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Determination of sun protection factor and antioxidant properties of six Chilean Altiplano plants

[Determinación del factor de protección solar y propiedades antioxidante de seis plantas del Altiplano Chileno]

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Abstract: In the present study, we investigated sun protection factor and antioxidant properties of *Parastrephia lepidophyla* Cabr., *Fabiana squamata* Phil., *Ephedra chilensis* K.Presl., *Lampaya medicinalis* Phil., *Baccharis tola* Phil., and *Azorella compacta* Phil. The ethanol extracts were tested regarding their *in vitro* free radical scavenger ability and sun protection factor (SPF). Due to its antioxidant and photoprotective properties, *B. tola* is a promising candidate for use in cosmetic formulations. To evaluate the regenerative capacity of the *B. tola* extract, the planarian regeneration assay (*Dugesia tigrina*) was performed. Identification of phenolic compounds in *B. tola*, was performed by HPLC-ESI-MS/MS. Based on freeze-dried extracts of *B. tola*, a facial cream and a biphasic lotion with repairing tip action were formulated. These two formulations were evaluated by additional assays including organoleptic tests, measurement of pH, centrifugation and patch test to check a potential hypersensitivity (skin irritation) which can be induced by the products as well as a sensory survey. Stability studies, carried out over 12 months, prove that formulations were stable over time. It can be concluded that both products are innovative and shown solar protection, antioxidant and regenerative properties.

Keywords: antioxidant, sun protection factor, chilean altiplano plants, *Bacharis tola*

Resumen: En el presente estudio, hemos investigado el factor de protección solar y propiedades antioxidantes de *Parastrephia lepidophyla* Cabr., *Fabiana squamata* Phil., *Ephedra chilensis* K.Presl., *Lampaya medicinalis* Phil., *Baccharis tola* Phil., y *Azorella compacta* Phil. Los extractos etanolicos fueron sometidos a ensayos como: evaluación *in vitro* de la actividad atrapadora de radicales libres y factor de protección solar (FPS). Debido a las propiedades antioxidantes y fotoprotectoras, *B. tola* es un candidato ideal para ser usado en formulaciones cosméticas. Se evaluó la capacidad regenerativa de *B. tola* en ensayos de planaria (*Dugesia tigrina*). Se identificó polifenoles por HPLC-ESI-MS/MS en *B. tola*. Se formularon una crema facial y una loción bifásica reparadora de puntas del cabello, en base a extractos liofilizados de Ñaca, a los cuales se le realizaron controles organolépticos, evaluación de pH, centrifugación, test de parche para evaluar la posible reacción de sensibilidad que pueden ocasionar los productos, comprobando que estos no producen irritación dérmica y posterior encuesta sensorial. Posteriormente, se realizaron estudios de estabilidad a lo largo de 12 meses, demostrando ser estable en el tiempo. Se puede concluir que ambos productos son innovadores y que muestran factor de protección solar, propiedades antioxidantes y regeneradoras.

Palabras clave: antioxidante, factor de protección solar, plantas del atiplano chileno, *Bacharis tola*

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INTRODUCTION

Skin is the main biological border protecting us against environmental injuries. Due to this barrier function, skin is a potential target organ for oxidative stress induced by external insults such as ultraviolet (UV) irradiation, ionizing radiations and numerous deleterious chemicals. Such oxidative stress may be a key initiator in the pathogenesis of skin cancer and photoageing-mediated deleterious process (Carbonare *et al*., 1992) likely due to a dysregulation of skin redox status. In this context, a prolonged exposure to UV light results in a severe decrease in the antioxidant capacity of skin. Moreover, an increased formation of reactive oxygen intermediates has been reported (Auner *et al.*, 2005).

There has been an increasing interest in the use of antioxidants in sunscreens to provide supplementary photoprotective activity. The rationale of this is that antioxidants from natural sources may provide new opportunities for the treatment and prevention of UV-mediated diseases (Bonina *et al*., 1996; Saija *et al*., 1998; F'guyer *et al*., 2003).

Medicinal plants of the Northern Chilean and Southern Peruvian Highlands, which are commonly known as the "Altiplano", have been employed for the treatment of skin diseases for more than 2000 years (Villagrán *et al.*, 2003). According to a global trend in cosmetic industry looking for replacement of synthetic substances by natural ingredients, several studies have been conducted to evaluate the properties of some plants that grow in adverse conditions like in the Chilean altiplano (Pratim *et al.*, 2006). Indeed, these plants should synthesize secondary metabolites to grow and survive under severe conditions such as high temperature and scarce amount of water.

It has been reported that plants having the ability to absorb different radiation levels may be used to make sunscreens (Kaur & Saraf, 2010). Therefore, it is expected that extracts obtained from these plants should fulfill the following characteristics of a solar filter: (a) absorb the radiation ranging between 290-320 nm; (b) be stable under both cosmetics manufacture and use conditions; (c) be compatible with the excipients and the material of packaging for sun products, and (d) have none or the lowest toxicity possible (Lopez, 2007).

Both *in vivo* (i.e. the block test) and *in vitro* techniques have been used to evaluate solar filters. Among these procedures, one of the most applied *in vitro* methods is UV-spectrophotometry, because it is simple, fast, cost-efficient and risk-free method. In addition, it should be noted its great precision, sensitivity and applicability (Souza *et al.*, 1986).

On the other hand, since plant extracts can stimulate (or inhibit) tissue proliferation, such extracts might be of great medical usefulness. In this context, facilitating tissue regeneration may have a potential benefit during wound healings, while a delayed tissue remodeling may decrease the potential ability of cancer cells to metastasize (Afaq *et al.*, 2009).

Therefore, the aim of this work was to examine 6 plants of the Chilean altiplano and to select, on the basis of antioxidant results and organoleptic characteristics, the extract showing the best properties to further make two final cosmetic products with sun protection factor, antioxidant and regenerative properties.

MATERIALS AND METHODS

Chemicals and reagents

Ethanol, isopropyl myristate, propylene glicol, polysorbate 80, sorbitan monooleate, methyl paraben, Trolox and DPPH were purchased from Merck (Germany). Cetyl alcohol, sodium benzoate, triethanolamine were purchased from Reutter (Chile). Cyclomethicone, dimethiconol, phenyltrimethicone were purchased from Dow Corning (USA). Steararic acid was purchased from Winkler (Chile).

Plant materials

The plant species were collected near Colchane, at 3500 meters above sea levels (m.a.s.l.) between November 2009 and July 2010, 1st region of Chile. The plant species were: Tola (*Parastrephia lepidophyla* Cabr.), Kipa macho (*Fabiana squamata*), Pingo-pingo (*Ephedra chilensis*), Lampaya (*Lampaya medicinalis* Phil.), Ñaca (*Baccharis tola* Phil.) and Llareta (*Azorella compacta*).

The plants were identified and samples were deposited in the Herbarium of the Concepcion University. The plant materials (leaves and stems) were first dried at room temperature (20° C), reduced to a coarse powder (100 g), then they were packed in soxhlet apparatus separately and extracted with

ethanol (70 - 80º C) during 12 h. After extraction, the organic extract were lyophilized and concentrated. The extracts were protected from direct light and stored at 4º C until its use.

Measurement of sun protection factor (SPF)

Sample stock solutions were made by transferring 0.5 g of sample to a 100 mL volumetric flask and diluted to volume with ethanol. A 1.0 mL aliquot was transferred to 25 mL volumetric flask and diluted to volume with ethanol, to obtain a final concentration of 0.2 mg/mL, which represents the standard concentration

The absorption spectra of samples in solution were obtained in the range of 290 to 320 nm using 1 cm quartz cell and reading against ethanol as a blank. Three determinations were made at each point, and values of solar protection factor (SPF) were obtained by applying the Mansur equation (Soares *et al*., 2009).

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times abs(\lambda)$$

Where *CF* is correction factor (= 10), determined with Known SPF, so that a solution containing 8% of homosalate give SPF=8. *EE* (λ) the erythemal efficiency spectrum; *I* (λ) the solar simulator spectrum as measure with a calibrated spectroradiometer; and abs (λ) the spectroradiometer measure of sunscreen product absorbance, were estimated within a range of 290-320 nm in 5 nm increments.

Free radical scavenging activity

Free radical scavenging activity of plant extracts was determined by using a stable free radical, namely DPPH (1,1-diphenyl-2-picrylhydrazyl), according to a slightly modified method of Blois, 1958. Indeed, since DPPH has an unpaired electron its delocalization, by reaction with an antioxidant substance gives a violet color to the DPPH solution. By donating a hydrogen radical, DPPH is stabilized producing a decrease in absorbance (Castañeda *et al.*, 2008; Molyneux, 2004). DPPH solution was prepared at the concentration of 0.024 mg/mL of DPPH in ethanol. During assays, 1 mL of the crude extract (0.5 and 1 mg/mL) was mixed with 1 mL DPPH solution. Simultaneously, a control (Trolox®) was prepared without sample extracts. The mixture was incubated at room temperature for 30 min and further reading on a TECAN micro plate reader plate at 515 nm.

The percentage of free radical scavenging activity was expressed as percent inhibition from the given formula:

$$\text{\% inhibition of DPPH radical} = \frac{\text{Abs. of control} - \text{Abs. of sample}}{\text{Abs. of control}} \times 100$$

The assay was performed in triplicate.

Determination of the regenerative capacity of Baccharis tola Phil

For the regeneration assay, the heads of 13 adult planarians *Dugesia tigrina* were removed in the region behind the auricles using blades, and the decapitated animals were immediately used (Calevro *et al*., 1998). The bodies of planarians were placed in wells, 70% ethanol was added in the first well serving as a control. In the rest of the wells, 5 µL of each plant extract solutions were added at a final concentration of 2 mg/mL. Planarians were observed every day, using a magnifying glass to check the regeneration of a completely new head. Before start the test, the animals were fed 1 day to ensure that they have enough nutrients to regenerate.

HPLC-DAD *analysis of Baccharis tola* Phil

The Amberlite-retained phenolic enriched extract was analyzed by HPLC-DAD. The system used for analysis was an Agilent Technologies 1260 Infinity equipment (Agilent Technologies, Santa Clara, United States) consisting of a 1260 Quat Pump, a 1260 DAD VL UV diode array detector, 1260 TCC column oven, 1260 ALS autosampler and a OpenLab software. A MultoHigh 100 RP 18-5µ (250 x 4.6 mm) column (CS-Chromatographie Service GmbH-Germany) kept at 25° C was used. The HPLC analysis was performed using a linear gradient solvent system consisting of 1% formic acid in water

(A) and methanol (B) as follows: 75% A to 40% A over 10 min, followed by 40% A to 30% A from 10 to 20 min, 30% A to 40% A from 20 to 25 min and 40% A to 75% A from 25 to 28 min. The flow rate was 1 mL/min and the volume injected was 20 µL. The compounds were monitored at 254 nm and UV spectra from 200 to 600 nm were recorded for peak characterization.

***Identification of phenolics* by HPLC-ESI-MS/MS in *Baccharis tola* Phil**

Experimental conditions were the same as previously reported for HPLC-DAD analysis. Data were recorded on a HPLC-ESI-MS/MS system which consisted of the HPLC HP1100 (Agilent Technologies Inc, CA-USA) connected through a split to the mass spectrometer Esquire 4000 Ion Trap LC/MS(n) system (Bruker Daltonik GmbH, Germany). Ionization was performed at 3000 V assisted by nitrogen as nebulizing gas at 24 psi and as drying gas at 365º C and a flow rate of 6 L/min. Negative ions were detected using full scan (m/z 20-2200) and normal resolution (scan speed 10,300 m/z/s; peak with 0.6 FWHM/m/z). The trap parameters were set in ion charge control (ICC) using manufacturer default parameters, and maximum accumulation time of 200 ms. Collision induced dissociation (CID) was performed by collisions with helium background gas present in the trap and automatically controlled through SmartFrag option.

Emulsion Preparation

O/W emulsions were prepared according to a direct emulsification process (Roussos, 1983) and the following standardized formula was obtained. Oily phase consisted of stearic acid (4%), cetyl alcohol (3%) isopropyl myristate (7%) and surfactant sorbitan monooleate (0.28%) was heated up to 70 ± 1° C. At the same time, aqueous phase consisting of water (q.s) was heated to the same temperature and then the following components were further added: Ñaca extract (3%), propylene glycol (3%), methylparaben (0.05%), sodium benzoate (0.1%) and polysorbate 80 (2.52 %). Afterwards, aqueous phase was added to the oil phase drop by drop. Stirring was continued for about 15 min until complete aqueous phase was added; 2 drops of honey essence were added during this stirring time to give good fragrance to the formulation. After the complete addition of the aqueous phase, the speed of agitation maintaining constant for complete homogenization; until the emulsion cooled to room temperature. Once the emulsion was formed, the triethanolamine was added for pH adjustment.

Biphasic lotion preparation

The raw material was calculated and weighed to prepare 60 mL, which correspond to 100% of the total preparation. The oil phase A is containing 50% (30 mL) and the aqueous phase B, is containing the additional 50% (30 mL).

Preparation of oil phase A: 23% cyclomethicone, 23% dimethyconol and 4% phenyl trimethicone were added to a beaker in the same order in which are described.

Preparation of aqueous phase B: A 4% of Ñaca extract was placed into a beaker and 15% propylene glycol was added to dissolve it. Then, the solution was heated in a water-bath to increase the temperature and water was added slowly in the solution to avoid precipitation of the mix. Subsequently, the solution was filtered with sterile gauze to remove any precipitate.

Once both phases were ready, the phase A was added slowly and with agitation on phase B, to produce the mixture of both phases. In addition, triethanolamine and essence of honey were added under stirring. Thereafter the mix was set aside to view the separation of phases.

Organoleptic and physico-chemical parameters

Organoleptic characteristics

Color: N-Normal/M-Modified/IM-Intensely modified
Odor: N-Normal/M-Modified/IM-Intensely modified.
Spread ability and touch: A–pleasant touch, easy application/D–unpleasant touch, sticky/MD–very unpleasant touch, very sticky, compromises skin application.

pH value

The pH of each formulation was determined by a Hanna pH-meter at room temperature. As acceptance criteria, formulations with variations of pH value higher than 15%, comparing with the initial value, were reproved.

Apparent Viscosity
The samples were analyzed in duplicated in Brookfield viscometer (spindle 05), at 100 rpm. The apparent viscosity values were registered after 24 h of emulsion preparation and every 15 days during 3 months.

Microbiological analysis
Samples were seeded on Müeller–Hinton agar. The Petri dishes were incubated at 37° C and bacteria colonies were numbered after 24, 48 and 72 hours.

Sensorial evaluation
Every individual was provided with a perform questionnaire previously prepared to test the cream sensory values. This form consisted of seven parameters to be evaluated and the score of every parameter was composed by 11 values ranging from −5 to +5, representing a scale of very bad to very good respectively. This form was asked to be completed independently by each individual on day 28.

Stability studies
Samples (emulsion and biphasic lotion) in duplicated, were submitted to stability studies. Both cosmetic forms (5.0 g) were submitted to centrifugation at 3000 rpm for 30 min. Thermal stress, this assay is taken into account several temperature conditions and times of analyses. Samples were submitted for 24 h to 7 cycles of low temperature (4 ± 2° C) and high temperature (40 ± 2° C) during 14 days. Finally, samples were stocked for 12 months at 40 ± 2° C (accelerated stability) and at room temperature (25 ± 2° C) avoiding exposure to light. (Vieira *et al.,* 2009)

Patch test
Patch tests were performed on the forearms of 30 healthy subjects 20-45 years old, who participated in this study after having given their informed consent. The exclusion criteria were as follows: presence of any dermatitis and/or other skin or allergic diseases, smokers and previous treatment of forearms' skin with cosmetic formulations such as moisturizers, sunscreens or anti-ageing cosmetics. During the test period, the subjects were allowed to be washed normally, but they were instructed not to use any other skin care products on their arms. The patch (Bandage disc) for the right forearm was saturated with 0.2 g of base while the patch for left forearm was saturated with 0.2 g of formulation. Each was applied to the 5 cm x 4 cm marked regions separately on each forearm. The regions were covered with adhesive tape 3M® after application. The patches were removed after 48 hours and the forearms were rinsed with physiological saline. After 48 hours, scores were recorded for the presence of erythema (skin redness) using a scale recommended by the International Contact Dermatitis Research Group ICDRG (Table 1).

Table 1
Score and evaluation of Patch test according to ICDRG recommendations

Score	Result	Interpretation
(-)	Absence of reaction	Negative
(+?)	Weak Erythema	Uncertain
(+)	Erythema with some papules	Possible
(++)	Erythema, papules and vesicles	Probable
(+++)	Intense Erythema, papules and vesicles	Very probable
IR	Different types of reaction (vesicles, blister, necrosis)	Irritant reaction

IR= irritant reaction

RESULTS AND DISCUSSION

The SPF is a quantitative measurement of the effectiveness of a sunscreen formulation. After obtained the freeze-dried extracts, SPF was assessed following standard procedures reported by Maske *et al.*, 2013. The SPF values of extracts were in the range of 4.2 to 8.5, (Table 2). These results shown that Ñaca and Lampaya were the species with the highest SPF values. To note that commercial products containing benzophenone-3 (4%), octyl methoxycinnamate (7.5%) avobenzone and octocrylene gave similar SPF values to that obtained with these plant preparations.

Table 2
Sun protection factor values of extracts from different plant species of Chilean Altiplano

Plant species Scientific name	Vernacular name	**Sun protection factor**
Parastrephia lepidophyla	Tola	5.1
Fabiana squamata	Kipa macho	4.2
Ephedra chilensis	Pingo-Pingo	6.0
Lampaya medicinalis	Lampaya	10.7
Baccharis tola	Ñaca	18.5
Azorella compacta	Llareta	4.5

Figure 1
Free radical scavenger activity of ethanol extracts of six plants of Chilean Altiplano, (a) 0.5 mg/mL y (b) 1.0 mg/mL.

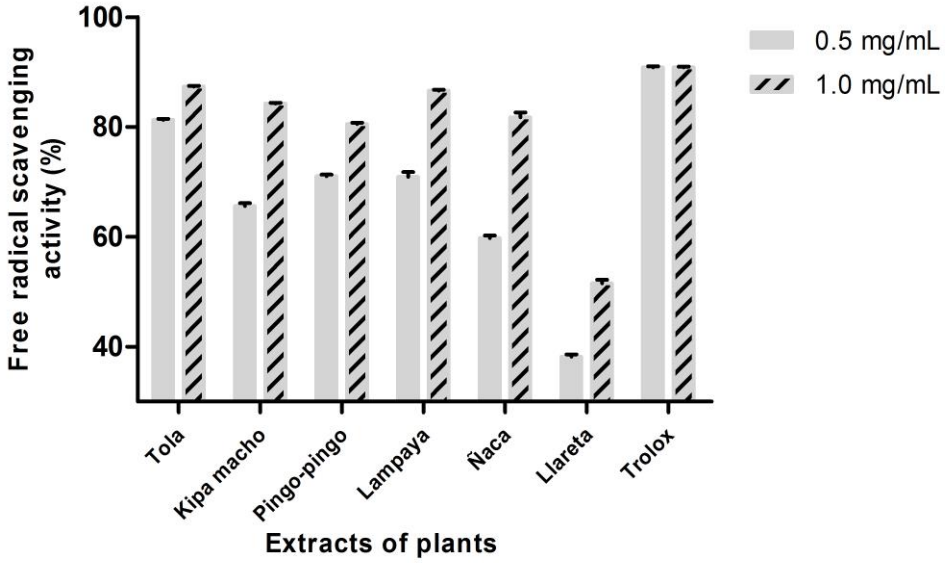
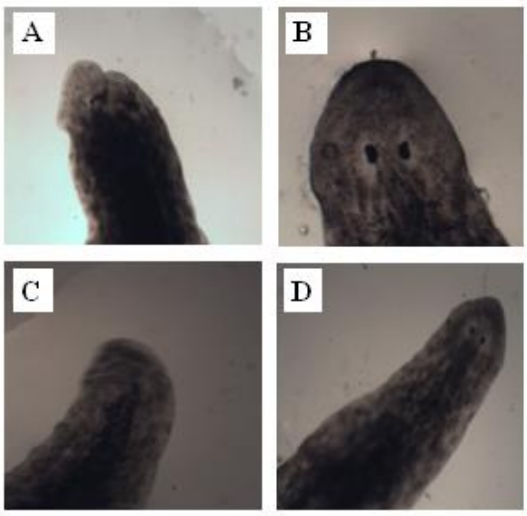


Figure 1 shows that most of extracts have a strong dose-dependent free radical scavenger activity. When they were tested at a concentration of 1 mg/mL, such antioxidant activity (more than 80%) was significantly different ($p < 0.0001$) when compared to that obtained by Trolox®, a well-known antioxidant. At low doses (0.5 mg/mL), only 5 out of 6 extracts retained their antioxidant activity as compared to Trolox®. Based on these results, the Ñaca extract was chosen to carry out the cosmetic formulations. In addition, those made with other plant extracts displayed inadequate organoleptic characteristics, a critical issue in a cosmetic product.

Studies on Planarian regeneration were conducted to evaluate the repair activity of Ñaca extract on tissues. Figure 2 shows that regeneration occurred at early days, for instance, the appearance of eyes took only 3 days (Figure 2a,b), while the normal Planarian regeneration is 7 days (Figure 2c,d). Since the pure extract has the property of tissue regeneration, it may be inferred that the cosmetic preparation has repairing tips action.

Figure 2
Planarian regeneration assay (*Dugesia tigrina)*. Effect of Ñaca at day 3 (A) and day 7 (B). Control, at day 3 (C) and day 7 (D).

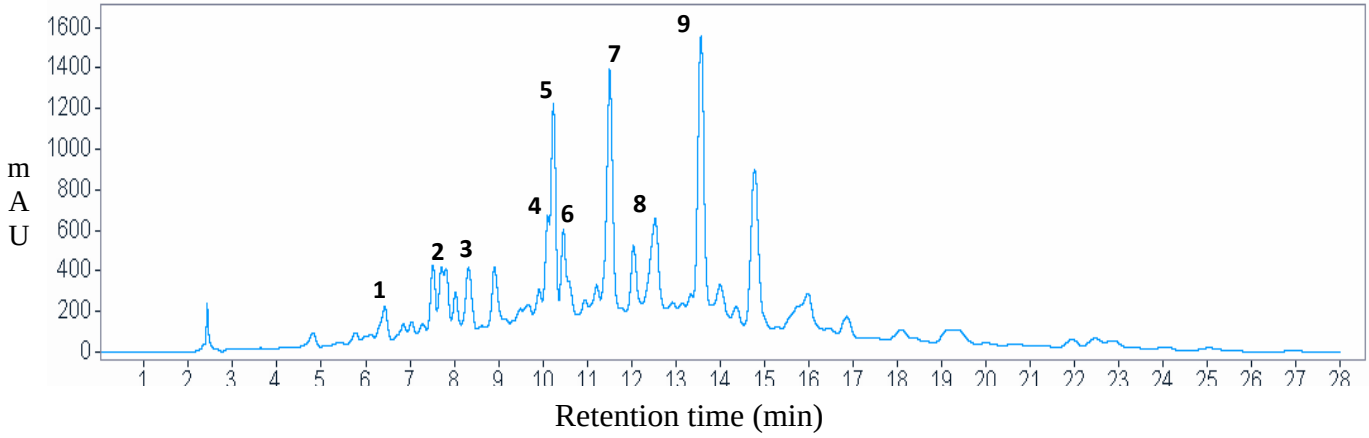


With regard to the HPLC-DAD-MS-MS analyses, the compounds were identified or tentatively identified on the basis of UV spectra and MS fragmentation patterns in comparison with published data of the literature. A representative chromatogram of the extract at 254 nm is presented in Figure 3. A total of 9 compounds were tentative identified. The HPLC-ESI-MS/MS analysis was carried out in negative ionization mode. Their identities, retention times and observed molecular and fragment ions in the chromatogram (Figure 3) are presented in Table 3. The retention time of the peak **1** was compared against standard corresponding to caffeoyl quinic acid (Compound **1**). The peak **2** at Rt 7.7 min presented an UV max at 355 nm, compatible with a flavone. The MS/MS spectrum showed peaks assignable to $[M-H-90]^-$, $[M-H-120]^-$, $[M-H-180]^-$ and/or $[M-H-210]^-$, in agreement with flavonoid C-glycosides with a C-linked hexose and C-pentose or C-linked dihexose (Quispe *et al.*, 2014). Compound **2** presents a $[M-H]^-$ ion at 593 atomic mass unit (amu) and the fragmentation data is in agreement with that of Vicenin II (apigenin-6,8-di-C-glucoside) (Barreca *et al.*, 2011). Compound **3** showed an ion at 563.6 amu. The spectrum presented peaks assignable to $[M-H-90]^-$, $[M-H-120]^-$, $[M-H-180]^-$ and $[M-H-210]^-$, compatible with a C-linked hexose and C-pentose. The compound **3** was identified as Schaftoside (Quispe *et al.*, 2014). Compound **4**, rutin pentoside, gave mass spectral fragmentation patterns consistent with a quercetin derivative. The nature of the quercetin flavonoid core was revealed by key ions at *m/z* 301 and 300. The molecular ion peak at *m/z* 741.5 from which a small, but significant, ion at *m/z*

609.2 revealed the loss of 132 Daltons indicative of pentoside cleavage. This indicated that the compound was rutin with an additional attached pentoside, (Khallouki *et al*., 2015). Peak **6** eluting at 10.5 min was characterized as to be quercetin-di-hexoside (compound **6**) with a parent ion at *m/z* 625.3. The obtained fragment ions at *m/z* 463.0 and 300.8 were likely due to a sequential loss of two hexose ([M-H-hexose]$^-$ and [M-H-hexose-hexose]$^-$) moieties. Compounds **5** and **7** with ions at *m/z*: 515.4 and 515.2 were identified as dicaffeoyl quinic acid isomers according to the fragmentation ions (Jaiswal *et al*., 2010). These compounds have been previously found by Zampini *et al*., 2009 in the water infusion and water-soluble portion of the methanolic extract of *B. incarum*. Compound **8** was tentatively assigned as caffeoyl-feruloylquinic acid with a pseudomolecular ion at *m/z* 529.2 and fragmentation ions at 367.0 and 352.8 (Farrell *et al*., 2011). Finally, compound **9** was observed at 13.3 min the tentative identification showed a tricaffeoyl quinic acid, with an ion at *m/z* 677.4, while the loss of 162 uma presented a fragmentation at 515.1, the following loss of 162 uma generated an ion at 353.0 corresponding to caffeoyl quinic acid (Jaiswal *et al*., 2010).

Figure 3

HPLC-DAD Cromatogram of the amberlite-retained from *Baccharis* infusion. Ultraviolet detection at 254 nm. Compounds: Caffeoyl quinic acid (**1**), Apigenin-di-C-hexoside (Vicenin II) (**2**), Apigenin-C-hexoside-C-pentoside (Schaftoside) (**3**), Rutin pentoside (**4**), Dicaffeoyl quinic acid (isomer) (**5**), Quercetin-dihexoside (**6**), Dicaffeoyl quinic acid (isomer) (**7**), Caffeoyl feruloylquinic acid (**8**), Tricaffeoyl quinic acid (**9**).



Regarding organoleptic characteristics shown in Table 4, the color, odor and spread ability and touch are normal. Color, odor, spread ability and touch alterations were not observed in the preparations, indicating adequate stability, even in the high temperature condition. Odor and color are crucial properties of cosmetics, and the more skillfully they are harmonized the more attractive and valuable the cosmetic becomes. Spread ability indicates the area on which a semi-solid topical formulation spreads on application to the skin. This parameter plays a key role in determining both the efficacy and the consumer acceptance of the product. A poor spread ability may result in an uneven distribution of the formulation on the skin, thus affecting the amount of the dose applied and the efficiency of active ingredient skin permeation. When considering physical-chemistry properties, none of the samples showed instability indexes nor sedimentation, flocculation and creaming, a well-known characteristic of emulsions. They neither showed separation of phases indicating that they are stable preparations (Gennaro, 2000; Aulton, 2001). Given the pH of the skin, a cosmetic for topical application should have a pH value in the range of 4.5 and 5.9 (Orlandi, 2004). Since the pH of the

emulsion was 5.3, that means it complies with such requirement. Since the viscosity value of the Ñaca extract remained quite unchanged during 3 months, it can be concluded that such emulsion should be considered as stable. The emulsion showed pseudoplastic behavior, since viscosity decreased with increasing shear stress. This thixotropic behavior is considered desirable for topical preparations as it improves spreading of the product on the skin. Finally, the two cosmetic preparations were micro-organisms free, being therefore suitable for further use.

Table 3
Identification of compounds in the Amberlite-retained fraction of Baccharis infusion by HPLC-DAD-ESI-MS data

Peak	Rt (min)	UV max (nm)	$[M-H]^-$	MS/MS	Tentative identification
1	6.4	328, 298sh, 244	353.2	190.5 (100), 178.6 (36), 134.5 (6)	Caffeoyl quinic acid [(a)]
2	7.7	331, 271	593.6	575.2 (10), 503.2 (30), 473.2 (100), 383,1 (7), 353.6 (31)	Apigenin-di-C-hexoside (Vicenin II) [(b,c)]
3	8.3	336, 272	563.6	545.0 (21), 503.1 (62), 473.2 (100), 443.3 (45), 383.4 (21), 353.6(17)	Apigenin-C-hexoside-C-pentoside (Schaftoside) [(b,c)]
4	9.8	-	741.5	609.2 (66), 591.2 (25), 475.2 (18), 343.0 (20), 300.9 (67), 300.0 (100), 255.3 (5), 271.2 (11)	Rutin pentoside [(b,c)]
5	10.0-10.2	330, 300sh, 248	515.4	353.0 (100), 173.0 (3)	Dicaffeoyl quinic acid (isomer) [(b,c)]
6	10.5	-	625.3	463.0 (15), 342.8 (13), 300.8 (100), 299.9 (11)	Quercetin-dihexoside [(b)]
7	11.5	330, 300sh, 248	515.2	353.0 (100)	Dicaffeoyl quinic acid (isomer) [(b,c)]
8	12.5	-	529.2	367.0 (21), 352.8(100)	Caffeoyl feruloylquinic acid [(b,c)]
9	13.3	332, 299sh, 247	677.4	515.1 (100), 353.0(3)	Tricaffeoyl quinic acid [(b,c)]

Identification according to: [a]Compound conclusively identified by comparing retention time, absorption and mass spectra with that of an authentic standard; [b]Confirmed by fragmentation pattern; [c]Confirmed by reference.

When the development of two products was completed, a sensory evaluation test was conducted to study their acceptability by potential customers (n=30). The emulsion has a pale yellow color, a honey fragrance and a soft texture. The biphasic lotion has a colorless phase (Silicones) and a yellow water phase, smell of honey and a soft and oiled texture. The survey showed the following results: regarding the emulsion, 100% of people found the cream color nice and they would purchase the product; about 80% found a product of attractive appearance; and 53% found a pleasant aroma. A vast majority (96%) describes a soft product after application. With regard to the biphasic lotion, 81% of people considered it look gorgeous and 62.5% found a pleasant aroma. Most of people (96.9%) really like the color, and as in the previous product, 100% of the study participants would buy the product.

No variation in characteristics during temperature stress was observed during stabilities studies, and the pH remained stable. Secondly, no variation in the stability of cosmetic products, the life span and the compatibility of the formulation with the packaging material was seen. Regarding long duration stability, when exposed to environmental changes of temperature and humidity, the pH of the emulsion and the biphasic oil remained stable

throughout the time. It was concluded then that the formulations have 12 months as proven validity period. Under shelf and stove, both formulations kept their organoleptic characteristics and homogeneity. So the packaging material was considered as suitable.

Table 4
Evaluation of organoleptic characteristics of facial cream.

Storage condition		Organoleptic characteristic		
Time (months)	**Temp. (°C)**	**Color**	**Odor**	**Spread ability and touch**
0	25 40	Pale yellow	Honey	Viscous, easy to spread
1	25 40	N N	N N	A A
2	25 40	N N	N N	A A
3	25 40	N N	N N	A A
6	25 40	N N	N N	A A
12	25 40	N N	N N	A A

Color and odor: N-Normal
Spread ability and touch: A–pleasant touch, easy application

The last assay was associated with a potential type of allergy caused by hypersensitivity. To this end, the patch test was employed to investigate this adverse reaction. The experimental exposure involves keeping in contact the patient's healthy skin with a suspicious substance during 48 h (Lopez *et al*., 2003). The freeze-dried extract of Ñaca was applied as 3% in the emulsion and 4% in the biphasic lotion. An initial kind of cutaneous hypersensitivity was discarded because the absence of reaction score, quoted as (-), during the observation time performed at 48 and 96 hours. Therefore, it can be concluded that the emulsion and the biphasic lotion, as well as other components of the products do not produce any type of undesirable skin reaction.

CONCLUSIONS

The present study shows that Ñaca extract has SPF property as well as antioxidant and regenerative activities. Nine phenolic compounds, supporting a putative antioxidant ability of Ñaca extract, were identified on the basis of UV spectra and MS fragmentation patterns. Two cosmetic products retaining all these qualities have been developed. They were subjected to several quality control assays and stability tests, showing a remarkable stability. They were also devoid of pathogen micro-organisms, a critical issue in the cosmetic legislation in Chile. A 100% acceptance was agreed by consulting people with regard to their organoleptic characteristics. Finally, the patch test indicated that both products may be safely used without any risk for the skin of consumers.

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