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ORIGINAL PAPERS

Novel UV filters from *Pentacalia pulchella* extracts with photoprotective properties and antioxidant activity

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Abstract
Most of the plant species found in the high mountain ecosystems of the tropics is unique and exceptional, because they have developed complex adaptations to survive in extreme environmental conditions, such as high levels of UVR and low temperatures of these ecological environments. In an exploratory study carried out on some plants of this ecosystem, we found that one of the most promising species was the *Pentacalia pulchella* (Kunth) Cuatrec. (Asteraceae) an endemic plant of Colombia, which grows between 2500 and 3500 m.a.s.l. Therefore, the objective of this work was to evaluate the photoprotective, antioxidant, and chemical composition of extracts from the leaves of *P. pulchella*. Extracts showed good absorption coefficients in UVA-UVB, high content of total phenols, with antioxidant activity comparable to that obtained with butylhydroxytoluene (BHT). Finally, the formulation labeled "7" with 10% extract presented adequate sensory characteristics for topical use, good in vitro photoprotection values in the UVA–UVB range (SPF (Sun Protection Factor): 7.3 ± 0.9, UVAPF (Ultraviolet A Protection Factor): 5.3 ± 0.6, λ_c 376), and antioxidant activity. Results obtained allow us to suggest that the extract of *P. pulchella* has a high potential as a source of new natural solar filters.

Keywords Natural sunscreens · *Pentacalia pulchella* · Photoprotection · UVA–UVB radiation

1 Introducción

In recent decades, a notable increase in the incidence of skin cancer has been observed around the world, which is mainly associated with habits of excessive exposure to ultraviolet radiation (UVR) from the sun [1–3]. In small doses, UVR is beneficial for health since they favor the production of vitamin D. However, overexposure can cause erythema, sunburn, carcinomas, cataracts, and other eye diseases, as well as photo-aging. Additionally, it has been proven that these radiations reduce the immune system's efficiency, since they modify the activity and distribution of cells responsible for the immune response [2, 4–7].

Likewise, solar radiation has a dual effect on plants; it can be beneficial and harmful. For this reason, environmental conditions have a great influence on the morphology and physiology of plants, since characteristics, such as size and shape, are modified by light, temperature, and drought, among others. These environmental conditions also modify its metabolism, leading to a decrease or increase of secondary metabolites [8–12]. One of the defense mechanisms identified against UVR damage is the production of polyphenolic compounds, among which the most characterized group are flavonoids. At present, numerous studies have helped understand not only their biosynthesis but also the metabolomic and genetic expression factors necessary for the biosynthesis of these molecules [12–15]. Studies have also been carried out that correlate the increased production of flavonoids when the plant is under environmental stress conditions. Then, high levels of UVR, low temperatures, pollution, and droughts can induce oxidative stress, which produces an increase in reactive oxygen species (ROS), generating alarm signaling processes in plants. Thus, they

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modify metabolism and gene expression, to respond to adverse environmental conditions [9, 11, 12, 15, 16].

In this sense, raw materials of natural origin could be a new source of bioactive compounds for skin sun protection. Numerous approaches and studies have been directed at groups of secondary metabolites, looking for photoprotective properties. Thus, compounds, such as carotenoids, mycosporins, and polyphenols, have been studied, obtaining promising results [17–20]. Regarding polyphenols, there is broad evidence in the literature of the benefits that these compounds can have when used as adjuvants in photoprotective formulations [11, 12, 21–26]. The additional advantages of polyphenols, when they are used as photoprotective bioactive, are as follows: their wide distribution in the plants that allows their easy availability, and their antioxidant potential that decreases the damage by oxidative stress induced by UVR [23, 27–29].

Furthermore, various polyphenols, such as epigallocatechin, resveratrol, caffeic acid, ferulic acid, quercetin, and kaempferol, among others, have also been found to have the ability to act as broad-spectrum sun filters, covering the UVA–UVB radiation range [11, 12, 30]. This evidence allows us to infer those polyphenols are promising compounds that could be used as new sun protection strategies. Colombia is a mega-diverse country, and new sources of photoprotection can be suspected in the plants of high mountain ecosystems. Therefore, we evaluated the chemical composition and photoprotective and antioxidant properties of the high-altitude plant *Pentacalia pulchella* (Kunth) Cuatrec. (Asteraceae).

2 Materials and methods

2.1 Chemicals

2,2-Diphenyl-2-picrylhydrazyl (DPPH•), polysorbate 80, gallic acid, and reference standards for LC and MS testing (caffeic acid, 3,4-dimethoxycinnamic acid, chlorogenic acid, ferulic acid, rutin, quercetin, and kaempferol) were obtained from Sigma Chemical Co. (St. Louis, MO). 2-thiobarbituric acid (TBA), butylated hydroxytoluene (BHT), and methyl linoleate (MeLo) were provided from Alfa Aesar (Ward Hill, MA, USA). Formic acid was obtained from Fermont (Monterrey, NL, México). Folin-Ciocalteu phenol reagent, methanol-HPLC, acetone, 37% hydrochloric acid, potassium chloride, sodium acetate, and sodium carbonate were obtained from Merck Chemical Supplies (Darmstadt, Germany). Carbomer 940 (Carbopol 940), triethanolamine (TEA), propylene glycol, and glycerin were obtained from LM. Productos químicos (Medellín, Colombia). All reagents were used as received.

2.2 Collection and extraction of plant material

The plant material (leaves) was collected in July 2013 in the municipality of Yarumal (Llanos de Cuiva), Antioquia-Colombia, (6° 49′50.6″ N; 75° 29′29.9″ W) at 2730 m.a.s.l. A specimen was deposited in the Herbarium of the University of Antioquia, Medellín, Antioquia, Colombia with voucher number HUA194797. Contract for Access to Genetic Resources and their Derivative Products N° 252, Resolution 0399—Ministerio de Ambiente y Desarrollo Sostenible, Colombia. The leaves were dried at room temperature for 30 days protected from light. Then, dry vegetal material (DVM) was minced using an electric mill (IKA, A11 basic S1). Briefly, 1 g of crushed DVM was degreased using 50 mL of hexane and magnetic stirring for 6 h (*ca.* 25 ± 2 °C). Next, the defatted DVM was subjected to extraction with 50 mL of solvent (methanol or acetone) and hydrochloric acid 37% (v/v) (0 or 0.25 mL) at room temperature (*ca.* 25 ± 2 °C) with magnetic stirring for 24 h. Then, the extracts were filtered and treated with activated carbon (0 or 1 g) to remove chlorophylls, filtered again through paper filters, and 0.45 µm membranes and dried on a rotary evaporator (IKA RV10 Basic, 40 °C). Finally, the dry extracts were dissolved in 100.0 mL of methanol and stored at -9° C. All assays were performed according to a full factorial design (2^3). Each assay was performed in triplicate (Table 1).

2.3 HPLC–DAD analysis

Analysis of the main components present in each of the extracts was carried out on an Agilent Technologies 1200 Series HPLC (Agilent, Palo Alto, CA, USA), equipped with a diode array detector (G1315B DAD), a degasser (G1379B), a binary gradient pump (G1312A), an autosampler (G1367B), and a column thermostat (G1316A). Reverse phase separation was performed on a Symmetry® column

Table 1 Extraction conditions according to a full factorial design (2^3)

Assay	Solvent	HCl[a] volume,	Activated carbon
1	Acetone	0.25 mL	0 g
2	Acetone	0.25 mL	1 g
3	Methanol	0.25 mL	0 g
4	Methanol	0.25 mL	1 g
5	Acetone	0.00 mL	0 g
6	Acetone	0.00 mL	1 g
7	Methanol	0.00 mL	0 g
8	Methanol	0.00 mL	1 g

[a]HCl: hydrochloric acid 37% (v/v)

of 4.6 mm × 75 mm, particle size 3.5 µm (Waters, Ireland) at constant temperature (30 °C), and a flow rate of 0.5 mL/min. For the separation, an elution gradient was used with a mobile phase composed of (A) formic acid 0.1% in Milli-Q water (v/v) and (B) formic acid in methanol HPLC grade 0.1% (v/v). Gradient program was 30% B (0.5 min), 30–90% B (6.5 min), 90% B (1 min), 90–30% B (1 min), and 30% B (2 min). The injection volume was 2 µL. Chromatographic analysis was monitored at 290, 310, 340, 380, and 520 nm, and the diode array detector was set in an acquisition range, at wavelengths comprised between 190 and 700 nm. Compounds were identified by their spectroscopic characteristics, such as absorption maxima, fragmentation patterns, and where possible by retention times compared to reference standards (caffeic acid, 3,4-dimethoxycinnamic acid, chlorogenic acid, ferulic acid, rutin, quercetin, and kaempferol).

2.4 UPLC-ESI-IT-MS^n analysis

Analysis of the main components present in each of the extracts was performed on a Thermo Scientific chromatograph equipped with an Accela autosampler and an Accela 600 quaternary pump coupled in-line to an LCQ Fleet ion trap mass spectrometer (Thermo Scientific). Reversed phase separation was performed as described in Sect. 2.3 and the injection volume was 10 µL. Effluent from the column was analyzed by ESI–MS in positive ion mode (LCQ Fleet ion trap from Thermo Scientific). The capillary voltage was set to 35 V, spray voltage was 4.5 kV, and tube lens at 80 V, Sheath Gas (nitrogen) flux was 45 (arbitrary units), and Aux Gas was 5 (arbitrary units). The capillary temperature was 300 °C. Data were acquired in a mass range of 100–1000 m/z. MS^n experiments were performed in the "Data Dependent Ion Tree" mode with a normalized collision energy of 35 (arbitrary units). Finally, data were analyzed using Xcalibur 2.1 (Thermo Scientific) and MZmine 2 (MZmine Development Team) software. Compounds were identified by their spectroscopic characteristics, such as molecular weight, fragmentation patterns, and where possible by comparison to reference standards (caffeic acid, 3,4-dimethoxycinnamic acid, chlorogenic acid, ferulic acid, rutin, quercetin, and kaempferol).

2.5 Antiradical capacity–DPPH assay

The antiradical ability of all extracts was determined using the DPPH• test as free radical [31, 32]. For each extract, different concentrations were evaluated, and the radical scavenging activity was calculated. The decrease in absorbance was determined at 515 nm at 0 min and every 30 s until completing 30 min. The initial concentration of DPPH• in the reaction medium was calculated from a calibration curve of DPPH measured at 515 nm, in concentrations of 2.5, 5.0, 7.5, 10, 25, 50, and 100 µM in methanol, prepared from the stock solution of 100 µM (equation $y = 1.146E2\,x - 4.192E\text{-}3$, $r = 0.9999$). Antiradical activity was expressed as effective concentration (EC_{50} = steady-state test concentration/DPPH concentration = t0). The results were compared against the antiradical activity of BHT.

2.6 Antioxidant activity—lipid (MeLo) model

The antioxidant capacity was measured by the inhibition of lipid peroxidation in MeLo, through the production of conjugated diene hydroperoxide (CDH) and thiobarbituric acid reactive substances (TBARS), using the method described by Mejía-Giraldo et al. [33]. Solutions of MeLo 10 mM (negative control), MeLo plus BHT 0.02% w/v (positive control), and MeLo plus 0.02% (w/v) of each extract were exposed to accelerated oxidation by heating at 40 ± 5 °C for 5 days in test tubes. After accelerated oxidation, each sample was dissolved in 1 mL of ethanol. To quantify the concentration of CDH produced during oxidation, the samples were diluted with ethanol in a ratio of 1:25 and measured spectrophotometrically at 234 nm, using an extinction coefficient of 29,000 M^{-1} cm^{-1}. The peroxidation level was expressed as mmol CDH kg^{-1} MeLo. Also, the lipid peroxidation level was determined as TBARS and expressed as mmol malondialdehyde kg^{-1} MeLo (mmol MD kg^{-1} MeLo) produced in the oxidation. For the quantification of TBARS, briefly, 50 µL of each sample was mixed with 350 µL of ethanol, 100 µL of BHT 0.2% (w/v) in ethanol, and 500 µL of TBA 0.37% (w/v) in HCl (0.25 mM) in a test tube. The resulting mixture was heated for 30 min in a sand bath at 90 ± 5 °C (the temperature of the sand bath was controlled using a temperature probe). The solution was rapidly cooled in an ice bath and the flocculated material was precipitated by centrifugation at 3000 rpm for 10 min. The absorbance of the samples was measured at 535 nm and corrected for non-specific turbidity by subtracting the absorbance at 600 nm. Finally, the peroxidation level was expressed as mmol of malondialdehyde (MDA) per kg MeLo using a molar extinction coefficient of 156,000 M^{-1} cm^{-1}.

2.7 Total phenolic contents: Folin–Ciocalteu assay

The total phenolic contents (TPC) in all extracts were measured using the modified Folin–Ciocalteu colorimetric method [34, 35]. Briefly, 100 µL of extract solution and 525 µL of deionized water were added to a test tube. Then, 125 µL of Folin–Ciocalteu reagent was added to the solution and it was allowed to react for 5 min. Subsequently, 1250

µL of 20% sodium carbonate solution was added and mixed. The blue color developed for 90 min, and the absorbance was read at 760 nm. TPC was calculated from a gallic acid calibration curve prepared at concentrations of 0, 1, 2, 4, 6, 8, and 10 mg/L in water, from a stock solution of 100 mg/L, and expressed as milligrams of gallic acid equivalents (EAG) per gram of dry extract ($y = 0.124\,x + 1.581\text{E-}2$, $r = 0.9997$).

2.8 UVA–UVB absorption coefficient

Appropriate dilutions of each extract were made with methanol. Subsequently, the absorption spectrum was carried out in the UV–visible (200–700 nm), in a quartz cuvette (1 cm step length) for each extract. The absorption coefficients (absorbance (A)/(mg of dry extract/mL)) were calculated at 290, 310, 340, and 380 nm [35, 36]. All spectrophotometric measurements were acquired using a Thermo Scientific Evolution 60S UV–visible spectrophotometer.

2.9 Preparation of sunscreen gel

For evaluating the photoprotection and photostability of the extract, eight gel photoprotector formulations were prepared with each of the extracts from assays 1–8, and were named in the same way as assays 1–8. The extracts of *P. pulchella* were brought to dryness, in a rotary evaporator, with reduced pressure, until constant weight, to ensure the removal of the extraction solvent, and redissolved in a mixture of ethanol–propylene glycol–water (40:30:30), for a final concentration of 50% (w/w). Then, the solution was incorporated into a gel formulation [carbomer 940 (2.0% (w/w)], triethanolamine [2.0% (w/w)], glycerin [2.0% (w/w)], polysorbate 80 [0.5% (w/w)], and distilled water quantity sufficient (qs) 100), for a final concentration of 10% (w/w) of dry extract.

2.10 In vitro determination of photoprotective efficacy

Sunscreen gel was accurately applied (0.75 mg/cm^2) to roughen polymethyl methacrylate (PMMA) plates (Helioplate HD6, Labsphere, Inc. North Sutton, NH, USA) spreading evenly over the entire surface with a gloved finger. The film was allowed to stabilize protected from light at room temperature (*ca.* 25 ± 2 °C) for 15 min. UV diffuse transmittance measurements (290–400 nm) were performed with a spectrophotometer equipped with an integrating sphere (UV Transmittance Analyzer UV-2000S, Labsphere, North Sutton, USA). In vitro photoprotection efficiency in UVB was evaluated according to SPF (Sun Protection Factor) parameter and in vitro photoprotection efficiency in UVA according to UVAPF (Ultraviolet A Protection Factor), critical wavelength (λ_c), and the UVA/UVB parameters. All the calculations were performed according to the models proposed by ISO 24443 (2012) Determination of sunscreen UVA photoprotection in vitro [37]. For each assay, three plates were prepared and nine measurements were taken for each plate. As a blank, the gel without extract was used and a commercial sunscreen SPF 25 was evaluated as a positive control [37–39].

2.11 Photostability of sunscreens

The photostability of the sunscreen gel was evaluated using the method described by Jarzycka et al. [37–42]. Plates previously prepared (see above Sect. 2.10) were irradiated for 2 h with a solar simulator apparatus (Solarbox 1500e; Erichsen, Germany) equipped with a xenon arc lamp (1500 W) and special UV glass filters cutting off radiation below 290 nm. The light source emission was maintained at 650 W/m^2 in accordance with global solar spectral irradiance. SPF, λ_c, and UVA/UVB ratio parameters were measured at time zero and every 30 min for 2 h, and the UVAPF was measured at time zero and at 2 h. The degree of photostability was expressed as the percentage of effectiveness, after exposure, of both protection factors: the SPF in vitro (% SPF_{eff}) and the UVA-PF (% $UVAPF_{eff}$), and calculated according to Eqs. (1) and (2), respectively. Three plates were prepared, and measurements were made nine times for each plate.

$$\%\ SPF_{eff} = SPF_{in\ vitro}\text{after irradiation}/SPF_{in\ vitro}\text{before irradiation} * 100, \quad (1)$$

$$\%UVAPF_{eff} = UVAPF_{in\ vitro}\text{after irradiation}/UVAPF_{in\ vitro}\text{before irradiation} * 100. \quad (2)$$

2.12 Sensory analysis

Gel formulations were evaluated for the attributes of color, odor, appearance, and texture by three individually selected sensory evaluators. The gel samples were spread on white paper sheets and evaluated under white light. Descriptive and acceptance analysis of each of the attributes described was carried out, under the criteria of acceptability of a cosmetic product. In the evaluation of the appearance and texture, all the mechanical, geometric, and surface attributes of the formulations, perceptible through touch and vision, were evaluated [43, 44].

2.13 Statistical analyses

The results are expressed as the means ± SD. All data were analyzed by one-way or three-way analysis of variance (ANOVA) followed by Tukey tests when appropriate using R Development Core Team (2011), R: A Language and Environment for Statistical Computing and Microsoft Excel. p values of less than 0.05 ($p < 0.05$) were considered significant.

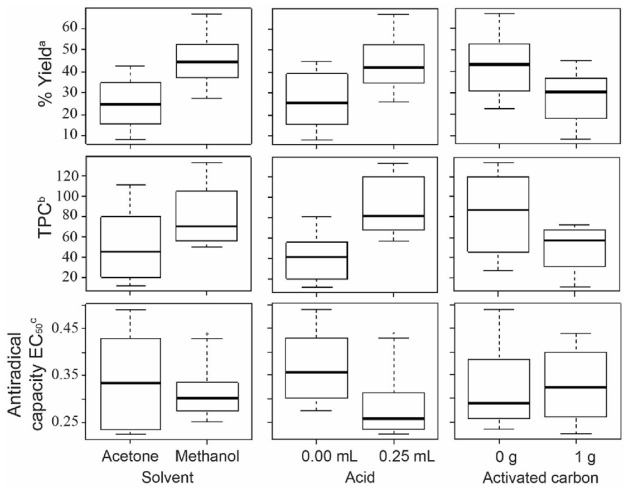

Fig. 1 Main effects of solvent, acid, and activated carbon on extraction effectiveness. [a]% Yield extraction (% dry extract), [b]TPC: Total phenolic content (mg gallic acid equivalents per g dry extract). [c]EC_{50}: Efficient concentration 50 (g dry extract per mmol DPPH•)

3 Results and discussion

According to the analysis of variance of three factors, we found a significant effect was found in the triple interaction (solvent: acid: carbon; $p = 0.04$) for the yield percentage. Figure 1 shows the global effect of solvent, acid, and activated carbon on the response variables of yield percentage, TPC, and antiradical capacity. According to the results, it was observed that methanol and the addition of acid increased the overall yield percentage of the extracts, and the addition of activated carbon decreased it. The assays with acidified methanol (tests 3 and 4) and the assays without acidification (tests 7 and 8) presented the highest yields. In addition, the treatment with activated carbon (assays 2, 4, 6, and 8) significantly decreased the yield percentage, which was much more noticeable in assay 6 (9.06 ± 0.39% of dry extract) (Table 2). This shows that the carbon is adsorbing chlorophylls and other types of compounds, and it was observed with the change in color of the extract solutions.

Regarding the antioxidant capacity measured by the DPPH• free radical method and the inhibition of lipid peroxidation in MeLo by the extracts, we observed a significant effect of the three evaluated factors (solvent: acid: carbon; $p < 0.05$). Assays 4, 5, and 6 presented the highest EC_{50} values (0.41 ± 0.05, 0.46 ± 0.03, and 0.40 ± 0.0 g of dry extract/mmol DPPH, respectively), while the EC_{50} values for assays 1, 2, 3, and 8 were the lowest values (0.2–0.3 g dry extract/mmol DPPH), and did not present a statistically significant difference between them ($p > 0.05$). Although these values present a statistically significant difference with respect to

Table 2 Extraction yield, in vitro antioxidant capacity, and TPC of *P. pulchella* extracts

	% Yield	TPC[¥]	EC_{50}[†]	CDH[₸]	MDA[£]
Assay 1	40.87 ± 2.43 a	246.86 ± 14.24 a	0.25 ± 0.02 a	ND	0.58 ± 0.03 a,b
Assay 2	29.43 ± 2.83 b	217.50 ± 2.44 a	0.23 ± 0.01 a	ND	0.35 ± 0.08 a
Assay 3	62.80 ± 3.46	210.52 ± 15.84 a,b	0.26 ± 0.01 a,b	ND	0.35 ± 0.05 a
Assay 4	42.73 ± 2.74 a	161.83 ± 16.64 c	0.41 ± 0.05 c,d	ND	1.44 ± 0.25 c
Assay 5	22.84 ± 0.53	133.52 ± 16.58 c	0.46 ± 0.03 c	ND	0.63 ± 0.11 a,b
Assay 6	9.06 ± 0.39	132.88 ± 9.35 c	0.40 ± 0.01 d	ND	1.17 ± 0.13 b,c
Assay 7	44.51 ± 0.31 a	159.10 ± 28.48 c	0.31 ± 0.01 b	ND	0.74 ± 0.22 a,b
Assay 8	30.52 ± 3.10 b	171.39 ± 7.80 b,c	0.29 ± 0.01 a,b	ND	1.10 ± 0.23 b,c
BHT*	–	–	0.11 ± 0.01[‡]	ND	0.31 ± 0.04 a
MeLo*	–	–	–	11.74 ± 2.73	10.22 ± 0.48

Results are expressed as the mean value ± standard deviation ($n = 3$). Values in the same column followed by different letters are significantly different at the 5% level. *BHT* Butylated hydroxytoluene, *MeLo* Methyl linoleate, *ND* No detect. [¥]TPC: total phenolic content, mg gallic equivalents per g dry extract. [†]EC_{50}: efficient concentration 50, g dry extract per mmol DPPH. [₸]CDH: conjugated diene hydroperoxide, mmol CDH per kg MeLo. [£]MDA: malondialdehyde, mmol MDA per kg MeLo

*Controls obtained under the same experimental group from two previous publications [35, 45]

BHT (0.11 ± 0.01 g of antioxidant/mmol DPPH, $p < 0.05$), if it is considered that they are crude extracts without an exhaustive purification process, they are considered quite good. In addition, the benefits of using plant extracts rich in polyphenols in dermocosmetics have been reported, not only for their antioxidant capacity but also for their anti-inflammatory, immunomodulant, photoprotective, and anti-cancer effects [23, 27, 46–48]. Concerning the results obtained in the lipid model, we observed that in the MeLo assay with BHT and each extract (assays 1–8), there was a protective effect both in the first stages of peroxidation, due to the absence of CDH, as in the final stages due to a decrease in the formation of MDA with respect to MeLo without preserving. Additionally, the assays 2, 3, 5, and 7 did not show significant difference ($p < 0.05$) compared to BHT, concerning the inhibition of MDA formation. Therefore, an antioxidant capacity similar to BHT was demonstrated against the prevention of MeLo peroxidation.

Moreover, the analysis of variance for TPC assays showed that the interaction between solvent:acid:carbon was significant ($p = 0.027$). Furthermore, Fig. 1 shows that methanol as an extraction solvent and the addition of HCl increased the overall value of TPC. However, TPC values decrease when activated carbon is added due to the low selectivity of activated carbon that not only adsorbs chlorophylls but may also be absorbing other types of compounds, such as polyphenols, as previously reported in *B. antioquensis* extracts, a plant of the same family Asteraceae [45]. In previous studies carried out by us and various publications have shown that the addition of acids, mainly HCl, increases the yield of polyphenol extraction [33, 45, 49, 50], as observed in this study (Table 2 tests 1, 2, 3, and 4). The reason why we discarded the extracts where HCl was used was due to the poor sensory characteristics of these formulations, since the incorporation of these extracts was not adequate in the hydrogel, presenting brown colorations and large dispersed particles. This not only affects the characteristics of texture and color but also the effectiveness since there is not complete solubilization of the extract in the cosmetic matrix. Assays 1, 2, and 3 presented the highest TPC values, (246.86 ± 14.24, 217.50 ± 2.44, 210.52 ± 15.84 EAG/g dry extract, respectively), among which there was no statistically significant difference. The other assays (4–8) presented values between 132.88 ± 9.35 and 171.39 ± 7.80 EAG/g dry extract, and did not present a statistically significant difference between them. Furthermore, we observed a high correlation between TPC and antioxidant activity ($R^2 = 0.7336$).

Concerning the absorption capacity of the extracts in the UVA–UVB region, the analysis of variance showed that the interaction between the solvent, the acid, and the activated carbon was not significant ($p > 0.05$). In Fig. 2, the absorption coefficients of the extracts obtained in the different assays are shown, with a statistically

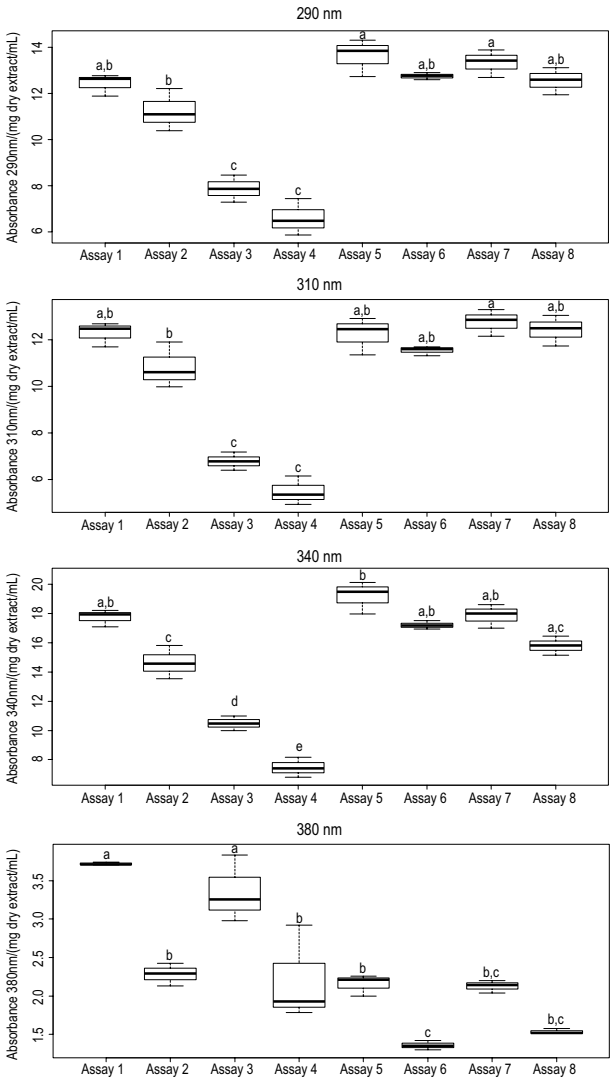

Fig. 2 UVA–UVB absorption coefficients of *P. pulchella* extracts. Data are the means of three replicates with standard deviation shown by vertical bars. Bars topped by different letters are significantly different at the 5% level

non-significant reduction in the absorption coefficients in the presence of activated carbon (assays 2, 4, 6, and 8), for the tests without activated carbon (assays 1, 3, 5, and 7) for the absorption coefficients obtained at 290 nm and 310 nm. On the other hand, there was significant differences between tests 1 and 2 and between 3 and 4 at 340 nm, and between 1 and 2, 3 and 4, 5 and 6 at 380 nm. These values decrease when activated carbon is added due to the low selectivity of activated carbon that not only adsorbs chlorophylls but may also be absorbing other types of compounds, such as polyphenols, that absorb at these wavelengths. Additionally, the reduction of the absorption coefficients was also notable when HCl was added. However, the solvent:carbon and acid:carbon interaction did

Table 3 In vitro photoprotection and photostability of extracts of *P. pulchella*

		SPF	UVAPF		λ_c	UVA/UVB	
Gel		0.92±0.00	0.0		-	-	
CSS† SPF 25		25.18±1.11	3.0±0.0		356	0.430	
Time, min		0	30	60	90	120	Gel spread on paper
Assay 1	SPF	6.3±0.1	4.5±0.3	4.0±0.2	3.5±0.2	3.3±0.1	
	UVAPF	5.0±0.0	-	-	-	3.0±0.0	
	λ_c	375	377	378	378	379	
	UVA/UVB	0.800	0.770	0.751	0.736	0.729	
	% SPF_{eff}	**100.0 %**	**71.4%**	**63.5 %**	**55.6 %**	**52.4 %**	
	%$UVAPF_{eff}$	**100.0 %**	-	-	-	**60.0 %**	
Assay 2	SPF	4.8±0.4 a	4.0±0.2	3.4±0.2	3.0±0.1	2.8±0.2	
	UVAPF	4.0±0.0	-	-	-	2.0±0.0	
	λ_c	374	376	376	377	377	
	UVA/UVB	0.794	0.756	0.733	0.718	0.711	
	% SPF_{eff}	**100.0 %**	**83.3 %**	**70.8 %**	**62.5 %**	**58.3 %**	
	%$UVAPF_{eff}$	**100.0 %**	-	-	-	**50.0 %**	
Assay 3	SPF	3.4±0.2 b	3.0±0.2	2.9±0.2	2.8±0.2	2.7±0.2	
	UVAPF	3.0±0.0 a	-	-	-	2.3±0.6	
	λ_c	380	381	381	382	382	
	UVA/UVB	0.843	0.822	0.816	0.809	0.800	
	% SPF_{eff}	**100.0 %**	**88.2 %**	**85.3 %**	**82.4 %**	**79.4 %**	
	%$UVAPF_{eff}$	**100.0 %**	-	-	-	**76.7 %**	
Assay 4	SPF	3.3±0.2 b	3.0±0.2	3.0±0.1	3.0±0.1	3.0±0.1	
	UVAPF	3.0±0.0 a	-	-	-	3.0±0.0	
	λ_c	381	383	383	383	383	
	UVA/UVB	0.808	0.821	0.820	0.814	0.810	
	% SPF_{eff}	**100.0 %**	**90.9 %**	**90.9 %**	**90.9 %**	**90.9 %**	
	%$UVAPF_{eff}$	**100.0 %**	-	-	-	**100.0 %**	
Assay 5	SPF	4.5±0.2 a	3.3±0.1	3.1±0.1	3.0±0.1	3.0±0.2	
	UVAPF	3.7±0.6 a	-	-	-	2.3±0.6	
	λ_c	375	376	377	377	377	
	UVA/UVB	0.830	0.783	0.773	0.764	0.759	
	% SPF_{eff}	**100.0 %**	**73.3 %**	**68.9 %**	**66.7 %**	**66.7 %**	
	%$UVAPF_{eff}$	**100.0 %**	-	-	-	**62.2 %**	
Assay 6	SPF	3.0±0.1 b	2.4±0.1	2.3±0.1	2.2±0.1	2.2±0.1	
	UVAPF	3.0±0.0 a	-	-	-	2.0±0.0	
	λ_c	374	375	375	375	375	
	UVA/UVB	0.794	0.755	0.741	0.735	0.724	
	% SPF_{eff}	**100.0 %**	**80.0 %**	**76.7 %**	**73.3 %**	**73.3 %**	
	%$UVAPF_{eff}$	**100.0 %**	-	-	-	**66.7 %**	
Assay 7	SPF	7.3±0.9	6.4±0.5	5.2±0.5	5.1±0.4	4.9±0.5	
	UVAPF	5.3±0.6	-	-	-	4.3±0.6	
	λ_c	376	379	379	379	379	
	UVA/UVB	0.824	0.819	0.813	0.807	0.810	
	% SPF_{eff}	**100.0 %**	**75.6 %**	**71.1 %**	**68.9 %**	**67.1 %**	
	%$UVAPF_{eff}$	**100.0 %**	-	-	-	**81.1 %**	
Assay 8	SPF	3.4±0.2 b	2.8±0.1	2.7±0.1	2.6±0.1	2.5±0.2	
	UVAPF	3.0±0.0 a	-	-	-	2.0±0.0	
	λ_c	375	377	377	377	378	
	UVA/UVB	0.793	0.787	0.783	0.780	0.777	
	% SPF_{eff}	**100.0 %**	**82.4 %**	**79.4 %**	**76.5 %**	**73.5 %**	
	%$UVAPF_{eff}$	**100.0 %**	-	-	-	**66.7 %**	

Results are presented as the mean value ± SD ($n = 3$). Values for the same parameter followed by different letters have a statistically significant difference with a confidence level of 5%. †CSS: commercial sunscreen SPF 25, which contains 8.5% octocrylene, 7.0% ethylhexyl methoxycinnamate, 5.0% benzophenone-3, and 4.0% octyl salicylate as active ingredients

not show a significant effect on the absorption coefficients ($p > 0.05$), contrary to the solvent:acid interaction where there was a significant effect ($p < 0.05$). Furthermore, no correlation was found between the TPC values and the absorption coefficients.

Regarding the preparation of gel formulations, there was difficulty in incorporating the extracts from trials 1, 2, 3, and 4. It was related to the addition of HCl in these extracts, which produced browned colored extracts, difficult to clean up with activated carbon. Moreover, they did not exhibit adequate sensory characteristics for topical use, which was much more evident in assays 1 and 2 (Images Table 3). These sensory properties are essential for cosmetic products, not only the efficiency and safety are relevant, characteristics, such as spreadability, texture perception, and visual appearance, among others, play a role in improving consumer acceptability. Therefore, because trials 1–4 did not meet the minimum sensory requirements, they were discarded.

On the other hand, the pH of the formulations where the extracts were incorporated was adjusted between 5.5 and 7.5 (suitable for topical formulations), and the amount of triethanolamine (TEA) added was that necessary to neutralize the carbomer (homopolymer of acrylic acid), in order to reach a pH close to 6–7, at which pH the polymer reaches its highest viscosity. Furthermore, additional base (TEA) was not necessary in the formulations where the extracts made with HCl were incorporated (assays 1–4), which indicates that the residual HCl in the extracts was not significant and only slightly decreased the pH values. Therefore, the pH of the formulations was measured and showed values between 5.9 and 6.6 (Base gel: 6.7, Gel assay 1: 6.1, Gel assay 2: 5.9, Gel assay 3: 6.3, Gel assay 4: 6.0, Gel assay 5: 6.4, Gel assay 6: 6.5, Gel assay 7: 6.5, Gel assay 8: 6.6).

The hydrogel formulations prepared with the extracts from tests 1–2 showed high SPF and UVAPF values (Table 3); these photoprotection effects are associated with high TPC values (Table 2) and absorption coefficients that contribute to efficacy (Fig. 2). However, the lack of solubility of the extracts affects the efficiency, since the large dispersed solid particles do not contribute significantly to the absorption of UVR, and on the contrary the contribution in the refraction and scattering of light is minimal. In addition, the particulate material in formulations 1–4 may be the reason for the poor correlation between the TPC and the SPF ($R^2 = 0.3844$) and the absorption coefficients at 290, 310, 340, and 380 nm and SPF ($R^2 = 0.0534$, $R^2 = 0.0107$, $R^2 = 0.0278$, $R^2 = 0.6442$, respectively). Moreover, the variability in SPF values confirms the need for optimal selection

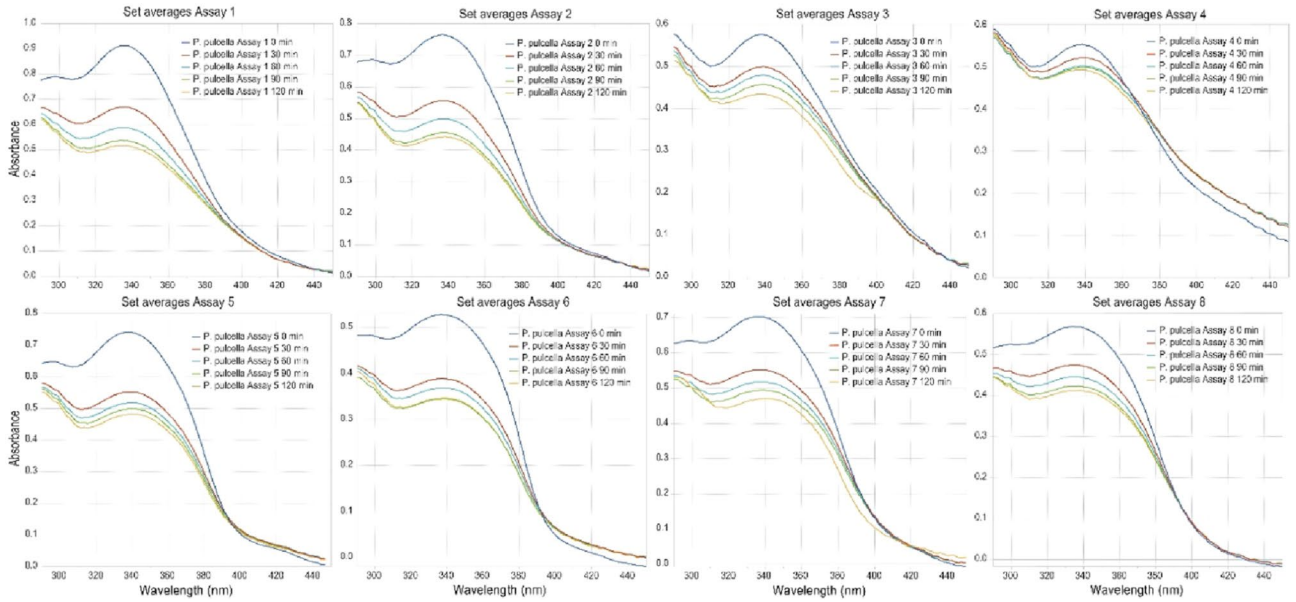

Fig. 3 Changes in the UVA–UVB spectra in the photostability study of the *P. pulchella* assays

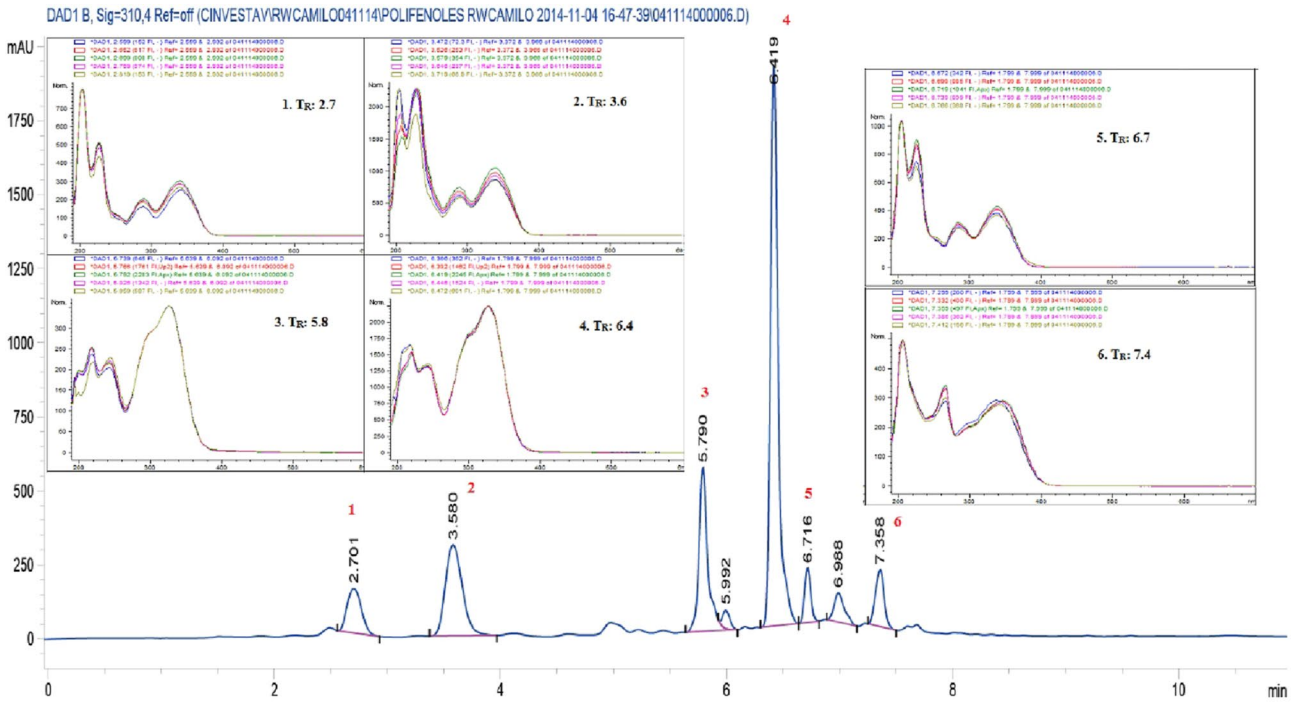

Fig. 4 HPLC–DAD chromatographic profile of the methanolic extract (assay 7) of *P. pulchella*

of the extract in the sunscreen formulation to minimize the effect of dispersed solid particles.

On the other hand, the gels containing the extracts from tests 5, 6, and 7 showed sensory characteristics according to a product for topical application (Images Table 3). Moreover, since assays 5–7 did not present dispersed solid particles, we can infer that the photoprotective effect (SPF and UVAPF) obtained on these formulations can be attributed to the polyphenolic compounds identified for *P. pulchella* with UVA–UVB absorption capability (Figs. 3 and 4).

In addition, the extracts of *P. pulchella* showed a broad spectrum in the UVA–UVB range, according to the

European Commission, ISO, and FDA parameters [37, 51, 52]. All λ_c were higher than 370 nm, and the UVAPF values were greater than one third of the SPF values, which allows declaring broad-spectrum UVB–UVA protection in the formulations (Table 3). In addition, the UVA/UVB ratio in assays 1, 2, 6, and 8 were between 0.6 and 0.8, which is equivalent to three stars (★★★) higher level of protection in the UVA range and for assays 3, 4, 5, and 7 were > 0.8, which is equivalent to four stars (★★★★) maximum protection level, according to *Boot's star* rating system [53]. On the other hand, both the λ_c values and the UVA/UVB ratio for all the assays did not change significantly in the photostability study. In no case they were below 370 nm and 0.7, respectively (Table 3) [52, 54, 55]. Table 3 and Fig. 3 show the results of the evaluation of the photostability of the extracts of *P. pulchella* in the gel formulations. Taking into account that for a formulation to be considered photostable, it must retain at least 80% of the effectiveness of the SPF and UVAPF (% SPF_{eff} and% $UVAPF_{eff}$) after 2 h of irradiation [38, 42], it can be observed that the formulations 1, 2, 3, 5, 6, and 8 were the most photo-unstable in both UVA and UVB with %SPF_{eff} values between 52.4% and 79.4% and% $UVAPF_{eff}$ between 50.0% and 76.7%, and formulation 7 presented instability in UVB, with a %SPF_{eff} of 67.1% and a slight loss of efficacy in UVAPF with %$UVAPF_{eff}$ of 81.1%. In Fig. 3, it can also be observed that the decrease in absorbance is another indication of the instability of these extracts in gel formulations. It was observed that the most photostable formulation was the one that contained the extract from assay 4 (%SPF_{eff} = 90.9% and %$UVAPF_{eff}$ 100%, Table 3 and Fig. 3). However, the values of SPF (3.3 ± 0.2) and UVAPF (3.0 ± 0.0) were not the highest compared to assays 1, 2, 5, and 7. Therefore, it was determined based on antioxidant activity, photoprotection, stability, and sensory characteristics (color, appearance, and texture), that the most promising extracts were those obtained in assays 5 and 7. However, taking into account the percentage of extraction performance (assay 7 = 44.51 ± 0.31%, assay 5 = 22.84 ± 0.53%) and the results of the photoprotection values in the UVA–UVB range (SPF: 7.3 ± 0.9, UVAPF: 5.3 ± 0.6, λ_c 376), the formulation containing the extract from assay 7 was the most prominent.

For the identification of the main components of the methanol and acetone extracts of *P. pulchella*, an evaluation by HPLC–DAD and HPLC-ESI-MS^n-IT was carried out. All the extracts (assays 1–8) presented similar chromatographic profiles; for this reason only the results and discussion of the data of the methanolic extract (assay 7) will be presented. Figure 4 shows the chromatographic profile obtained for the methanolic extract (assay 7) at a wavelength of 310 nm and Table 4 shows some spectroscopic characteristics of these compounds. **Compound 2** retention time (RT) 3.6 (Supplementary Information, Fig. S2, Sch. S2) and **compound 6** RT 7.4 (Supplementary Information, Fig. S6, Sch. S5) were identified as 3-*O*-caffeoylquinic acid (chlorogenic acid) and kaempferol 3-*O*-rhamnopyranosyl-(1 → 6)-glucopyranoside,

Table 4 Spectral characteristics of the compounds identified in extract of *P. pulchella*

Peak	RT min	UV spectra λ_{max} (nm)	$[M+H]^+$	Major MS^2, MS^3, and MS^4 fragments	Compound
1	**2.7**	203, 227, 288, 339	517	MS^2: 517 → 499, 481, 471, 355, 193; MS^3: 355 → 193, 163; MS^4: 193 → 165, 149, 133 MS^4: 163 → 145, 135, 117	Caffeoylquinic acid hexose
2	**3.6**	208, 230, 288, 338	355	MS^2: 355 → 163; MS^3: 163 → 145, 135, 117	3-*O*-caffeoylquinic acid (chlorogenic acid)
3	**5.8**	218, 243, 301sh, 326	523	MS^2: 523 → 505, 487, 343, 193; MS^3: 505 → 487, 193; MS^3: 487 → 469, 343, 325, 297, 193; MS^3: 343 → 315, 297; MS^3: 193 → 178, 165, 133	Feruloyl maleate 4-*O*-8 (5,7-dihydroxy-coniferyl alcohol)
4	**6.4**	220, 242, 303sh, 326	505, 507	MS^2: 505 → 487, 343, 193; MS^2: 507 → 489, 345, 193; MS^3: 487 → 469, 451, 343, 325, 297, 193; MS^3: 343 → 315, 297; MS^3: 193 → 178, 165, 149, 133	Feruloyl maleate 4-*O*-8- (7-hydroxy-coniferyl alcohol)
5	**6.7**	206, 226, 284, 339	595	MS^2: 595 → 433, 287; MS^3: 433 → 287; MS^3: 287 → 269, 259, 241, 213, 165, 153, 133, 121	Kaempferol 3-*O*-glucopyrano-syl-(1 → 2)-rhamnopyranose
6	**7.4**	206, 266, 299sh, 347	595	MS^2: 595 → 449, 433, 287; MS^3: 449 → 287; MS^3: 287 → 269, 259, 241, 213, 165, 153, 133, 121	Kaempferol 3-*O*-rhamnopyrano-syl-(1 → 6)-glucopyranose

respectively. These two compounds presented similar retention time and fragmentation patterns previously reported for the *B. antioquensis* plant (Asteraceae) [45].

Compound 1, with a RT of 2.7 min and a protonated molecular ion $[M+H]^+$ at m/z 517 (MS^1), presented a product ion of m/z 355 with the same spectral characteristics of **compound 2** (chlorogenic acid) (Supplementary Information, Fig. S2, Sch. S2). According to these data, it was tentatively identified as **caffeoylquinic acid hexose (chlorogenic acid—hexose)**, a compound previously reported (Supplementary Information, Fig. S1, Sch. S1) [56].

Compound 3, with a retention time of 5.8 min and a protonated molecular ion $[M+H]^+$ at m/z 523 (MS^1) (Supplementary Information, Fig. S3, Sch. S3), presented a product ion at m/z 505 with similar spectral characteristics to (**feruloyl maleate 4-*O*-8-(7-hydroxy-coniferyl alcohol**) (**compound 4,** RT 6.4) (Supplementary Information, Fig. S4, Sch. S3) and was tentatively identified as **feruloyl maleate 4-*O*-8 (5,7-dihydroxy-coniferyl alcohol)**, due to biosynthetic evidence that coniferyl alcohol (phenylpropanoid derived from shikimic acid) may exhibit additional hydroxylation at position 5. This fact is confirmed since the fragments of the feruloyl maleate portion presented an identical fragmentation pattern to **feruloyl maleate 4-*O*-8-(7-hydroxy-coniferyl alcohol)**. In this sense, Rohde A. et al. (2004) [57], in a metabolomic study of phenylpropanoids carried out in *Arabidopsis thaliana* by MS^n in negative mode, reported feruloyl maleate coupled 4–*O*–8 to coniferyl alcohol with MS^1 m/z 505, MS^2 m/z 389, and MS^3 m/z 341, and 193, compared to standard.

Compound 5, with a retention time of 6.7 min, was identified as kaempferol **3-*O*-glucopyranosyl-(1 → 2)-rhamnopyranose** (Supplementary Information, Fig. S5, Sch. S4), and its spectral characteristics differ significantly from **compound 6**, which presented the same molecular ion and was identified as **kaempferol 3-*O*-rhamnopyranosyl-(1 → 6)-glucopyranose** (RT 7.4 min) (Supplementary Information, Fig. S6, Sch. S5) since, in the experiment MS^2 from m/z 595, the initial loss of rhamnose (Y_1^+ m/z 449) was not observed. Still, instead, the loss of glucose (Y_1^+ m/z 433) was initially presented and then the loss of rhamnose, without observing the characteristic ion Y* of diglycosides with interglycosidic bond 1 → 6. To determine the type of interglycosidic union (1 → 6 or 1 → 2) in the sugar residue, two spectral facts were taken into account that have been reported by Cuyckens and Claeys [58], that confirm the union 1 → 2. First, the proportion of the Y_1^+/Y_0^+ ions is lower in the 1 → 6 junctions. The second, much more significant fact is the absence of the Y* ion (m/z 449), which would correspond to the loss of internal sugar, in this case, rhamnose, to produce by rearrangement an aglycone *O*-glucose ion. This rearrangement has been reported as characteristic of di-glycosylated flavonoids with 1 → 6 bonding, but not for 1 → 2 interglycosidic bonds. In addition, the Y_0^+ ion at m/z 287 (Supplementary Information, Fig. S5, and S6, Sch. S4 and S5) was identified as kaempferol compared to standard with the same fragmentation pattern described for kaempferol 3-O-ramnopyranosyl-(1 → 6)-glucopyranose from *B. antioquensis* [45].

The characterization of the extracts of *P. pulchella* showed that all the identified compounds are structurally phenylpropanoids derived from hydroxycinnamic acid with a C6–C3 skeleton, such as caffeic acid and ferulic acid (compounds 1, 2, 3, and 4), as well as flavonoids derived from chalcone, with a characteristic skeleton in their structure C6–C3–C6, like Kaempferol (compounds 5 and 6). In this sense, C6–C3 compounds, such as hydroxycinnamic acid derivatives (*p*-coumaric acid, ferulic acid, caffeic acid, etc.) have been reported as good attenuators of UVB radiation, with ε_{max} in the 310–325-nm waveband. Likewise, the majority of flavonoids (*i.e.,* apigenin, luteolin, quercetin, kaempferol, etc.) present absorption bands in the UVA–UVB between 280 and 355 nm, allowing characteristics of a wide spectrum of protection [11, 12, 16, 27, 30, 40, 46, 48]. In addition, currently one of the most used filters in sunscreen preparations on the market, due to its protective effect on UVB, in addition to its stability is Ethylhexyl Methoxycinnamate (INCI name) or Octinoxate (USAN name), authorized by the European Commission and FDA. For this reason, the compounds present in the extracts of *P. pulchella* are recognized in the literature as defense metabolites of plants against the harmful effects of UVA–UVB radiation, since they can act as solar filters and as substances with high antioxidant activity, which was confirmed by the efficacy tests carried out in this study.

4 Conclusion

On basis of the antioxidant activity, photoprotection, stability, sensory characteristics (color, appearance, and texture), and yield percentage of extraction, the extract identified as assay 7 (acid-free MeOH extract and without treatment with activated charcoal) was identified as the most promising extract of *P. pulchella.* It demonstrates a remarkable antioxidant and broad photoprotective capacity, complying with the requirements of the European Commission, ISO, and FDA. This was also supported by identifying secondary metabolites present in the extracts, and their polyphenolic structural characteristics, with chromophore groups capable of absorbing UVR in the UVA–UVB region and with free radical scavenger and antioxidant properties. Although more studies are needed to improve the photostability of formulations, for example, through the use of micro- and nanoencapsulation and quenching mechanisms, our findings

suggest *P. pulchella* plant as source of potential natural UV filters and antioxidants for skin protection.

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Author contributions JCM-G conceived and performed the experiments, analyzed the data, and drafted the paper. RW revised and edited the paper. MAP-M supervised the experiments, revised the paper, and gave the final approval of the manuscript and funding acquisition. All the authors read and approved the manuscript as submitted.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest among them or with the parent institution.

Data availability Not applicable.

Code availability Not applicable.

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