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Development of a topical gel containing dried extract of *Ipomoea pes-caprae brasiliensis* (L.) R. Br. (Convolvulaceae)

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Gel formulations containing *I. pes-caprae* spray dried extract (SDE) at 0.11 and 0.18%, were developed with three types of polymers Ce = Cellosize® QP 100, Ca = Carbopol® ultrez, and Ar = Aristoflex® AVC. The pH, sensory characteristics, weight loss and the viscosity profile of gels were analyzed at times (t) t_0, t_{90} and t_{180} (24 h, 90 and 180 days after their production, respectively), in ambient temperature (AT = 25 ± 2 °C), in the fridge (F = 5 ± 2 °C) and in the oven (OV = 40 ± 2 °C). Isoquercitrin assay were analyzed by High-performance liquid chromatography (HPLC), after 24h and 360 days (in AT and in the F). None of these formulations nor the spray dried extract (SDE) showed any level of cutaneous irritation in agarose overlay test. The greatest polymers were Ca and Ar in relation to their sensory characteristics and viscosity.

Key words: *Ipomoea pes-caprae*, gel, spray dried extract, stability, cytotoxicity.

INTRODUCTION

Ipomoea pes-caprae brasiliensis (L.) R. Br. (Convolvulaceae), popularly known in Brazil as “Salsa da Praia”, is a typical sandbank plant, which naturally occurs in tropical and sub-tropical coastal regions worldwide (Christman, 2000; Castellani and Santos, 2006). It has been used in folk medicine to treat wounds caused by jellyfish venom (Pongprayoon et al., 1991a, 1991b). Antinociceptive and anti-inflammatory properties (De Souza et al., 2000), among a wide range of pharmacological activities like antioxidant, antispasmodic, antihistaminic, insulogenic and hypoglycemic activities have been reported in an extended review (Manigaunha et al., 2010). From aerial parts, both methanol and hydro-alcoholic extracts have already revealed potent and significant anti-nociceptive and anti-inflammatory properties, in the abdominal contortions models and the pleurisy models, respectively (De Souza et al., 2000; Vieira et al., 2013). Among the vehicles for topical use, the choice for

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the choice for gels is mostly due to the absence of grease substances, the sensory of refreshment, as well as the tendency to spread easily by friction (Chorilli et al., 2006). Some of the most common products used to producing gels are the polymers as hydroxyethyl cellulose, acrylic acid polymers and ammonium acryloyl dimethyltaurate/ carboxyethyl acrylate co-polymers. The cellulose-based derivatives as hydroxyethyl cellulose, like Cellosize®, are an important class of polymers with many industrial applications.

It is an important non-ionic biodegradable polysaccharide that has interesting hydrophilic, rheological, as thickener or binder, and antibacterial properties, producing transparent films (Martinez-Richa, 2012). Carbopols® are insoluble acrylic acid polymers, which became stiff gels upon neutralization in aqueous medium. Carbopol® Ultrez presents better dispersion properties and a potential wide range of applicability in the pharmaceutical field, with high viscosity at low concentration, compatibility, bioadhesion and good user acceptance. Aristoflex® AVC correspond to a co-polymer of ammonium acryloyl dimethyltaurate/ carboxyethyl acrylate, forming transparent gels in aqueous systems, with good spreading and stability over pH from 4 to 9, promoting a soft sensation in the skin (Tamburic & Craig, 1995; Contreras et al., 2001). Taking into account the traditional use and the pharmacological results which have been related for ethanol extract of *I. pes-caprae* aerial parts, the present work was designed to develop topical gel formulations containing *I. pes-caprae* spray dried extract (SDE), which could be safe and proper to be used for the treatment of algesic and inflammatory skin diseases.

MATERIALS AND METHODS

Isoquercitrin (> 95% of purity by HPLC) was purchased from Sigma Aldrich (St. Louis, Missouri, USA). Colloidal silicon dioxide and Aristoflex® AVC were purchased from Pharma Special (São Paulo, Brazil). Cellosize® QP 100, imidazolinidilureia and Nipagin® were purchased from Via Farma (Ipiranga-SP, Brazil). Carbopol® Ultrez and propylene glycol were purchased from Galena (Campinas-SP, Brazil), 2,2'-azino-di-(3-ethyl benzthiazoline-6-sulphonic acid (ABTS) from Fluka (São Paulo, Brazil), sodium dodecyl sulphate (SDS) from Biomatec (Rio de Janeiro, Brazil), minimum essential medium (MEM), phosphate buffered saline (PBS) from Gibco (São Paulo, Brazil) and $NaBH_4$ from Nuclear (São Paulo, Brazil). The ethanol was analytical grade, purchased from Dinâmica (Diadema, São Paulo Brazil). All other solvents were analytical grade. Methanol and acetonitrile (J.T.Baker®, Phillipsburg, USA) were HPLC grade. High purity water (18 MΩ) was provided by an Easy pure water system (Waltham, Massachusetts, USA) fed with reverse osmosis water.

Plant material

The botanical material was collected from Esplanada beach (Jaguaruna, Santa Catarina, Brazil) in February 2007. A voucher was deposited at the Barbosa Rodrigues Herbarium (Itajaí, Santa Catarina, Brazil) under number V.C. Filho 009. The leaves, stems and other aerial parts (flower buds, seeds and flowers) were manually separated, cleaned and dried in an air oven at 35 ºC until moisture stabilization. After drying, plant material was ground in a hammer mill (outlet sieve = 3 mm) and the average size of the particles was determined by sieving (Allen et al., 2007).

Extractive solution

At first, the extractive solution (ES) was obtained by maceration over 7-day period, with 12.5% (*w/v*) plant ratio in alcohol 70 °GL, as previously related by Vieira et al. (2013). Before spray drying, ES was pre-concentrated under vacuum until 40% reduction of its original volume. The percentage of dried extractives in ES was determined gravimetrically at 105 °C, according to the method described in the Brazilian Pharmacopoea (Brasil, 2010). Each 5.0 g of samples were analyzed in quadruplicate.

Spray dried extract

The spray dried extract (SDE) of *I. pes-caprae* (SDE) were obtained in a Mini-Spray Dryer (Büchi 290, Flawil, Switzerland), with two components nozzle and co-current flow, nozzle aperture of 0.7mm, spraying pressure of 5 bar, flow of 4 mL min^{-1}, inlet temperature of 170°C, aspiration of 90% and air flow rate of 400 NL h^{-1}, by co-drying with 20% (*w*/*w*) of Aerosil® (in relation to dry residue of previous concentrated extractive solution). The residual humidity was determined in an infrared weight scale (MettlerToledo, LJ16, Switzerland) and expressed as the average percentage of three determinations. The morphology of the dried product was determined using scanning electron microscopy (SEM) performed on a PhilipsXL30 microscope, and representative samples of selected drying processes were put over a metallic support covered with colloidal gold under vacuum.

Gel formulations

To develop gel formulations, three types of polymers were used (Ce = Cellosize® QP 100, Ca = Carbopol® Ultrez and Ar = Aristoflex® AVC) in two concentrations each (Ce = 1 and 2%; Ca = 1 and 2%; Ar = 2.5 and 5%) and two SDE concentrations (0.11 and 0.18%). Each formulation was composed by SDE, propilenoglycol (0.2%), Nipagin® (0.2%), imidazolidinyl urea (0.3%) and purified water. The pH of all formulations was adjusted to 5.5 to 6.5 by adding citric acid or sodium hydroxide solution, respectively. The gels were stored in aluminum tubes, and placed in different conditions as described in the accelerated stability study.

Accelerated stability study

The pH, sensory characteristics, weight loss, viscosity profile of gels were analyzed at different times (t) (t_0, t_{90} and t_{180} corresponding to 24h, 90 and 180 days respectively, after their production), at ambient temperature (AT = 25 ± 2 °C), in a fridge (F = 5 ± 2 °C) and in an oven (OV = 40 ± 2 °C). Chromatographic profile and isoquercitrin contents, in the highest polymer and SDE concentrations, were analyzed by HPLC at time 0 (t_0) and after 360 days (t_{360}) and at AT and F. Gel formulations which demonstrated the best performance in the stability studies, as well the SDE itself, were evaluated by *in vitro* cytotoxicity through the agarose overlay

method.

Viscosity analysis

Gel samples (1 g) were analyzed in a rotational viscometer, cone-plate sensor Haake VT 550 PK 1 1°, 25 °C, coupled to a circulating water bath (25 ± 1 °C). Flow curves were analyzed to characterize the viscosity (mPa.s), flow index and thixotropy of the formulations.

HPLC analysis

A previous validated method (Vieira et al., 2013) for assay of isoquercitrin in SDE sample, that showed linearity in the range of 10 to 110µg mL^{-1} (r^2 > 0.99), repeatability and intermediate precision with relative standard deviation (RSD%) < 15%, and 97.57 to 107% of isoquercitrin recovery was used in this study in order to preview the chemical stability of gel formulations, after extraction steps. A Shimadzu LC-10AD HPLC system (Shimadzu, Tokyo, Japan) equipped with a binary pump and a SPD-M10A photo diode array detector, a CTO-10A column oven, and an automatic injection system was used. The mobile phase consisted of acidified water pH 3.2 as solvent A and acetonitrile: methanol (50: 50, *v/v*) as solvent B, gradient system of 75:25 for 30min, to 60:40 on 20min, 35 °C and flow rate of 0.8mL min^{-1}, through a C_{18} 5 µm 100 Å (250 x 4.6 mm) column (Phenomenex®, Torrance, USA), with detection at 254nm and injection of 20µL. All gel samples (1.0g) were extracted with the aid of centrifugation with methanol (5.0mL) and sodium chloride salt (0.1g). The supernatant was dried under hot air and the dry residue was dissolved with methanol (2.0mL) and filtered through 0.2µm membranes (Cromafil® PET-20 µm /15 mm) (Macherey-Nagel Inc. USA) before injection. The isoquercitrin assay in the samples was determined by external standardization, using an analytical curve built with the authentic sample of the marker.

The recovery of the extraction method was carried out by spiking the placebo with 200 µL of standard solution of isoquercitrin at 120 µg mL^{-1}, to obtain a final concentration, in the sample solution of 12µg mL^{-1}, within the linearity of the method.

In vitro citotoxicity

The agarose overlay method was used as an *in vitro* test to evaluate the cutaneous irritation of developed gel formulations. For that, L929 cells were obtained from the Rio de Janeiro cell bank. These were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 1% PSN (peniciline, steptomicine and neomicine) antibiotic and 0.5% antifungal amphotericin B, at 37 °C with 5% CO_2 and 95% air. Cells were plated at 1000 cells / well in DMEM, remaining incubated for 24h at 37 °C with 5% CO_2 and 95% air. After this time, the medium was replaced with DMEM serum medium absent FCS containing 0.01% neutral red as vital dye. Cells remained in the oven for 3h at 37 °C and 5% CO_2 for appearance of red staining. The culture medium was removed and replaced again by mixing 1:1.2 agarose medium and MEM medium (mixture overlay) maintained under heating at 40 °C to prevent solidification methods. The mixture was placed in overlay plates (6 sterile wells containing 1 x 10^6 cells) and these were maintained in a 37 °C oven and 5% CO_2 for 24h. After that, the samples were applied in triplicate on disks with 0.5cm diameter and positive controls (latex and SDS) and negative control (solution 73 saline) were incubated for 24h at 37 °C and 5% CO_2. Besides SDE and developed gel formulations, propylene glycol, and other herbal gel acquired from the market were also evaluated. The degree of irritation was evaluated by the zone of lysis (no incorporation of vital dye), with the aid of microscopic visualization and calipers. The evaluation criteria were followed according to United States Pharmacopoeia (2006).

RESULTS AND DISCUSSION

The batch of herb raw material used for this study had 38.46% of stems and 61.54% of leaves, with an average particle size of 0.537mm. The 12.5 % (*w/v*) hydroalcoholic solution presented a dry residue of 2.35± 0.11 % (*w/w*). The extract SDE was obtained with an arithmetic mean yield of 61 ± 2.83%, taking into account the total solids into the feed solution of spray drying. The moisture content was 5.92 ± 0.063%, fulfilling the acceptance criteria for hygroscopic powders such herbal derivatives (List and Schmidt, 1989). SDE was characterized as a fine powder, with a yellow-brown color. The SEM (Figure 1) showed agglomerated and spherical particles with smooth surfaces with a size range between 2 and 10 µm.

In this study, all the formulations maintained pH values from 5.5 to 6.5, which could be appropriate for topical use. However, the presence of SDE caused a strongly darkening in all of the samples stored in OV (t_{180}). This effect was mostly evident in the Ce gels, including AT (t_{90}) and F (t90), which not only suffered a darkening but also a heterogeneous aspect in AT (t_{90}) and OV (t_{180}). The weight loss ranged from 0.4% (Ca 1A/AT t_{90}) and 18.13% (Ar 2.5C/OV t_{180}). OV was the condition that most influenced weight loss of the formulations. Comparatively, there was a tendency to greater dehydration of the formulations containing Aristoflex® AVC gel, and most formulations containing Cellosize® QP 100 showed less tendency to dehydration, regardless of storage conditions. Probably, this behavior is associated with the water-retaining properties of this polymer, since all formulations contain propylene glycol as humectant. The viscosity was stable up to 90 days at AT, nevertheless tending to be reduced in F and OV (t_{90}), and higher at AT and OV (t_{180}) (Figure 2). All the gel formulations showed pseudoplastic flow and a characteristic thixotropic profile until the end of the study. All formulations increased the viscosity, depending on the polymer concentration, and decreased from 8 to 15% with the presence of SDE.

The viscosity had not suffered great modifications over 90 days at AT, but in the F and OV the viscosity of all formulations reduced around 24%. In the end of 180 days, this effect was reproduced in the formulations at F, with a viscosity reduction of 16%. However, after 180 days, the viscosity increased for all the formulations with Carbopol® 2% and SDE in AT, in the F and OV. As this effect was not direct correlated with the weight loss of formulations, some other kind of polymeric interactions

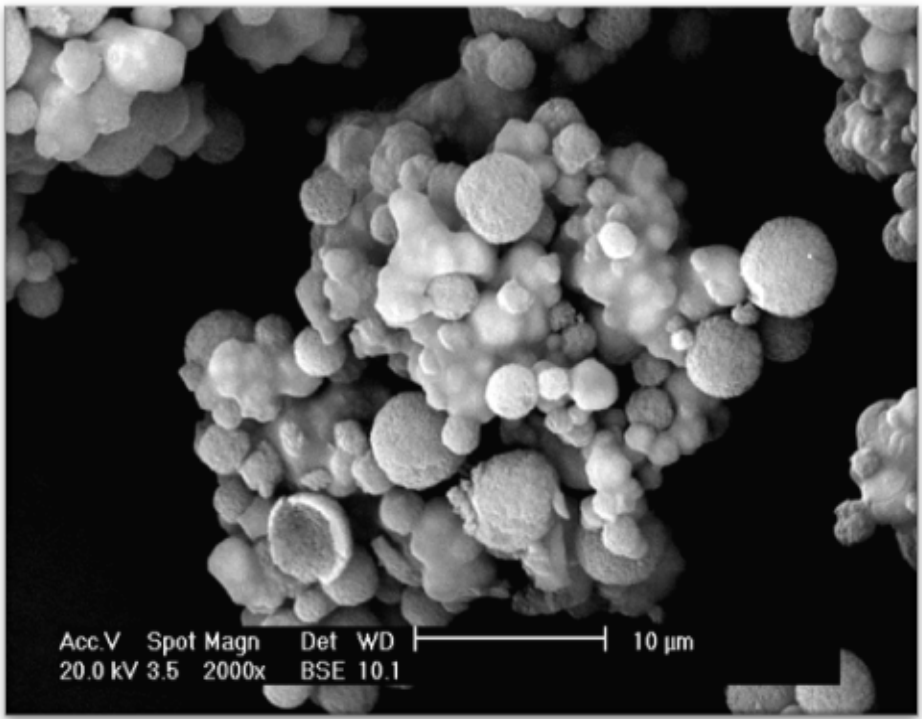

Figure 1. Microscopic image of the SDE samples of *I. pes-caprae* obtained by scanning electron microscopy.

might be related. There are formulations in which a decrease of viscosity was observed during the storage, probably due to the entrapment of bubbles air into the gel. Formulations of Cellosize® base plus SDE showed a heterogeneous aspect after 90 and 180 days in the OV and F conditions. This phenomenon did not allow the viscosity measurements and might be related to interactions between the hydroxyethyl cellulose and SDE. The extraction and quantification methods for HPLC analysis of isoquercitrin (Iso) in the SDE of *I. pes-caprae* (Vieira et al., 2013) were adapted for the Ca and Ar gels in AT and Ce in F (Figure 3). The Iso content was reduced to 6.5% in the Ca in AT (t_{360}). In the Ar gels, stored in F (t_{360}), new peaks were observed in the chromatogram (Figure 3), suggesting degradation products. Only the Ce gel stored in the F (t_{360}) maintained 75% of Iso at the end of this study (Table 1), but with a significant decrease in viscosity. The results of the content of isoquercitrin are related to the marker recovery difficulties. The recovery was 98.2% isoquercitrin (RSD% = 0.99%) for the gel with Carbopol® Ultrez, 73.54% (RSD% = 3.4%) for Aristoflex® gel and only 44.6% (RSD% > 15%) for the gel with Cellozise® 100 QP, due to the nature of the gel suggesting that studies using other clean-up techniques must be employed in this formulation. Only after 1 year, in the fridge, it was possible to measure the concentration of Iso from the gel with Cellozise® QP 100, but at the expense of the loss of viscosity.

The cytotoxicity test diffusion by agarose overlay is indicated to emulsions and gels in aqueous continuous phase, related to potential irritation risk (ANVISA, 2004). Samples of propylene glycol, *I. pes-caprae* dry extract and gels containing or not SDE were evaluated. All samples showed no irritation halo, indicating that there is no degree of mucosa/skin irritation. The positive controls showed halos 4, classified as severe reactivity. The phytopharmaceutical gel, analyzed as the reference gel with anti-inflammatory properties (negative control), showed halos 3, classified as “moderate reactivity”. Therefore, gels containing Ar and Ca with SDE were approved in the issue of security by cytotoxicity test. The results in this work were prompt to show a greater feasibility for the production of gels containing 0.18% dry extract of *I. pes-caprae* from Ca and Ar bases, taking into account their visual characteristics and viscosity. In addition, none of them as well as the SDE, showed any level of cutaneous irritation. Further studies of stability and degradation should be done with herbal raw material and intermediate products such as dry extracts and concentrated extractive solution, in order to elucidate their chemical stability as well as other possible vehicles or formulations should be evaluated for stability improvement.

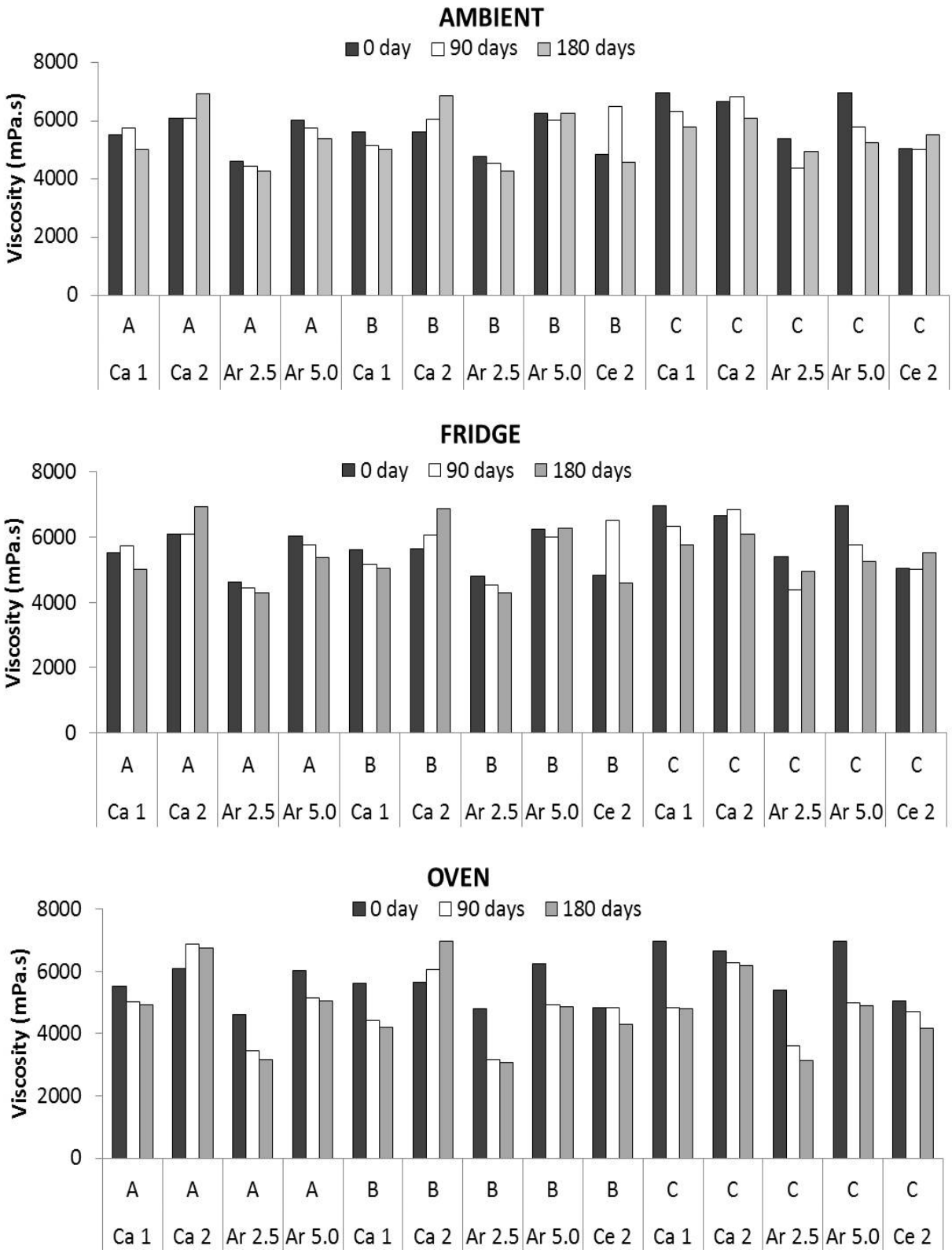

Figure 2. Viscosity measurements of gel formulations in ambient temperature (AT), in the fridge (F) and oven (OV). Ar = Aristoflex® AVC; Ca = Carbopol® ultrez and Ce = Celulosize® QP100. A = 0.11% of SDE; B = 0.18% of SDE; C = 0.0 of SDE. 1, 2, 2.5 and 5 are the polymer concentrations (%).

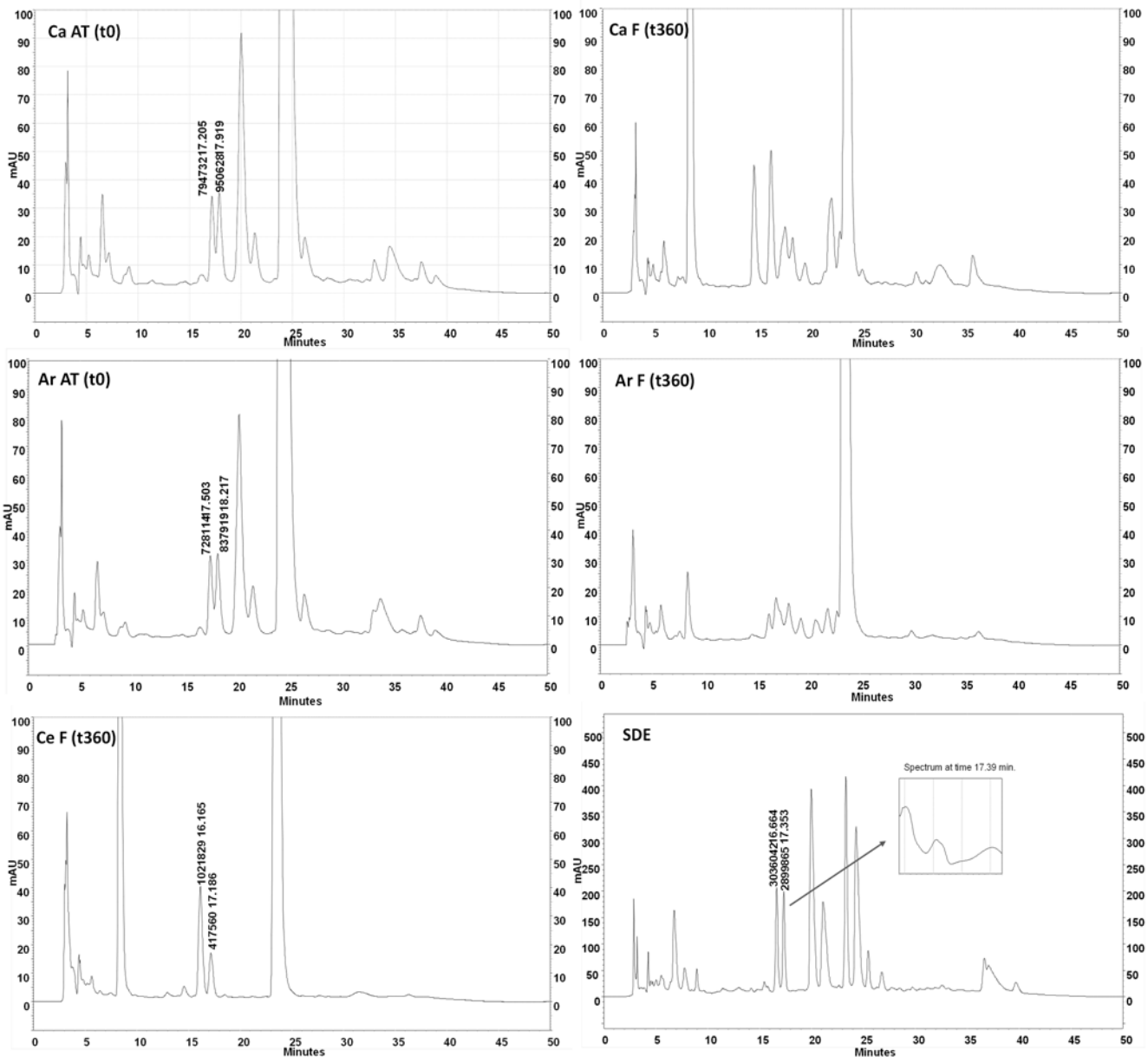

Figure 3. Chromatographic profile of SDE (solution at 10 mg/mLpeak at 18 min = isoquercitrin), Ca (2%), Ar (5%) and Ce (2%) gels containing 0.18% of SDE, monitored at 254 nm.

conclusion

In conclusion, this work provides useful information about physical and chemical stability of gels containing a dry extract of an herbal derivative. Despite that some of the commercial topical formulas are available in the market, with cosmetic or therapeutic purposes; this work demonstrated how difficult it is to obtain a gel formulation with an acceptable quality. The problems were mostly related with the viscosity, as well the chemical marker content over the time.

Conflict of interests

The author(s) have not declared any conflict of interests.

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Table 1. Isoquercitrin concentration (Iso) in the gels containing 0.18% of spray dried extract of *I. pes-caprae* over stability study by HPLC.

Gel	Ambient temperature		Fridge
	Iso (μg/mL) at T_0	Iso (μg/mL) at T_{360}	Iso (μg/mL) at T_{360}
Aristoflex® AVC	94.67 (7.41)	6.42 (3.27)	ND
Carbopol® ultrez	99.33 (8.04)	6.17 (8.13)	ND
Celulosize® QP100	13.58 (26.79)	13.67 (4.86)	73.33 (4.27)

ND = not detectable; T_0 = time after 24 h; T_{360} = storage for 360 days; In parenthesis, relative standard deviation (RSD%)

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