorporated had shown altered rheological behavior, which may influence the physical and chemical stability (Isaac *et al.,* 2008). The formulations with extract (F1, F3, F5 and F7) showed lower viscosity when compared to formulations without extract. This can be attributed to the reduction of solvency of polymeric chains upon addition of electrolyte, which results in the reduction of the adsorbed layer thickness (Tadros, 2004). Yapar, Ynal and Erdal (2013) observed viscosity reduction of the formulations after incorporating the plant extract in their studies, whereas they found that the formulation was stable even after incorporation of extract. Similarly, stable formulations were developed by incorporation of extracts, such as *Prunus padus* bark extract (Hwang *et al.,* 2014), and *Opuntia ficus indica* Mill. in different cosmetic vehicles (Ribeiro *et al.,* 2015).

The preliminary stability studies were useful to screen the prepared formulations (Lima *et al.,* 2008). From the above results all the formulations can be considered stable and can further proceed to accelerated stability testing. However, the accelerated stability studies were carried out for F3, F4, F5 and F6 formulations.

### *Accelerated stability testing*

The stress to which formulations are exposed to in accelerated stability studies is lower when compared to preliminary stability studies. The accelerated stability is useful to estimate the shelf life of the products (Anvisa, 2005).

F3 and F5 pH values were changed after 90 days' exposure, but remained between 6.30 and 6.83 (Table III), which can be considered as acceptable to avoid the risk of skin irritation (Yapar, Ynal, Erdal, 2013). Mahmood *et al.* (2013) showed that formulations containing green tea extract stored at 40 °C showed a slight variation in pH due to degradation of the compounds. The reasons for the change in pH were not well understood, but one of the most accepted reasons is the dilution effect of polyphenols based on interactions with emulsifiers (Zillich *et al.,* 2015). The change in the pH values in our formulations is within in the variation of 10% in all formulations, thus according to Anvisa (2005) the formulations can be considered stable.

The relation between storage temperature and emulsion stability is well established (Ribeiro *et al.,* 2015). In the emulsions stored at 4 °C, no change in the color was observed (Table III). However, the results of F4 and F6 showed significant variations in the apparent viscosity, as shown in Table III. There were no significant differences in the minimal apparent viscosity of F3 and F5 formulations, indicating good stability. The formulation F4 and F6 showed significant change in viscosity, but pH did not change. These results showed that apparent viscosity decreased when the extract was added, whereas the stability of emulsions containing the extract did not show significant difference when compared to emulsions without extract.

The flow rate and consistency were calculated using the Power Law model. The flow index analysis indicates the non-Newtonian behavior of the emulsions. The flow index values were below "1", indicating pseudoplastic behavior of the emulsions (Ferrari, Rocha-Filho, 2011). The formulations containing polymers usually show pseudoplastic behavior (Yapar, Ynal, Erdal, 2013).

**TABLE III -** Results of accelerated stability tests of O/W emulsions with and without 0.5% of *Kalanchoe brasiliensis* extract

| Test | F3 | | | | F4 | | | | F5 | | | | F6 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Initial 24h | 90 days | | | Initial 24h | 90 days | | | Initial 24h | 90 days | | | Initial 24h | 90 days | | |
| | | 4°C | 25°C | 45°C | | 4°C | 25°C | 45°C | | 4°C | 25°C | 45°C | | 4°C | 25°C | 45°C |
| Characteristic | H | H | SM | SM | H | H | SM | SM | H | H | SM | SM | H | H | SM | SM |
| pH | 6.36 ± 0.12 | 6.70 ± 0.09* | 6.35 ± 0.22 | 6.58 ± 0.11* | 7.02 ± 0.10 | 7.68 ± 0.13* | 7.39 ± 0.25* | 7.63 ± 0.11* | 6.83 ± 0.09 | 6.74 ± 0.05* | 6.82 ± 0.07* | 6.80 ± 0.07 | 7.33 ± 0.05 | 7.66 ± 0.09* | 7.68± 0.08* | 7.17 ± 0.06* |
| Minimal apparent viscosity (cP) | 9774 ± 680 | 11339 ± 236 | 11380 ± 1111 | 8620 ± 1094* | 20.791± 554 | 22980 ± 392 | 22764 ± 1089 | 20236 ± 1363* | 15139± 524* | 16305 ± 1032 | 17337 ± 1548 | 12163± 1.939* | 24881 ± 2091 | 26653± 889* | 24980 ± 2154* | 23077 ± 1165* |
| Flow index | 0.23 ± 0.02 | 0.23 ± 0.01 | 0.24 ± 0.02 | 0.24 ± 0.02 | 0.19 ± 0.02 | 0.19 ± 0.01 | 0.19 ± 0.01 | 0.18 ± 0.07 | 0.22 ± 0.01 | 0.28 ± 0.02 | 0.21 ± 0.01 | 0.25 ± 0.01 | 0.19 ± 0.17 | 0.17 ± 0.06 | 0.21 ± 0.02 | 0.21 ± 0.01 |
| Consistency index | 38266 ± 3396 | 39925 ± 15227 | 44035 ± 5985 | 33632 ± 5289 | 87295± 5433 | 96965 ± 9761 | 95455 ± 5269 | 73848 ± 28406 | 61099 ± 2674 | 64517 ± 5435 | 70350 ± 7554 | 46428 ± 7984 | 105152± 9103 | 100450 ±37847 | 102895 ± 7886 | 94244 ± 5743 |

Notes: * p-value ≤ 0.05; H – Homogeneous; SM – Color Slightly Modified; F3 and F5 are formulations with 0.5% *Kalanchoe brasiliensis* extract; F4 and F6 are vehicle.

Marcon, Wagemaker and Maia Campos (2014) evaluated the rheological properties of formulations with and without pearl extract. They exhibited low variations in apparent viscosity during the stability study at room temperature and during heat stress conditions at 37 °C and 45 °C. The results indicate that all the formulations were physically stable.

All the above mentioned parameters were evaluated for all the formulations. The results indicate that F3, F4, F5 and F6 were stable. However, F3 and F4 were statistically compared to F5 and F6 formulations and the results indicate that, F3 and F4 are significantly more stable than F5 and F6. Therefore, we chose F3 e F4 to evaluate the clinical efficacy.

## Evaluation of *in vivo* moisturizing properties

The main function of the skin is to act as a barrier, prevent water loss and protect the body against external factors. More knowledge about the mechanisms of the effect of different ingredients on the skin is required, because the actives and the excipients may both have unexpected influences on the structure and function of the skin (Kligman, 2011). To authenticate the efficacy of cosmetic products, non-invasive biophysical techniques have been used in cosmetology to evaluate products under real time conditions of use (Gianeti, Mercurio, Maia Campos, 2013). The skin hydration was measured by using a Corneometer® CM 825 which measures the capacitance, one of the basic measurements for all dermatological and cosmetic applications.

The clinical consequences of potential differences in the efficacy of moisturizers include differences in hydrating properties, effects on visible dryness symptoms, and the possibility of reduced risks of dermatitis (Kligman, 2011). At the beginning of analysis (basal), the mean volunteer hydration was under 40 arbitrary units (Figure 1). This profile is related to dry skin. The results show a significant increase ($p < 0.05$) of skin hydration after the application of formulations on the skin and this effect lasted for 5 h (Figure 1).

After application of product, there was a significant difference ($p < 0,05$) between F3 (with 0.5% of extract) and F4 (without extract) formulations moisturizing efficacy

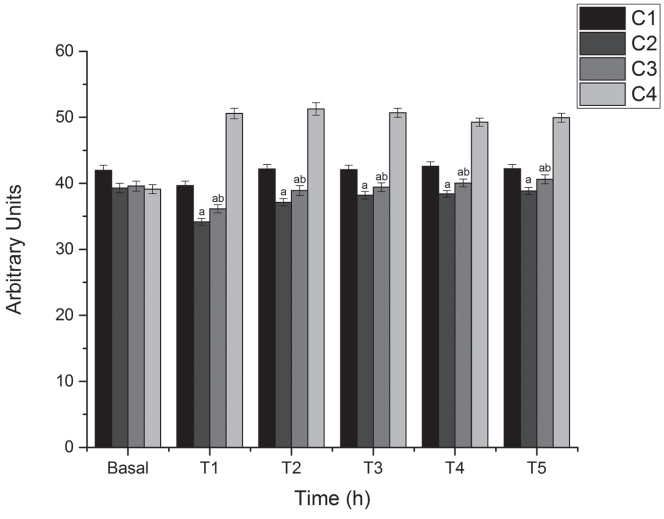


**FIGURE 1 -** *Stratum corneum* hydration values measured before and at 1, 2, 3, 4 and 5 hours after single application of formulations (Mean ± SE). Notes: a = p-value < 0.05 for Anova + Tukey post-test vs. negative control. b = p-value < 0.05 for Anova + Tukey post-test vs. C2. C1 – Negative control; C2 – vehicle; C3 – Vehicle + 0.5% *Kalanchoe brasiliensis* extract; C4 – Positive control. T1 – After one hour; T2 – After two hours; T3 – After three hours; T4 – After four hours; T5 – After five hours.

after 5 h of test, proving the moisturizing potential of *K. brasiliensis* extract. Some of the reports in the literature show that *K. brasiliensis* contains several polysaccharides, flavonoids, ascorbic acid and other elements (Mourao *et al.,* 1999). Thus, the increase in skin moisture can be attributed to its chemical composition.

Akhtar *et al.* (2014) studied formulations containing 3.0% *Crocus sativus* (Saffron) concentrated extract and the vehicle. In this study, O/W emulsion containing extract significantly improved the skin moisture. This increase in skin moisture content was due to the phenolic compounds quercetin and kaempferol, which have been proved to be potent moisturizers. These results corroborate with the results of our study.

A commercial moisturizer which contains moisturizing complex made by skin hydration factors was used as a positive control in this study and showed significant hydration of stratum corneum after the first hour, and maintained the moisturizing effect for at least 5 h after application (Figure 1). This effect was expected because the market formulation has different moisturizers in it.

We noted a statistically significant variation in TEWL and barrier function compared with initial measurements, notably a decrease in TEWL was observed (Figure 2). According to Akhtar *et al.* (2014), the presence of phenolic compounds can decrease the TEWL. Our formulations were able to maintain the water loss at under 10 g/mm$^2$·h in all the volunteers, which indicates very healthy skin as observed by Courage-Khazaka, and collaborated in the maintenance of skin health. Other studies attributed the decrease in TEWL and skin protection barrier to polysaccharides and flavonoids present in the plant extract (Faria, Damasceno, Ferrari, 2011; Yotsawimonwat *et al.,* 2010).

The protective effect of skin observed in C3, and the consequent reduction in TEWL, was higher than in C1 (negative control) at 1, 3 and 5 h of experiment, however, the vehicle was statistically significant at 1, 3 and 4 h when compared with C1.

Dal`Belo *et al.* (2006) noted that formulations supplemented with freeze-dried *Aloe vera* extract significantly increased the water content of the stratum corneum but with no change in the TEWL when compared with the vehicle. We observed that with our formulations, despite using a freeze-dried extract, change in TEWL was observed when compared with the vehicle. Gianeti, Mercurio and Maia Campos (2013) evaluated the moisturizer efficacy of formulations containing green tea extracts and found significant skin moisturizing effects, but the TEWL was not statistically significant. In this sense, we hypothesize that formulations containing *K. brasiliensis* extract act through a humectant and occlusion mechanism.

The clinical efficacy results showed that *K. brasiliensis* is a potential moisturizing extract indicating that this extract can be used as raw material for the development of moisturizing cosmetics.

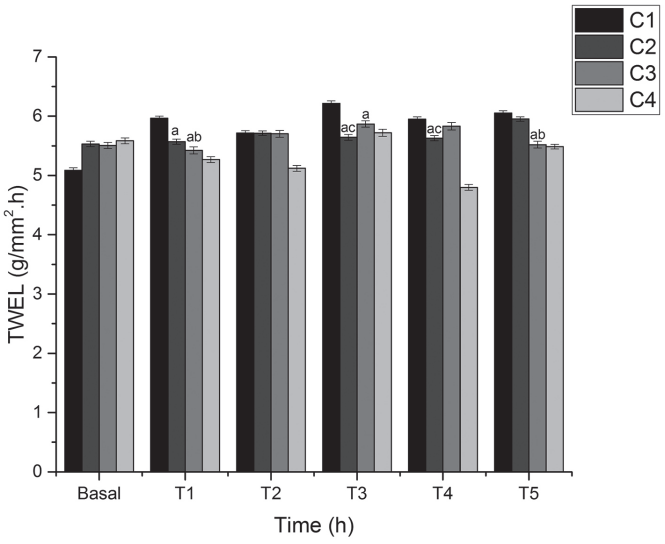


**FIGURE 2 -** Transepidermal water loss (Mean ± SE) measured before and 1, 2, 3, 4 and 5 hours after a single application of formulations. Notes: a =p-value < 0.05 for ANOVA + Bonferroni post test vs. negative control; b=p-value < 0.05 for ANOVA + Bonferroni post test vs. C2. C1 – Negative control; C2 – vehicle; C3 – vehicle + 0.5% *K. brasiliensis* extract, and C4 – Positive control. T1 – After one hour; T2 – After two hours; T3 – After three hours; T4 – After four hours; T5 – After five hours.

## CONCLUSIONS

The study collaborated with the cosmetic market trends, contributing to produce a cosmetic product containing plant extract from Brazilian *Caatinga* biome. As well as this, a polymer was identified that is still little used on the market, capable of stabilizing the systems containing botanical extracts. The O/W emulsion containing 0.5% of *K. brasiliensis* leaves extract significantly increases water content in the stratum corneum for 5 h, and decreases TEWL when compared to vehicle formulation, demonstrating the moisturizing effect of *K. brasiliensis* leaves extract. This work presents a new raw material for the cosmetics industry with moisturizing properties, which can be useful to develop other products with moisturizing proprieties. Overall, we have shown the *K. brasiliensis* extract's efficacy as a raw material, and the formulations containing it have potential use in the field of cosmetics.

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