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Original Article

Quantification and *in vitro* photo-protective studies of phenolic compounds from *Baccharis papillosa* Rusby

[Cuantificación y estudios de fotoprotección *in vitro* de compuestos fenólicos de *Baccharis papillosa* Rusby]

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Abstract

Context: The ethanolic extract of the leaves from *Baccharis papillosa*, a plant used in Bolivian folk medicine, presents high UVB/UVA absorption spectrum, and therefore, it could have photo-protective potential.

Aims: To isolate, identify and quantify the compounds of an enriched extract in phenolic compounds obtained from the ethanolic extract of *Baccharis papillosa* in different seasons and geographical altitudes, and evaluate its photo-protective potential.

Methods: The enriched extract in phenolic compounds was submitted to phytochemical analysis for compound isolation. The enriched extract and isolated compounds were identified by NMR, and monitored by HPLC and spectroscopic methods. The enriched extract with photo-protective potential was analyzed to determine its Spectroscopic Sun Protection Factor (SSPF), its Broad Spectrum Index (BSI) and its photo-protective activity on *Escherichia coli* bacteria.

Results: Six flavonoids and two cinnamic acid derivatives were isolated and identified. Four of them are reported in *B. papillosa* for the first time in this study. The highest concentration of total flavonoids was observed in spring and at the highest altitude. The major compound, drupanin, was the main responsible of the high UVB (290-320 nm) absorption spectrum. The high presence of flavonoids in the extract explains the absorption spectrum in the UVA (320-400 nm) region.

Conclusions: The phenolic compounds enriched extract has photo-protective properties comparable to standard commercial synthetic sunscreens and presents an attractive BSI.

Keywords: Broad Spectrum Index (BSI); cinnamic acid derivative; flavonoids; photo-protective activity; Spectroscopic Sun Protection Factor (SSPF).

Resumen

Contexto: El extracto etanólico de las hojas de *Baccharis papillosa*, planta utilizada en la medicina popular boliviana, presenta un alto espectro de absorción UVB/UVA por lo que podría presentar potencial fotoprotector.

Objetivos: Aislar, identificar y cuantificar los compuestos de un extracto enriquecido en compuestos fenólicos obtenido a partir del extracto etanólico de *Baccharis papillosa* en diferentes épocas del año y altitudes geográficas y evaluar su potencial fotoprotector.

Métodos: El extracto enriquecido en compuestos fenólicos fue sometido a análisis fitoquímicos para aislamiento de compuestos. El extracto enriquecido y los compuestos aislados fueron identificados por RMN, y monitoreados por HPLC y métodos espectroscópicos. El potencial fotoprotector del extracto enriquecido se analizó mediante la determinación de su Factor de Protección Solar Espectroscópico (SSPF), su Índice de Amplio Espectro (BSR) y su actividad fotoprotectora sobre bacterias *Escherichia coli*.

Resultados: Se aislaron e identificaron seis flavonoides y dos derivados del ácido cinámico, de los cuales, cuatro de ellos se reportan en este estudio por primera vez en esta especie. La mayor concentración de flavonoides totales se observó en primavera y a mayor altura. El compuesto mayoritario, drupanina, fue el principal responsable del alto espectro de absorción UVB (290-320 nm) del extracto enriquecido. La alta presencia de flavonoides en el extracto explica el espectro de absorción en la región UVA (320-400 nm).

Conclusiones: El extracto enriquecido en compuestos fenólicos tiene propiedades fotoprotectoras comparables a filtros solares sintéticos comerciales estándar y presenta un amplio espectro de protección solar.

Palabras Clave: derivado del ácido cinámico; factor de protección solar espectroscópico; flavonoides; fotoprotección; índice de amplio espectro.

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INTRODUCTION

The *Baccharis* genus (*Asteraceae*) has around 400 species widely distributed in South America. In Bolivia, there are around 30 *Baccharis* species, which grow mainly in the Andean highlands over 3000 meters above sea level. These plants suffer high exposure to different environmental stress conditions like cold weather, low rainfalls (arid soils) and high UV solar radiation (Zaratti et al., 2003). This is due to the geographical altitude and latitude at which the plants grow (tropic geographic conditions). Because of those conditions, the Bolivian highland plants developed diverse adaptive mechanisms; among them, photoprotective secondary metabolites produced by some plants (Andersen and Markham, 2005; Grotewold, 2006)

The chemical research of *Baccharis* genus showed mainly cinnamic acid derivatives, flavonoids and diterpenes as major secondary metabolites. These compounds reported diverse pharmacological activities (Calle et al., 2017; Enríquez et al., 2018; Gene et al., 1996; Nichols and Katiyar, 2010). *Baccharis* genus plants are used in folk medicine in Bolivia attracting the attention of our research group that studied six *Baccharis* species: *Baccharis latifolia* (Enríquez et al., 2018; Loza Almanza et al., 2011), *B. papillosa* (Escobar et al., 2009; Loza Almanza et al., 2011), *B. boliviensis* (Calle et al., 2012; 2017), *B. densiflora* previously identified as *B. pentlandii* (Sotillo et al., 2021; Tarqui et al. 2012), *B. tola* Syn *B. santelices* (Villagómez et al., 2006) and *B. lepthophylla* (Almanza et al., 2000; Salcedo Ortiz et al., 2013). In addition, some studies suggested that the concentration of flavonoids in these plants is linked to the UV-exposure (Peñaranda, 2020). *B. papillosa*, previously identified as *B. obtusifolia*, reported the presence of phenolic compounds, coumarins, steroids and a sesquiterpene lactones (Escobar et al., 2009; Zdero et al., 1989). Moreover, other studies showed no acute toxicity for one oral dose of its ethanolic extract (Loza Almanza et al., 2011), as well as no cytotoxicity on colon cancer cells (Rodrigo et al., 2010).

It is well known that flavonoids and derivatives of cinnamic acid absorb UV radiation (Calle et al., 2012; Escobar et al., 2009; Nichols and Katiyar, 2010; Peñaranda et al., 2020). Several studies made in *Arabidopsis mutants* proved that large amounts of flavonoids, anthocyanins and sinapic esters were found in *A. mutants* tolerant and resistant to UV radiation (Landry et al., 1995; Li et al., 1993). The ultraviolet radiation that passes through the ozone layer and reaches the surface of Earth is about 5% of the sun-emitted radiation. The intensity of this radiation varies depending on the altitude above sea level, the zenith angle and the geographic position (Zaratti et al., 2003). The UV radiation is composed of about 5–10% of UVB radiation (280–320 nm) and the rest corresponds to UVA radiation (320–400 nm) (Fleming, 2008). The effects of the UV radiation to human skin are accumulative and dose dependent. The UVB radiation is responsible for producing dermal erythema, skin inflammation, and cell DNA damage. On the other hand, UVA radiation produces skin detriment, collagen and elastin reduction, and photoaging. Both UVB and UVA radiation contribute to photocarcinogenesis (Camacho, 2001; Rigel et al., 2006).

Because of the ultraviolet radiation risks to skin damage, many different formulations of sunscreens have been developed. The sunscreens contain an active substance (called filters) that may reflect, absorb, or refract the UV radiation preventing the skin UV damage. Chemical filters are molecules that absorb the UV radiation and re-emit it as lower energy radiation (thermic, visible, or fluorescent light) by the molecule's relaxation processes (Moreno and Moreno, 2010; Sánchez-Saldaña et al., 2002). Those molecules could be natural products from plants. Depending on the sunscreen properties, the most common classification for its photo-protective effectiveness is the Sun Protection Factor, SPF. The SPF is defined as the ratio between the time that a person can be exposed to the sun before getting a dermal erythema, with and without the protection of a sunscreen (Calderón, 2001; Camacho, 2001; Ribeiro, 2004; Sánchez-Saldaña et al., 2002). According to the SPF classification it is possible to find sunscreens with minimum SPF (2–12), moderate SPF (12–30) and high SPF (>30) (Camacho, 2001). The SPF is commonly measured in clinical trials with humans. However, these trials are rather costly, time consuming and may be considered controversial by ethical reasons. Because of this, different *in vitro* techniques for measuring the effectiveness of the sunscreen photoprotection, like the spectroscopic sun protection factor, have been developed and used for new sunscreen formulation developments (da Silva et al., 2005; Garcia Forero et al., 2019; Mansur, 1986a; 1986b).

There is a considerable interest in the use of naturally occurring plant products, including phenolic compounds, for the prevention of UV-induced skin photodamage, primarily, for preventing the risk of skin cancer (Nichols and Katiyar, 2010). A previous study on Chilean highland plants showed that *B. tola* could be a good candidate for a sunscreen photoprotection cream based on its SSPF as well as its antioxidant properties (Gajardo et al., 2016). In this sense, the aim of this study was to analyze the photo-protective

activity of an extract (EPP) from *B. papillosa* that was selected on a preliminary UVA/UVB absorption spectra study from five Bolivian *Baccharis* (*B. latifolia*, *B. papillosa*, *B. boliviensis*, *B. densiflora* and *B. tola*). For this, the EPP was subjected to a phytochemical analysis to identify their major phenolic components. Then, 3 of them were quantified at different altitudes and different seasons of the year to determine the relation between their concentration in the leaves and these abiotic factors. Finally, the following EPP photoprotective properties were determined: its Spectroscopic Sun Protection Factor (SSPF), its Broad-Spectrum Index (BSI), its Specific Extinction Coefficient compared with commercial photo-protectors and its photo-protective activity on *Escherichia coli* bacteria.

MATERIAL AND METHODS

Apparatus and chemicals

UV absorption spectra were obtained in an UV/Vis spectrometer (Cecil), CE 7400, 7000 series, using methanol (Sigma-Aldrich) as solvent. Nuclear Magnetic Resonance spectra were recorded on NMR equipment, of 300 MHz (Bruker) using $CDCl_3$ and DMSO d_6 (Sigma-Aldrich) as solvents. HPLC chromatograms were obtained in Agilent 1100 Series equipment with a quaternary pump, a diode array detector, DAD, and a RP-Silica C18 250 * 4.6 mm E10174 column. All solvents used were HPLC grade and the ultra-pure water was obtained by an ultrafiltration equipment (Satoorius Stedi m brand). The extractions and separations were performed with commercial solvents previously purified by distillation. The fractionations were carried out using size exclusion chromatography on Sephadex LH-20 and absorption chromatography columns on Silica gel 60 (63-200 mesh) and Silica gel 60 G (Merck). The photoprotective assay on bacteria was developed using a UVB lamp (Spectroline) at 312 nm.

Plant material

The aerial parts of the five *Baccharis* species were collected in the surroundings of La Paz city (Lluto and/or Cota Cota). *B. papillosa* was particularly collected in Lluto at an altitude of 4182 m.a.s.l. (16°37′23″ S, 68°01′8″ W; slope 38%), 4000 m.a.s.l. (16°35′25″ S, 68°00′5″ W; slope 30%), 3825 m.a.s.l. (16°35′21″ S, 68°01′10″ W; slope 26%) and from Cota Cota at an altitude of 3600 m.a.s.l., during the years of 2013 and 2014. The collected specimens were identified as *Baccharis latifolia*, *B. papillosa* subsp. *papillosa*, *B. boliviensis*, *B. densiflora* and *B. tola*. The specimen of *B. papillosa* subsp. *papillosa* was deposited in the National Herbarium of Bolivia and certified by the specialist Esther Valenzuela.

Extraction

For the preliminary evaluation, the milled and dry plant material (100 g of each *Baccharis* spp. aerial parts) was extracted by maceration in ethanol for 15 minutes (1:10 w/v). The plant was dried at room temperature, on a table in a ventilated room with no direct sunlight. The extracts' solvents were evaporated in a rotavapor until dryness under reduced pressure. Then, some small portions were dissolved in methanol for phytochemical and spectroscopic preliminary analysis.

For isolation of compounds and the other developed analysis, 2000 g of leaves of *B. papillosa* were subjected to a similar extraction process giving a dry ethanolic extract. To obtain an enriched extract in flavonoids and derivatives of cinnamic acid, a portion (5 g) of ethanolic extract was fractionated on Sephadex LH-20 column employing ethanol as solvent. The fractions were controlled by TLC plates and UV spectroscopy. The fractions with the highest intensities in their UV absorption spectra and that showed yellow spots in TLC plated revealed with H_2SO_4 were selected. The last fractions were selected and grouped together, giving an extract enriched in flavonoids and cinnamic derivative compounds. The extract enriched in phenolic compounds with photo-protective potential (EPP) was used for the rest of the analysis.

Isolation

The 8 major compounds **(1-8)** were isolated using adsorption chromatographic columns (CC). Silicagel 60 G was used for VLC fractionations, and Silicagel 60 (63-200 mesh) was used for CC separations, employing a gradient elution with petrol ether, ethyl acetate and methanol. The isolated compounds were identified by comparison with inhouse standards and analysis of the 1D and 2D NMR spectra as: 5,7-dihydroxy-3,4'-dimethoxyflavone **(1)**; 3-prenil-4-hydroxy-cinnamic acid **(2)**; 5,7,3′-trihydroxy-3,4'-dimethoxy-flavone **(3)**; 5,7,4'-trihydroxy-3-methoxyflavone **(4)**; 5,7,3',4'-tetrahydroxy-3-methoxyflavone **(5)**; 3,4-dihydroxycinnamic acid **(6)**; 3,5,7-trihydroxy-4′-methoxy-flavone **(7)** and 3,5,7,4′-tetrahydroxyflavone **(8)**.

Compound 1 (5,7-dihydroxy 3,4′-dimethoxyflavone)

Yellow crystals, Mp. 218-221 °C; $C_{17}H_{14}O_6$ (PM 314.3 g/mol); **UV** (c. 0.02 g/L. in MeOH); λ_{max} 267.0 (absorbance 1.25); λ_{max} 348.0 (absorbance 1.07), **^{1}H NMR** (300 MHz, in CCl_3D), δ 6.24 (1H, *d*, *J* = 2.0 Hz, H-6), 6.38 (1H, *d*, *J* = 2.0 Hz, H-8), 8.04 (2H, *dd*, *J* = 2.1, 9.0 Hz, H-2′, H-6′), 7.0 (2H, *dd*, *J* = 2.1, 9.0 Hz, H-3′, H-5′), 3.78 (3H, *s*, OCH_3), 3.87 (3H, *s*, OCH_3); **^{13}C NMR**

(300 MHz in CCl_3D), δ 156.0 (C-2), 138.6 (C-3), 178.6 (C-4), 156.9 (C-5), 98.8 (C-6), 164.0 (C-7), 93.8 (C-8), 161.6 (C-9), 105.1 (C-10), 122.5 (C-1′), 129.9 (C-2′), 113.9 (C-3′), 161.6 (C-4′), 113.9 (C-5′), 129.9 (C-6′), 59.8 (3-OCH_3), 55.1(4'-OCH_3); **HPLC** (Rt 28,26 min, λ = 370 nm).

Compound 2 (3-prenyl-4-hydroxycinnamic acid), drupanin

White crystals, Mp. 149 – 151 °C; $C_{14}H_{16}O_3$ (PM 232.3 g/mol); **UV** (c. 0.02 g/L. in MeOH); λ_{max} 236.0 (absorbance 0.97); λ_{max} 315.0 (absorbance 1.5); **^{1}H NMR** (300 MHz, in CCl_3D), δ 7.16 (1H, *d*, *J* = 2.0 Hz, H-2), 6.69 (1H, *d*, *J* = 8.2 Hz, H-5), 7.30 (1H, *dd*, *J* = 2.0, 8.2 Hz, H-6), 7.5 (1H, *d*, *J* = 15.9 Hz, H-1′), 6.12 (1H, *d*, *J* = 15.9 Hz, H-2′), 3.2 (1H, *d*, *J* = 7.2 Hz, H-1′′), 5.21 (1H, *dddd*, *J* = 7.2, 7.2, 1.3, 1.3 Hz, H-2′′), 1.65 (1H, *s*, H-4′′), 1.6 (1H, *s*, H-5′′); **^{13}C NMR** (300 MHz in CCl_3D), 125.8 (C-1), 129.64 (C-2), 128.58 (C-3), 157.21 (C-4), 114.0 (C-5), 127.3 (C-6), 146.1 (C-1′), 115.0 (C-2′), 170.0 (C-3′), 28.0 (C-1′′), 121.7 (C-2′′), 133.1 (C-3′′), 25.5 (C-4′′), 17.5 (C-5′′); **HPLC** (Rt 22.91 min, λ = 315 nm).

Compound 3 (5,7,3′-trihydroxy-3,4'-dimethoxyflavone)

Yellow crystals, Mp. 229 – 230 °C; $C_{17}H_{14}O_7$ (PM 330.3 g/mol); **UV** (c. 0.02 g/L. in MeOH); λ_{max} 254 (absorbance 0.85); λ_{max} 359 (absorbance 0.78), **^{1}H NMR** (300 MHz, in CCl_3D), δ 6.18 (1H, *d*, *J* = 2 Hz, H-8), 6.32 (1H, *d*, *J* = 2 Hz, H-6), 7.57 (1H, *d*, *J* = 2.1 Hz, H-6′), 6.89 (1H, *d*, *J* = 8.2 Hz, H-6′), 7.60 (1H, *dd*, *J* = 8.2, 2.1 Hz, H-5′, H-2′), 3.71 (3H, *s*, OCH_3), 3.88 (3H, *s*, OCH_3); **^{13}C NMR** (300 MHz in CCl_3D), δ 159.9 (C-2), 142.7 (C-3), 182.7 (C-4), 165.4 (C-5), 102.8 (C-6), 168.0 (C-7), 97.9 (C-8), 160.9 (C-9), 109.0 (C-10), 127.1 (C-1′), 118.7 (C-2′), 149.8 (C-3′), 153.6 (C-4′), 114.8 (C-5′), 125.2 (C-6′), 64.0 (3-OCH_3), 59.8 (4′-OCH_3).

Compound 4 (5,7,4′-trihydroxy-3-methoxyflavone)

Yellow crystals, Mp. 293 – 294 °C; $C_{16}H_{12}O_6$ (PM 300.3 g/mol); **UV** (c. 0.02 g/L. in MeOH); λ_{max} 267.0 (absorbance 1.07); λ_{max} 359.0 (absorbance 1.00), **^{1}H NMR** (300 MHz, in CCl_3D), δ 6.23 (1H, *d*, *J* = 2.1 Hz, H-6), 6.36 (1H, *d*, *J* = 2.1 Hz, H-8), 7.96 (2H, *dd*, *J* = 2.0, 8.9 Hz, H-2′, H-6′), 6.91 (2H, *dd*, *J* = 2.0, 8.9 Hz, H-3′, H-5′), 3.76 (3H, *s*, OCH_3); **^{13}C NMR** (300 MHz in CCl_3D), 156.5 (C-2), 138.3 (C-3), 178.7 (C-4), 156.9 (C-5), 98.8 (C-6), 163.8 (C-7), 94.0 (C-8), 159.6 (C-9), 105.1 (C-10), 121.6 (C-1′), 130.3 (C-2′), 115.6 (C-3′), 161.4 (C-4′), 115.6 (C-5′), 130.3 (C-6′), 60.0 (3-OCH_3); **HPLC** (Rt 20.53 min, λ = 370 nm).

Compound 5 (5,7,3′,4′- tetrahydroxy-3- methoxyflavone)

Yellow crystals, Mp. 263 – 267 °C; $C_{16}H_{12}O_7$ (PM 316.3 g/mol); **UV** (c. 0.02 g/L. in MeOH); λ_{max} 255.0 (absorbance 1.04); λ_{max} 360.0 (absorbance 1.01), **^{1}H NMR** (300 MHz, in CCl_3D), δ 7.43 (1H, *dd*, *J* = 1.4, 8.4 Hz, H-2′), 6.9 (1H, *d*, *J* = 8.4 Hz, H-3′), 7.53 (1H, *d*, *J* = 1.43 Hz, H-6′), 6.18 (1H, *d*, *J* = 1.4 Hz, H-6), 6.4 (1H, *d*, *J* = 1.4 Hz, H-8), 3.77 (1H, *s*, OCH_3), 12.7 (1H, *s*, OH); **^{13}C NMR** (300 MHz in CCl_3D), 156.8 (C-2), 138.1 (C-3), 178.3 (C-4), 156.0 (C-5), 99.1 (C-6), 164.9 (C-7), 94.1 (C-8), 161.7 (C-9), 104.5 (C-10), 121.0 (C-1′), 121.2 (C-2′), 116.2 (C-3′), 149.2 (C-4′), 145.7 (C-5′), 115.8 (C-6′), 60.1 (3-OCH_3); **HPLC** (Rt 14.03 min, λ = 370 nm).

Compound 6 (3,4-dihydroxycinnamic acid), caffeic acid

White crystals, Mp. 211 – 213 °C; $C_9H_8O_4$ (PM 180.2 g/mol); UV (c. 0.02 g/L. in MeOH); λmax 295.0 (absorbance 0.7); λmax 327.0 (absorbance 0.91); **^{1}H NMR** (300 MHz, in CCl3D), δ 6.97 (1H, d, J = 1.9 Hz, H-6), 6.73 (1H, dd, J = 8.0, 2.5 Hz, H-6, H-2), 6.86 (1H, dd, J = 8.0, 1.9 Hz, H-5, H-2), 7.51 (1H, d, J = 15.7 Hz, H-8), 6.19 (1H, d, J = 15.9 Hz, H-7); **^{13}C NMR** (300 MHz in CCl3D),δ 128.8 (C-1), 116.3 (C-2), 147.2 (C-3), 149.8 (C-4), 117.5 (C-5), 124.3 (C-6), 148.1 (C-7), 116.5 (C-8), 170.0 (C-9).

Compound 7 (3,5,7-trihydroxy-4′-methoxyflavone)

Yellow crystals, $C_{16}H_{12}O_6$ (PM 300.3 g/mol); **UV** (c. 0.04 g/L. in EtOH); λ_{max} 265.0 (absorbance 0.75); λ_{max} 366.0 (absorbance 0.88); **^{1}H NMR** (300 MHz, in CCl_3D), shows 6 aromatic protons forming two spin systems: the first is a *meta* spin system at δ_H 6.37 *br.s,* H-6 and δ_H 6.21 *br.s*, H-8, coupled in ring A. Ring B presents a spin system *ortho* to δ_H 6.96 *d* (*J* = 8.8 Hz) and to δ_H 8.10 *d* (*J* = 8.8 Hz) corresponding to the protons H-5' and H-3' coupled to H-6' and H-2'; **^{13}C NMR** (300 MHz in CCl_3D), 145.86 (C-2), 135.61 (C-3), 175.40 (C-4), 156.91 (C-5), 98.58 (C-6), 163.98 (C-7), 93.99 (C-8), 160.88 (C-9), 103.29 (C-10), 123.39 (C-1′), 129.35 (C-2′), 113.88 (C-3′), 160.57 (C-4′), 113.88 (C-5′), 129.35 (C-6′), 55.28 (4′-OCH_3); **HPLC** (Rt 26.98 min, λ = 370 nm).

Compound 8 (3,5,7,4′-tetrahydroxyflavone)

Yellow crystals, $C_{15}H_{10}O_6$ (PM 286.2 g/mol); **UV** (c. 0.04 g/L. in MeOH); λ_{max} 265.0 (absorbance 0.80); λ_{max} 366.0 (absorbance 0.98); **^{1}H NMR** (300 MHz, in CCl_3D), shows 6 aromatic protons forming two spin systems: the first is a *meta* spin system at δ_H 6.21 *d (J = 2.0),* H-6 and δ_H 6.40 *d (J = 2.0)*, H-8, coupled in ring A. Ring B presents a spin system *ortho* to δ_H 6.92 *d* (*J* = 8.9 Hz) and to δ_H 8.08 *d* (*J* = 8.9 Hz) corresponding to the protons H-5' and H-3' coupled to H-6' and H-2'; **^{13}C NMR** (300 MHz in CCl_3D), 146.72 (C-2), 135.69 (C-3), 175.90 (C-4), 156.87 (C-5), 98.16 (C-6), 164.01 (C-7), 164.01 (C-8), 160.92 (C-9), 103.35 (C-10), 122.41 (C-1′), 129.43 (C-2′), 115.10 (C-3′), 158.95 (C-4′), 115.10 (C-5′), 129.43 (C-6′); **HPLC** (Rt 19.49 min, λ = 370 nm).

Spectral evaluation of the UV absorption capacity of extracts and compounds

A comparison of the areas under the absorption spectral curves of the ethanolic extracts of the five *Baccharis* species, and the EPP of *B. papillosa*, was carried out using an UV/Vis spectrometer (Cecil), CE 7400, 7000 series, and methanol (Sigma-Aldrich) as solvent. The area under the spectral curves was calculated as in equation [1].

$$A = \sum[(\lambda_j - \lambda_i) * h_i] \quad [1]$$

Where A is the area under the absorbance curve; λ is a spectrum wavelength; λ_j is the subsequent wavelength; and *h* is the height (Absorbance) at λ_i. All the UV absorption spectra of *Baccharis* extracts were obtained at 80 ppm, while the spectra of compounds were obtained at 20 ppm. The areas underneath the curves were calculated in the following ranges: UV (280-400 nm), UVB (280-320 nm) and UVA (320 to 400 nm).

Sample collection in different seasons and altitudes

For sample collection, the Lluto region was selected, Mecapaca province, department of La Paz, Bolivia. The region was selected because it has a steep altitudinal gradient (between 3450 and 4200 m.a.s.l.), a high natural production of *B. papillosa*, and an absence of urban environmental pollution. The study was carried out every three months around December 21st of 2013 (summer), March 21st of 2014 (autumn), June 21st of 2014 (winter) and September 21st (Spring). Three study areas were located based on the most representative populations of *B. papillosa* (Zone 1: 4182 m.a.s.l.; Zone 2: 4000 m.a.s.l.; Zone 3: 3825 m.a.s.l.) where the height, slope, and coordinates were determined. The georeferencing of points was made with a GARMIN 64s GPS. The altitude was measured with an altimeter, model 39 (Barigo), and the slope was measured with a clinometer, PM5/360PC (SUUNTO).

Quantification of total flavonoids by UV spectroscopy method

The method used for the determination of total flavonoids by UV spectrophotometry, was an adaptation of the method of Rengifo-Penadillos (2013). The total flavonoid content was determined using an aluminum chloride colorimetric method with luteolin as standard. According to this method, 0.5 mL of ethanolic extract was mixed with 1.5 mL of distilled 96° ethanol. This was followed by the addition of 2.8 mL of distilled water, 0.1 mL of $AlCl_3$ solution (10% w/v), and 0.1 mL of potassium acetate solution (1 M). The solution was left still for 30 - 40 min and then subjected to spectral analysis in the range of 200 to 500 nm. The samples of which its absorbance turned pink, were measured at 406 nm. A luteolin's calibration curve was made by preparing solutions at 30, 60, 90, 120 and 150 ppm of the standard, following the same aluminum chloride reaction, and recording the absorbance at λmax = 406 nm, where luteolin showed good linearity (y = 0.0823x, r^2 = 0.9988). The total flavonoid content was calculated using luteolin's calibration equation. Note that all calibration curves were fitted to Beer-Lambert equation A = εbc.

Separation of components by HPLC

The HPLC method used was an adaptation of the method of Talhaoui et al. (2014). The compounds separation was carried out on a HPLC equipment (Agilent, 1100 Series), which consists of a vacuum degasser, manual injector, a quaternary pump, and a diode array detector, DAD, with multiple wavelength detection. The separations were carried out on an Eclipse Plus (reversed phase) C_{18} column (ZORBAX) of 4.6 × 250 mm d.i., 5 µm at 25°C. The wavelengths used were 370.4 nm, 310.8 nm, and 280 nm. The separation method used a gradient elution of phosphoric acid pH = 2 (solvent A) and acetonitrile (solvent B); 0 min, 25% B; 5 min, 28% B; 10 min, 30% B; 17 min, 32.5% B; 20 min, 36% B; 25 min, 42% B; 33 min, 44.8% B; 40 min, 53.5% B; 60 min, 100% B. The samples were run at a constant flow of 0.6 mL/min throughout the gradient. Then, 25 µL of sample was injected to the column. Samples were prepared by dissolving the extracts in 5 mL with HPLC grade methanol and filtered with a 0.45 µm PTFE membrane filter.

Quantification of compounds by HPLC

For the quantification by HPLC using the method described above, three compounds **(1)**, **(2)** and **(8)** were selected. These were the major compounds as can be assumed by the chromatograms of Fig. 3A-B. Calibration curves were performed by preparing solutions of compound **(1)** at 64, 100, 150, 200 and 250 mg/L and measuring its absorbance at λ_{max} = 348.0 nm obtaining the calibration equation (y = 26.469x, r^2 = 0.9945). For compound **(2)**, solutions were prepared at 500, 700, 1000, 1120 and 1400 mg/L, with λ_{max} = 315.0 nm and calibration curve (y = 28.96x + 13915, r^2 = 0.9807). For compound **(8)**, solutions were prepared at 250, 350, 450, 550, 650 and 760 mg/L, with λ_{max} = 366.0 nm, and calibration equation (y = 22.499x, r^2 = 0.9933).

Spectroscopic Sun Protection Factor (SSPF)

The SSPF of EPP was determined using Mansur Spectroscopic Method (Mansur, 1986a; 1986b). This method requires dissolving 0.2 µL of a sunscreen product in 1 mL of methanol. The SSPF is calculated using the equation [2] in the absorption spectrum of this solution.

$$SSPF = CF * \sum_{290}^{320} EE(\lambda) * I(\lambda) * abs(\lambda) \quad [2]$$

Where *CF:* is the Correlation Factor (= 10) determined according to known sunscreens, so that a cream containing 8% homosalate gives an SPF = 4; *EE(λ):* is the solar radiation effect in the respective wavelength; *I(λ):* is the solar radiation intensity as a function of wavelength; and a*bs(λ):* is the spectrophotometric absorbance of the sunscreen solution depending on the wavelength.

Three methanolic solutions of EPP were prepared to simulate sunscreen products at 5, 10 and 15% (w/v) to determine their SSPF. Each concentration represented an independent sample. They were diluted to a concentration of 0.2 µL/mL equivalent to 10, 20 and 30 mg/L, respectively. The spectroscopic absorbance was performed every 5 nm, from 290 nm to 320 nm. The obtained results were summed in Mansur's equation giving the SSPF *in vitro* using pure methanol as standard control.

Broad Spectrum Index (BSI)

To determine the BSI of the EPP, we used the method reported by Diffey (1994). This method requires determining a critical wavelength (*λc*), where the absorption spectrum area from 290 nm to λc, is 90% of the total absorption spectrum area between 290 and 400 nm. That is equation [3].

$$SBSR = \int_{290}^{\lambda_C} A(\lambda)d\lambda = 0.9 \int_{290}^{400} A(\lambda)d\lambda \quad [3]$$

Where A(λ): is the sunscreen absorbance at a given wavelength, λ. Once the λc value is determined, the corresponding rating from 0 to 4 stars is given by the follow classification: For $\lambda_c < 325$, the rating is 0 stars; For $325 \leq \lambda_c < 335$, the rating is 1 star; For $335 \leq \lambda_c < 350$, the rating is 2 stars; For $350 \leq \lambda_c < 370$, the rating is 3 stars and for $370 \leq \lambda_c$, the number of stars is 4. More stars imply broader spectrum index. The spectrum of three independent methanolic solutions (10, 20 and 30 mg/L) of EPP was used to determine the λc value. Note that the concentration is not important for determining the BSI.

Specific Extinction Coefficient ($E_{1,1}$) curve comparison between the EPP and commercial sunscreen active principles

The EPP $E_{1,1}$ was calculated by equation [4].

$$E_{1,1} = \varepsilon\left[{}^{L}/_{g \cdot cm}\right] \times 10\left[{}^{g}/_{L}\right] \times 1[cm] \quad [4]$$

Where ε [L/g• cm] is the Mass Extinction Coefficient and it is multiplied by the concentration of a solution of 10 g/L of analyte and an optical length of 1 cm. The EPP $E_{1,1}$ was calculated every 5 nanometers from 280 nm to 400 nm. These ε [L/g• cm] were calculated from calibration curves constructed for each nanometer used, varying the EPP concentration from 10 to 40 ppm. The slope of each curve corresponds to the $E_{1,1}$ for that specific wavelength. The $E_{1,1}$ values were graphed against their wavelengths, and these $E_{1,1}$ curves were compared to the curves reported by Lim and Draelos (Lim and Draelos, 2009) for different commercial sunscreen active principles. The Specific Extinction Coefficient ($E_{1,1}$) at the maximum wavelength and its standard error of mean (S.E.M.) is the result of four replicates.

Photo-protective assay on *Escherichia coli* bacteria

Bacteria

The bacteria used for the tests was *Escherichia coli* ATCC 23922 as a 0.5 McFarland standard (Catalogue No. TM50-TM60, December 2002).

Sample preparation

Fifty milligrams of EPP was dissolved with 50 µL of ethanol, then homogenized with 5 mL of Muller Hinton broth using a vortex. Ten milligrams of the standards, oxybenzone or para-aminobenzoic acid (HMBC or PABA) were dissolved with 50 µL of DMSO and homogenized with 5 mL of Muller Hinton broth separately. The first control sample was prepared mixing 50 µL of 96% ethanol with 5 mL of Muller Hinton broth while the second control sample was prepared mixing 50 µL of DMSO with 5 mL of the broth. All tubes were heated to 37°C before the test starts.

Test

The test was based on the method reported by Muela et al. (2000) and da Silva Fernandes et al. (2015). In each Muller Hinton broth, 10 µL of *E. coli* were added. Each sample was incubated for 45 min at 37°C. Then, 5 µL of the incubated sample was plated in 5 × 5 cm Muller Hinton agar Petri dishes in sextuplets. All the Petri dishes were half capped. Each uncapped half of the Petri dishes was irradiated with a UV lamp (Spectroline-B) at 312 nm for 12 seconds at 4.2 W/m^2. After irradiation, the Petri dishes were incubated at 37 °C for 18 hours. The number of colonies that survived the effect of irradiation were counted. The irradiation time was standardized with 50% survival in the control samples. This test was repeated four times to achieve 24 repetitions of each test.

Statistical analysis

The absorption spectra of the *Baccharis* species were recorded in triplicate. The variation of the absorbance at the maximum wavelength is expressed in terms of the standard deviation (S.D.). In the same way, the areas under the curves in the UV, UVB and UVA ranges are the average of three replicates. The variation of the areas is expressed in terms of the standard deviation (S.D.). ANOVA test followed by Tuckey's test ($p<0.05$) was performed to evaluate the statistical differences in the areas between the *Baccharis* species, using the software Microsoft Excel.

For the quantification of total flavonoids and of the individual compounds, three independent extractions were performed from the collected plant material, for each season and for each altitude. The total flavonoid

content was measured from each extract. The results report the average contents from these three extractions with its respective standard deviation (S.D.). ANOVA test followed by Tukey's test ($p \leq 0.05$) was performed between altitudes and between sampling seasons using the software SPSS v.8.

For the quantification of the spectroscopic sun protection factor, for the broad spectrum index BSI, and the Specific extinction coefficient, three samples of the EPP extract were weighted. The absorption spectra of the EPP were used for measuring the three parameters. The results report the average value and the standard deviation (S.D.). In the case of the specific extinction coefficient, the standard error of mean (S.E.M.) is presented.

The photoprotective assay in *Escherichia coli* was performed in sextuplets, with four repetitions each. ANOVA test with 95% confidence followed by Tuckey's test ($p<0.05$) was performed between the different photoprotection active principles and the controls. The statistical analysis was performed using the software Minitab, ver. 17.

RESULTS AND DISCUSSION

Spectral evaluation of the extract

The ethanolic extracts of five Bolivian *Baccharis* species (*B. latifolia*, *B. papillosa*, *B. boliviensis*, *B. densiflora* and *B. tola*) were submitted to a preliminary UV spectra analysis (Fig. 1). The results showed that all *Baccharis* presented phenolic compounds that absorb UVB and UVA radiation. However, the area under the curve of the UV spectra of the different ethanolic extracts at 80 ppm (Table 1) showed clearly that *B. papillosa* possess the highest absorbance area under the UVB and UVA region. As a result, *B. papillosa* was selected for further studies.

The ethanolic extract of the leaves of *B. papillosa* was separated by a Sephadex chromatography column to obtain the desired enriched extract in flavonoids and derivatives of cinnamic acid (EPP). Fifteen fractions were obtained and analyzed by UV spectroscopy. It was observed that the last fractions had the highest UV absorption. Therefore, these last fractions were selected and grouped to form the EPP.

The UV spectrum of the EPP in *B. papillosa* showed that the area under the curve of the UV region (280 – 400 nm), UVB region (280–320 nm), and UVA region (320–400 nm) is 3, 2.6 and 3.6 times higher than in its ethanolic extract, respectively (Table 1). This means that, effectively, the active principles of *B. papillosa* were enriched in the EPP.

Comparing the areas of the five *Baccharis*, it can be observed that the biggest area in the UV region belongs to *B. papillosa*. This species is followed by *B. latifolia*, *B. boliviensis*, y *B. tola* that have similar areas with no significant differences. Then, *B. densiflora* has the smallest area among all the *Baccharis* species. The same area order among the *Baccharis* species is kept for the UVB region. Note that *B. latifolia* has a quite constant absorption band between 265 and 345 nm. In the UVA region, *B. papillosa*, *B. latifolia*, *B. boliviensis*, and *B. tola* have the same areas with no significant differences. *B. densiflora* has the lowest UV, UVB, UVA absorption area among the studied *Baccharis* species. The UV spectra of *B. tola*, *B. boliviensis* and *B. densiflora* are more characteristic of phenolic components such as flavonoids. This may be assumed by the two characteristic absorption bands between 265–305 nm, and between 315–365 nm. Given the higher absorption bands of *B. papillosa*, it was selected for further studies.

Identification of phenolic compounds

The EPP was subjected to a phytochemical study to identify the compounds responsible of absorbing UVB and UVA light. Using different chromatographic techniques, eight compounds were isolated and identified as flavonoids and cinnamic acid derivatives. Their structures are shown in Fig. 2.

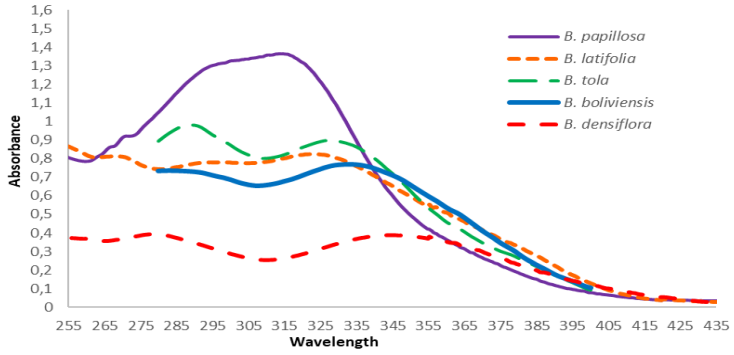

Figure 1. UV absorption spectrum of the ethanolic extract solution of five *Baccharis* spp. at 80 mg/L.

Average absorbance for each extract were *B. papillosa* (1.71 ± 0.26 at 315 nm, n = 3); *B. latifolia* (0.9 ± 0.1 at 323 nm, n = 3); *B. tola* (0.89 ± 0.04 at 332 nm, n = 3); *B. boliviensis* (0.76 ± 0.04 at 338 nm, n = 3); *B. densiflora* (0.4 ± 0.02 at 347 nm, n = 3).

Table 1. Areas under UVA, UVB and UV absorption curves of different *Baccharis* ethanolic extracts, and *B. papillosa* EPP.

Sample	Area UV 280–400 nm	Area UVB 280–320 nm	Area UVA 320–400 nm	Concentration (mg/L)	n
B. papillosa	104.3 ± 13.2[a]	59 ± 8.6[a]	45.8 ± 5.1[a]	80.0	3
B. latifolia	73.6 ± 5.1[b]	33.1 ± 2.7[b]	40.7 ± 2.6[a]	80.0	3
B. boliviensis	67.5 ± 3.5[b]	27.8 ± 1.5[b]	40.4 ± 2.0[a]	80.0	3
B. tola	75.5 ± 4.1[b]	35.3 ± 1.9[b]	41.1 ± 2.3[a]	80.0	3
B. densiflora	36.2 ± 1.7[c]	12.8 ± 0.7[c]	23.5 ± 1.1[b]	80.0	3
B. papillosa EPP	153.6 ± 6.4[d]	74.8 ± 3.1[d]	81.2 ± 3.2[c]	40.0	3

The table shows mean values of three replicates ± the standard deviation (SD). Different letters mean statistical area differences by ANOVA test followed by Tukey's test ($p \leq 0.05$) between *Baccharis* species in the UV, UVB, and UVA regions, respectively.

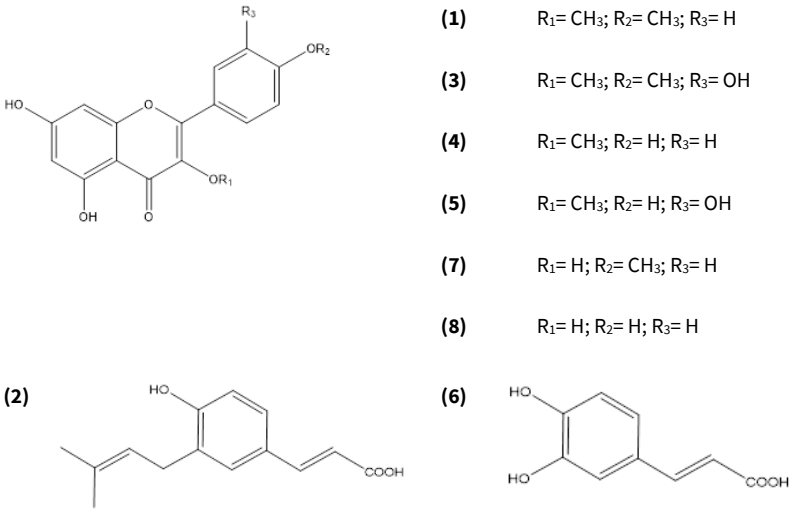

Figure 2. Phenolic compounds isolated from *Baccharis papillosa* ethanolic extract

Compounds **(1)** 5,7-dihydroxy 3,4'-dimethoxyflavone; **(2)** 3-prenyl-4-hydroxycinnamic acid; **(3)** 5,7,3'-trihydroxy-3,4'-dimethoxyflavone; **(4)** 5,7,4'-trihydroxy-3-methoxyflavone; **(5)** 5,7,3',4'-tetrahydroxy-3-methoxyflavone; **(6)** 3,4-dihydroxycinnamic acid; **(7)** 3,5,7-trihydroxy-4'-methoxyflavone; **(8)** 3,5,7,4'-tetrahydroxyflavone.

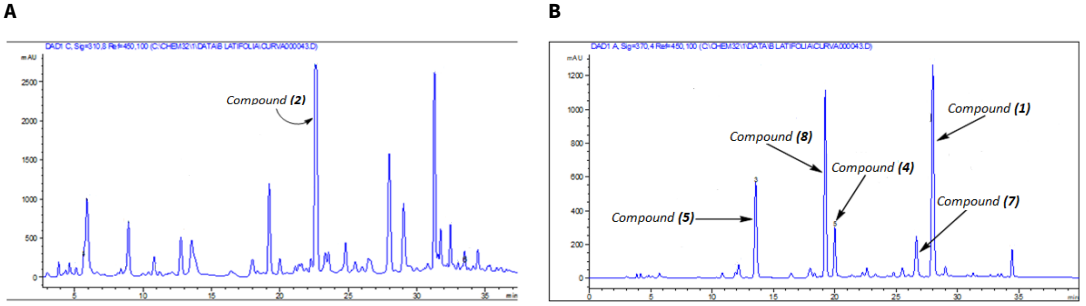

Figure 3. Chromatograms of EPP at 315 nm **(A)** and 370 nm **(B)**.

The peaks correspond to the compounds **(1)**, **(2)**, **(4)**, **(5)**, **(7)** and **(8)** were identified by their retention times of their individual chromatograms. The compounds retention times were: (1): Rt = 28.26 min, λ = 370 nm; (2): Rt = 22.91 min, λ = 315 nm; (4): Rt = 20.53 min, λ = 370 nm ; (5): Rt = 14.03 min, λ = 370 nm; (7): Rt = 26.98 min, λ = 370 nm; (8): Rt = 19.49 min, λ = 370 nm.

The compounds **(3)**, **(6)**, **(7)** and **(8)** are reported for the first time for the *B. papillosa* species in the present study. For more information about compound **(3)** see reference (CAS Common Chemistry, 2021), and for **(6)** see reference (ChemSpider, 2021; Cornard and Lapouge, 2006). The EPP and the isolated compounds were analyzed and monitored by HPLC at 315 nm (Fig. 3A), identifying the major compound as drupanin **(2)**. Also, the wavelength of 370 nm was used (Fig. 3B) for monitoring the isolated flavonoids. The com-

pound's retention time was determined by their individual chromatogram and UV spectra. The compounds **(3)** and **(6)** were not identified in the HPLC chromatograms due to the unavailability of the pure compounds.

Total flavonoid content at different seasons and altitudes

The total flavonoid content in the EPP and the content of three of the major isolated compounds were quantified during one year in the four different seasons and at three different altitudes of the Lluto region in La Paz, Bolivia. The total flavonoid contents were determined using the UV spectroscopic method by quelation with $AlCl_3$. On the other hand, the selected compounds were quantified by HPLC using a calibration curve using in-house prepared standards from the respective isolated and purified compounds.

The analysis of total flavonoids presented in Fig. 4 shows that the highest concentration of flavonoids in the leaves of *B. papillosa* is observed at the highest altitude in every season. This is in agreement with the photo-protective role of flavonoids in this species because at higher altitude the solar UV radiation is more intense (Lavola, 1998). In addition, the season which corresponds to the highest flavonoid concentration is Spring. This could be explained by different factors. September is characterized by higher availability of water than in previous months. Then, the solar UV radiation is higher than in winter and the plant is exposed to the attack of more herbivores (Mazza et al., 1999). Consequently, the plant could produce more flavonoids to protect the leaves against those hazards.

Quantification of major components

The major flavonoids (**1** and **8**) and drupanin **(2)** were quantified by HPLC from the same extracts, at the same altitudes and in the four seasons. The results are observed in Fig. 5. Drupanin **(2)** is in the highest concentration in spring and at the highest altitude, following the same behavior as the one of the total flavonoid content (Fig. 5B). This may be indicative that the plant uses drupanin as a photo-protective compound against UV irradiation damage (Almeida et al., 2020; Silva-Carvalho et al., 2015). Flavonoid **(1)** presents the highest content in summer and at the highest altitude (Fig. 5A). Flavonoid **(8)** presents the highest concentration in Spring at the lowest altitude of 3825 m.a.l.s. (Fig. 5C). It can be observed that the highest amount of drupanin and flavonoid production is during the months of highest UV radiation. However, the production of a higher amount of flavonoid **(8)** at lower altitudes may have to do with other functions of this flavonoid in the plant (Andersen and Markham, 2005; Grotewold, 2006).

Spectroscopic Sun Protection Factor (SSPF)

A person exposed to the sun without any protection will obtain an erythema on the skin after a certain period of time, depending on its skin phototype. The Sun Protection Factor (SPF) of a photo-protective preparation is a factor that indicates the number of times a person can be exposed to the sun without getting an erythema, while wearing on the exposed skin, a layer of sunscreen of 2 mg/cm² (Calderón, 2001; Ribeiro, 2004; Sánchez-Saldaña et al., 2002). The SSPF is the same factor but measured as a comparison of *in vivo* and spectroscopic measurements with respect to a preparation of 8% homosalate (w/w) as the reference. The Mansur Spectroscopic Method for measuring the SSPF is described in the methodology section (Mansur, 1986 a; b). The SSPF (equation [2]) was obtained from the UV spectra of diluted solutions from initial preparations containing 5 (A), 10 (B) and 15% (w/v) (C) of EPP in solvent as excipient. The preparations were diluted according to Mansur et al. and the spectra of the (A), (B), and (C) diluted EPP solutions are presented in Fig. 6.

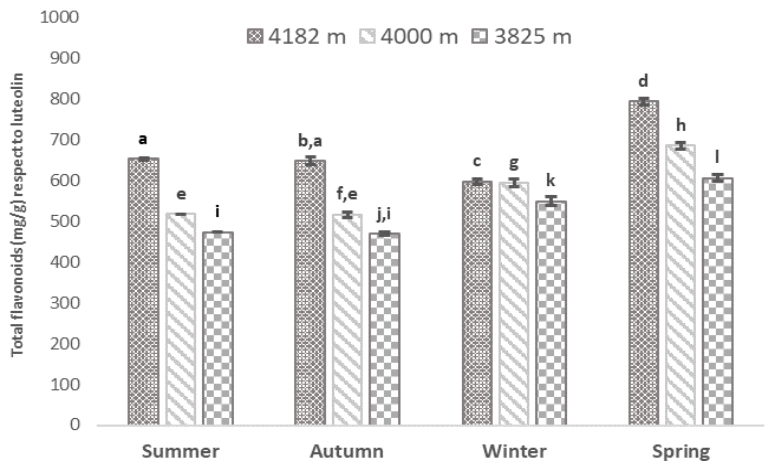

Figure 4. Total flavonoid content (mg/100 g dry weight, DW) in the ethanolic extract of the leaves of *B. papillosa*, in different seasons and at different altitudes.

Altitude 4187 m.a.s.l. (16°37'23" S, 68°01'8" W; slope 38%); Altitude 4000 m.a.s.l. (16°35'25" S, 68°00'5" W; slope 30%); and Altitude 3825 m.a.s.l. (16°35'21" S, 68°01'10" W).

Different letters mean statistical differences by ANOVA test followed by Tukey's test (p≤0.05) between altitudes and between sampling seasons. Each bar corresponds to the average of 3 replicates of the total flavonoids ± Standard deviation (S.D.)

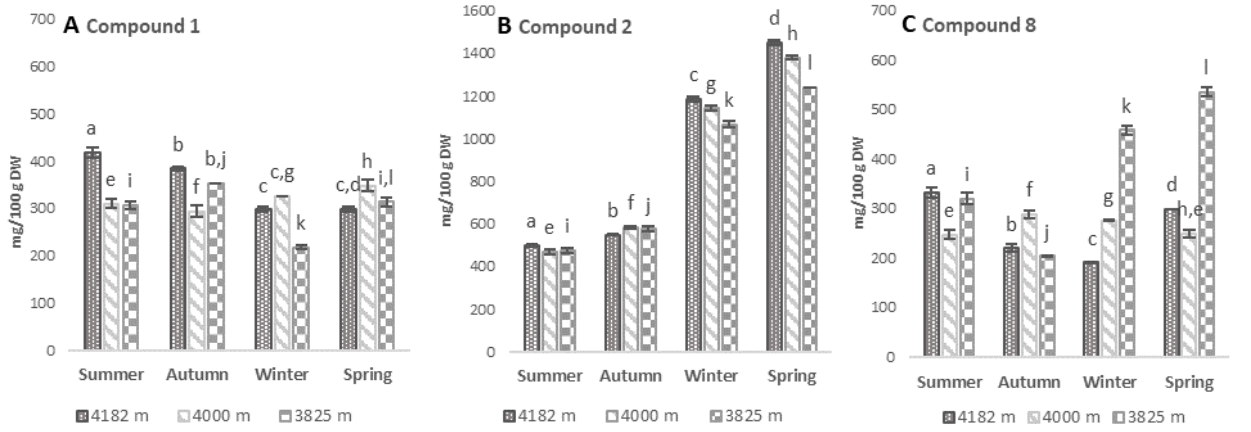

Figure 5. Contents of compounds **(1)**, **(2)** and **(8)** (mg/100g dry weight, DW) of the ethanolic extract of the leaves of *B. papillosa*, in different seasons and at different altitudes.

Different letters mean statistical differences by ANOVA and by Tukey's test ($p \leq 0.05$) between altitudes and between each season. DW: dry weight. The bar values indicate the average of three replicates of the phenolic compound content ± standard deviation (S.D.) These values are expressed for each season and all three different altitudes (4182, 4000, and 3825 m.a.s.l.)

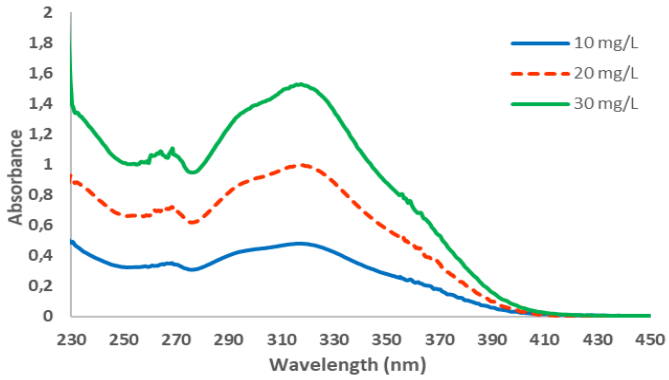

Figure 6. UV spectra of EPP solutions at 10 mg/L (A), 20 mg/L (B) and 30 mg/L (C) diluted according to Mansur et al. (1986a; 1986b) from initial reference solutions prepared at 5, 10 and 15% (w/v), respectively.

For 4 EPP solutions at 10, 20, 30 and 40 mg/L, the absorptivity value at 315 nm is 0.0512 ± 0.018 mg/L of EPP, $r^2 = 0.9996$.

The SSPF calculated for (A), (B) and (C) preparations are $SSPF_A = 4$, $SSPF_B = 9$ and $SSPF_C = 14$. These spectroscopic SPF values correspond to the minimum SPF found in sunscreens and cosmetics for UV protection. It was shown that a person may receive more than 25,000 minimum erythemal doses over their lives as a cumulative daily exposure to the sun. Thus, the EPP represent a good, daily, long term photoprotection of the skin to the cumulative lifetime solar UV radiation exposure (Nole and Johnson, 2004). Thus, this natural extract, EPP, could be very attractive to be used as an active principle in sunscreens, cosmetic products like make up and face creams for daily protection. Furthermore, note that the SSPF was calculated for a preparation of an active principle and a solvent transparent to light as excipient. Thus, more sophisticated formulations are expected to present higher values of SPF.

Broad Spectrum Index (BSI)

The SPF as well as the SSPF are usually determined in the UVB region. However, both ranges of radiation UVB and UVA have damaging effects on the skin. Because it is important to protect the skin from both ranges of irradiation, calculating the BSI is a useful indication of the degree of coverage of a photo-protective principle towards the UVA radiation range. The higher the number of stars given by the BSR, the higher the coverage of the range of the whole UV protection. The BSR for the EPP was calculated according to equation [3] (see Methods section). The critical wavelength (λ_c) is 365 nm. At this wavelength, the average area for a 20 mg/L EPP methanolic solution from $A_{290nm - \lambda c}$ was 58.9 ± 1.22 and the area $A_{290 - 400nm}$ was 65.9 ± 1.1. Then, at this wavelength, 90% of the total area of the EPP's UV spectrum was obtained. Hence, according to the BSR classification, for a wavelength of 365 nm, the corresponding EPP BSI was three stars. This result showed that the EPP has a good potential as broad spectrum photo-protective active principle because it will cover up to UVA II (320-365 nm) range. Nevertheless, it is necessary to compare the amplitude of the EPP UVA absorption spectrum with other sunscreen principles in the UVA range to have a better idea of its UVA photo-protection capacity.

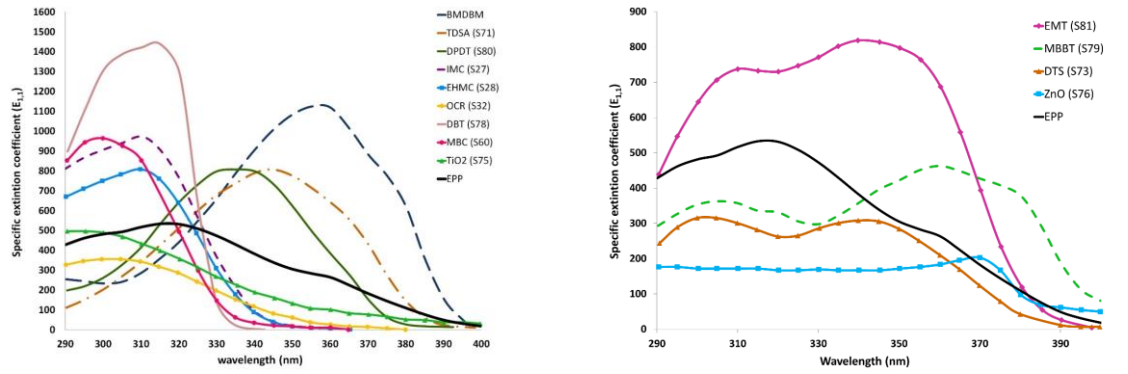

Figure 7. Specific extinction coefficient curves ($E_{1,1}$) as a function of wavelength of the *Baccharis papillosa* (EPP) and commercial sunscreen active principles for UVA and UVB range **(A)** and broad-spectrum commercial sunscreen active principles **(B)**. The maximum $E_{1,1}$ and its standard error at 315 nm is 511.7 ± 6.2 for four replicates.

The photoprotectors compared in the image are BMDBM: Avobenzone; TDSA: Terephthalylidene dicamphor sulphonic acid; DPDT: Dinatriumphenyldibenzimidazoltetrasulfonat; IMC: Isopentyl-4-methoxycinnamate; EHMC: Ethylhexyl methoxycinnamate; OCR: Octocrylene; DBT: Diethylhexyl butamido triazone; MBC: 4-Methylbenzylidene camphor; TiO2:Titanium oxide; EPP: *B. papillosa* extract enriched in phenolic compounds with photo-protective potential; EMT: Sepinov; MBBT: 2,2¢-Methylenebis[6-(2H-benzotriazol-2-yl)-4-(1,1,3,3-tetramethylbutyl)phenol; DTS: Drometrizole trisiloxane; ZnO: Zinc oxide.

Specific extinction coefficient ($E_{1,1}$) curve comparison

The $E_{1,1}$ curves of EPP and of commercial sunscreen active principles are shown in Fig. 7. The $E_{1,1}$ of the commercial sunscreen active principles were obtained from the curves reported by Lim and Draelos (2009), some of which are presented in Fig. 7. These curves are reproduced by permission of Taylor and Francis Group, LLC, a division of Informa plc. In Fig. 7A, the comparison between $E_{1,1}$ curves shows that the EPP's $E_{1,1}$ has higher values than the photoprotectors TiO_2, MBC, HMS, B-3 and B-4 through most of the UVB spectra (Lim and Draelos, 2009). However, the EPP has half of the $E_{1,1}$ values of MBC, EHMC, IMC y DBT approximately. On the other hand, the EPP's $E_{1,1}$ curve in the UVA range is lower than for DBM, TDSA and BMDBM (Lim and Draelos, 2009), except for the one of MA (Fig. 7A).

Comparing the $E_{1,1}$ curve of EPP with the ones of Broad Spectrum Photoprotectors (Fig. 7B), we can see that the EPP has higher values than DTS and ZnO in the whole UV spectrum. The EPP has also higher values than MBBT until 341 nm (UVB and UVA II range) even though EMT has the highest values until 370 nm. Analyzing these results, we can conclude that as a natural component, the EPP can be competitive with some of these sunscreens. Moreover, a formulation including zinc oxide would be very attractive.

Photoprotection on *Escherichia coli* bacteria

The photo-protective assay on *Escherichia coli* showed that the EPP has a photo-protective effect on the survival of *E. coli* bacteria compared to the survival of the unprotected bacteria in a medium containing only the solvents (ethanol and DMSO) used for dissolving the active photoprotectors. The EPP photoprotective effect was comparable to one of the controls PABA and oxibenzone positive standards.

E. coli bacteria grown in media with the *B. papillosa* extract (EPP), PABA and oxybenzone showed significantly greater survival than controls without treatment. This showed a photo-protection against the damage caused by UV-B in biological structures (Fig. 8).

It can be observed that there was no significant difference from the protection provided by the EPP compared to the commercial active principles PABA and oxibenzone. This supports the protective activity of the EPP for preventing UV photodamage.

The Sephadex column was found to be an effective technique for separating the phenolic compounds, responsible for the EPP UV absorption spectra, from bigger macromolecules as chlorophyl and other compounds. This allowed the enrichment of the flavonoids and cinnamic acid derivatives in one extract with photo-protective activity.

It was reported that altitude and environmental conditions play an important role in the production of secondary metabolites (Monschein et al., 2015). However, it is very difficult to separate the effects of both aspects (Körner, 2007). Our studies showed that the higher solar radiation found at higher altitudes would induce a higher production of flavonoids and cinnamic acid derivatives. In the same way, higher irradia-

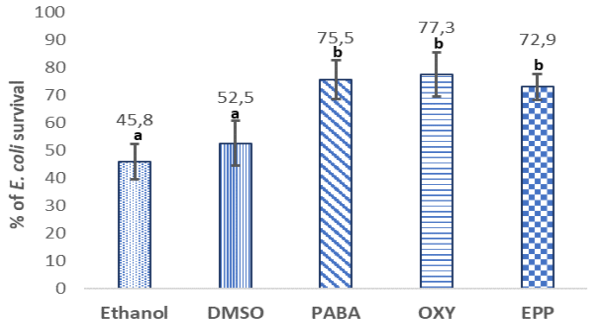

Figure 8. Photo-protective activity of EPP extract in survival of *E. coli* after irradiation with UV-B irradiation.

Data represent mean ± SD. Means with the same letter are not significantly different by ANOVA followed by Tuckey's test ($p < 0.05$). The samples presented are bacteria grown in media with para-Aminobenzoic acid (PABA), Oxibenzone (OXY), and *B. papillosa* extract (EPP). Control samples are bacteria grown in media with the solvent used for dissolving the photo-protective compounds: ethanol (EtOH), Dimethyl sulfoxide (DMSO). Each system has n = 4 replicates with 6 repetitions in each replicate (n total = 24 for each).

tion, as well as higher amounts of available water, would be obtained in spring and summer (Enríquez et al., 2018; Zaratti et al., 2003). Thus, this would influence the plant to produce higher quantities of phenolic content. These findings are important to increase the yield of production of the desired secondary metabolites, and thus decrease costs of production and availability of the active principles.

Nowadays, the Federal Drug Administration of USA considers that an optimum SPF is around 30 since a higher sun protection factor does not translate in important additional benefits. For example, an SPF of 30 blocks 96.7% of UV radiation, while a sunscreen with an SPF of 40 blocks 97.5% of the radiation (Camacho, 2001). Nevertheless, an SPF of 15 blocks 93.3% of the UV radiation (Pérez, 2012). Thus, the EPP from *B. papillosa* represents a convenient active photo-protective principle for a daily use to prevent cumulative skin damage from UV radiation. Moreover, it is a natural product, which is desired in an increasing natural product market.

All the tests performed for evaluating the photo-protective properties of the EPP showed positive results. There are fewer compounds with broad spectrum protection, which is why several components are needed during sunscreen formulations. The SPF is commonly measured for the UVB range. The SSPF calculated for the EPP confirms it prevents skin from obtaining an erythema under a prolonged time of exposure. Flavonoids have commonly UVA absorption giving an interesting broad spectrum protection. Drupanin has a high UVB absorption spectrum. The natural combination of both types of components obtained from the leaves of *Baccharis papillosa subsp. papillosa* offers the broad spectrum protection.

CONCLUSION

An enriched extract containing flavonoids and cinnamic acid derivatives was obtained from the leaves of *Baccharis papillosa*. Eight compounds were isolated from this extract. The compounds **(3)** 5,7,3′-trihydroxy-3,4′-dimethoxyflavone, **(6)** 3,4-dihydroxy-cinnamic acid, **(7)** 3,5,7-trihydroxy-4′-methoxyflavone, and **(8)** 3,5,7,4′-tetrahydroxyflavone are reported in *B. papillosa* for the first time. The phenolic content of the ethanolic extract of the leaves of *B. papillosa* measured at different altitudes and seasons of one year in Lluto, Bolivia, was performed. It was established that higher altitudes as well as seasons with higher UV radiation as spring and summer produced higher phenolic contents.

This EPP extract has an absorption spectrum in the range of the UVB-UVA region. According to its spectroscopic properties, the extract processes broad spectrum protection with a BSI of three stars. This means that the EPP covers a high portion of the UVA range (From 290 to 365 nm). For a formulation containing 15% (w/v) of EPP in solvent as an excipient, the spectroscopic sun protection factor, SSPF, equals to 9. This SSPF offers an interesting daily photo-protective activity for its use as an active component in sunscreens, makeup, and creams. Comparing different commercial sunscreen active principles, the EPP presents a competitive absorptivity along the UVB-UVA range. To further support the photoprotection activity found in the EPP, the survival of *E. coli* bacteria under UVB radiation showed that effectively, the EPP protects the biological tissue from photodamaging. The EPP has great potential as photo-protective principle and further studies are recommended.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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Contribution	Calle A	Curi-Borda CK	Gutierrez C	Salcedo L	Flores Y	Almanza GR
Concepts or ideas	x	x	x	x	x	x
Design	x	x	x	x	x	x
Definition of intellectual content	x	x				x
Literature search	x	x		x		x
Experimental studies	x	x	x	x	x	x
Data acquisition	x	x	x		x	x
Data analysis	x	x	x	x	x	x
Statistical analysis	x	x	x			
Manuscript preparation	x	x				x
Manuscript editing		x		x	x	x
Manuscript review	x	x	x	x	x	x

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