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Extracts from Dried Leaves of Alecrim-do-Campo (*Baccharis dracunculifolia*): Evaluation of Photoprotective Activity, Photoprotective Formulation, Antioxidant Potential and Nanoemulsion Stability as Candidates for Natural Sunscreen Filters

Extratos das Folhas Secas de Alecrim-do-Campo (Baccharis dracunculifolia): Avaliação da Atividade Fotoprotetora, Formulação Fotoprotetora, Potencial Antioxidante e Estabilidade da Nanoemulsão como Candidatos a Filtros Solares Naturais

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Baccharis dracunculifolia (commonly known as Alecrim-do-Campo) is a plant with ethnopharmacological significance due to its use by populations across various regions of Brazil. It is a native Brazilian plant, rich in flavonoids, that absorbs UV radiation, suggesting the potential for its use in natural sunscreen filters. This study aims to evaluate the sunscreen potential of Alecrim-do-Campo. Three extracts were obtained using different solvents. The dichloromethane and ethanolic extracts demonstrated the highest activity, as indicated by their higher Sun Protection Factor (SPF) values. The extracts underwent phytochemical screening, antioxidant activity testing, and quantification of total phenolic and flavonoid content, as well as GC-MS (Gas Chromatography-Mass Spectrometry) analysis. By correlating the antioxidant capacity with phenolic compound levels, it was found that the ethanolic extract of Alecrim-do-Campo contains the highest concentrations of phenolic compounds and exhibits strong antioxidant activity. A nanoemulsion containing the ethanolic extract was developed using the phase inversion method, showing promising stability results. GC-MS analysis revealed that the most abundant compound in the extracts is benzenepropanoic acid. Phytochemical screening confirmed the presence of flavonoids and phenolic compounds in both the dichloromethane and ethanolic extracts. The results suggest that *Baccharis dracunculifolia* is a promising candidate in the search for natural sunscreen filters.

Keywords: Flavonoids; sun protection factor (SPF); Pemulen TR-1 gel.

1. Introduction

There has been a considerable interest in the use of plants for protecting human skin against UV-induced damage. This interest stems from the fact that flavonoids and other phenolic compounds have been recognized as an important protective class against UV-induced damage. One of the current trends in cosmetology is the development of products with the highest possible degree of natural ingredients. Sunscreens mostly consist of a combination of inorganic filters, which reflect and disperse UV photons, and organic filters, which promote the absorption of radiation, thereby preventing direct reaction with epidermal cells. For a sunscreen to be considered effective, it must offer broad-spectrum absorption, safety, efficacy, and optimized filter concentrations. [1-3]

Baccharis dracunculifolia (commonly known as "Alecrim-do-Campo") is a plant with significant ethnopharmacological impact due to its traditional use by populations in different regions of Brazil.[4] It use has been reported for its antimicrobial activity and for treating skin disorders. *B. dracunculifolia* is a native plant of Brazil, typical of the Brazilian cerrado, with significant occurrence also in the southern region of the country.[4]

Alecrim-do-Campo is a widely studied plant due to its interaction with bees, as well as its diversity of phenolic compounds.[5-8] The main secondary metabolites identified in the of Alecrim-do-Campo are terpenoids, flavonoids and phenolic compounds derived from *p*-coumaric acid, such as artepilin C, which is also found in significant levels in green propolis

of Brazilian origin. Notably, Alecrim-do-Campo is one of the main plant species used by bees in the production of green propolis.[10]Nanoemulsions provide a promising approach for incorporation essential oils or plant extracts into pharmaceutical formulations, offering stability, biodegradability, and biocompatibility. As a result, nanoemulsions can be a potential vehicle for the topical application of essential oils or plants extracts. Bicarello *et al.* (2019)[11] prepared a nanoemulsion of essential oil of *Baccharis dracunculifolia* to evaluat its *in vitro* effects against *C. hominivorax* larvae. Thus, the objective of this study is focused on evaluating the photoprotective effects and preparing formulations using different extracts of Alecrim-do-Campo as a potential candidate for the development of natural sunscreens in topical products, aiming to prevent damage caused by UV radiation to the skin.

2. Experimental

2.1. General conditions

The solvents used in the extraction process were acquired from the brand VETEC, boasting a high degree of purity. Absorbance measurements were performed using a Genesys 105 UV-VIS spectrophotometer, equipped with a 1 cm quartz cuvette. The UVA-UVB sunscreen gel was obtained from BioFarma Manipulation Pharmacy, located in Itabirito, Minas Gerais.

2.2. Vegetable material

Leaf samples of *Baccharis dracunculifolia* were acquired in March 2023 from the natural products store Vida Alternativa, located in the municipality of Belo Horizonte, Minas Gerais State, southeastern Brazil. The store's pharmacist, specialized in medicinal plants, identified the species using an atlas of Brazilian medicinal flora. The species registration for this study was carried out under code number A9FBD24 in the National System of Management of Genetic Heritage and Associated Traditional Knowledge (SisGen).

2.3. Preparation of extracts from dried *Baccharis dracunculifolia* leaves

Three extracts were obtained through static maceration of grounds Alecrim-do-Campo leaves. The process was conducted at room temperature using the organic solvents hexane, dichloromethane and ethanol. Specifically, 25 g of dried plant leaves were immersed in 300 mL of each solvent and allowed to macerate for 10 days to ensure thorough extraction of leaf constituents. Subsequently, the solvents were evaporated using a rotary evaporator, and the resulting extracts were dried in a desiccator.

2.4. Preparation of solutions from dried *Baccharis dracunculifolia* leaves and determination of maximum absorbance

From the dried extracts, a mother solution was prepared at a concentration of 1 mg of extract per 1 mL of solvent (hexane or ethanol or dichloromethane) for each of the three types of extracts. Subsequently, diluted solutions were derived from this stock solution at concentrations of 0.02, 0.03, 0.04, 0.05, 0.07, and 0.1 mg mL^{-1}. UV-Vis absorption measurements were conducted using the Genesys 10S Spectrophotometer to determine the maximum absorbance in the ultraviolet B (UVB) region. Solutions were scanned in triplicate between wavelengths of 200 to 800 nm on the UV spectrophotometer, employing a quartz cuvette with a 1.0 cm optical path length. Hexane, ethanol, and dichloromethane were used as white references for their respective readings. Subsequently, the collected data were processed using Microsoft Excel. By applying the Mansur[12] method equation to absorbance readings between 290 and 320 nm, with intervals of 5 nm, the Sun Protection Factor (SPF) was determined. The SPF calculation was performed using a mathematical expression (Equation 1)[12], with the weighting factors listed in Table 1.

$$FPS = FC \times \sum_{290}^{320} \times EE(\lambda) \times I(\lambda) \times Abs(\lambda) \qquad (1)$$

Table 1. Weighting used to calculate the SPF by spectrophotometry[12]

Wavelength (nm)	EE × I (normalized)
290	0.0150
295	0.0817
300	0.2874
305	0.3278
310	0.1864
315	0.0839
320	0.0180
Total	1.0000

The dichloromethane and ethanol extracts exhibited the highest photoprotective activity. Consequently, a phytochemical screening was conducted to identify its chemical composition through gas chromatography-mass spectrometry (GC/MS). Furthermore, their antioxidant activity was evaluated. The extract was then utilized to develop a photoprotective formulation, incorporating Pemulen TR-1 Gel at 1% extract ratio, and formulated as a phase inversion nanoemulsion.

2.5. Antioxidant activity

2.5.1. DPPH spectrophotometric assay

The antioxidant activity was determined according to the procedure described in the literature[13] using the 1,1-diphenyl-

2-picrilhidrazyl (DPPH) reagent (0.28 mmol L^{-1}). Initially, the extracts were dissolved in ethanol to obtain stock solutions of 0.4 mg mL^{-1}. From these stock solutions, different aliquots were taken to obtain final solutions ranging from 0.002 to 0.172 mg mL^{-1} for the dichloromethane extract, and from 0.002 to 0.147 mg mL^{-1} for the ethanolic extract. Then, 1.25 mL of DPPH solution in ethanol was added to each sample, and the final volume was adjusted to 3 mL with ethanol. Absorbance (Abs_{sample}) was then measured at 517 nm using a spectrophotometer (UV-VIS Genesys 10S). The negative control was prepared by mixing 1.25 mL of the DPPH solution with 1.72 mL of ethanol ($Abs_{control}$). Ethanol (99.5%) was used as the reference sample for spectrophotometer calibration. Tests were carried out in triplicate and DPPH radical scavenging activity was calculated using the Equation 2.

$$(I\ \%) = \left(\frac{ABS_{control} - ABS_{sample}}{ABS_{control}} \right) \times 100 \qquad (2)$$

The extract concentration responsible for a 50% decrease in the initial activity of the DPPH (IC_{50}, μg mL^{-1}) was calculated by linear regression of the I % curves obtained for the extract different concentrations.

2.5.2. ABTS spectrophotometric assay

The ABTS assay, aimed at decolorizing the ABTS cationic radical (ABTS*+), was conducted in accordance with the procedure outlined by other authors.[14] For the preparation of the working solution, ABTS (7.4 mmol L^{-1}) was blended with potassium persulfate (2.6 mmol L^{-1}) and left to incubate at room temperature for 12–16 hours in darkness. On the day of experimentation, the solution was diluted with ethanol to achieve an absorbance of 0.70 (±0.02) at 734 nm. Extract concentrations were prepared as per previous descriptions. Subsequently, 1.6 mL of the ABTS solution was introduced to in the samples, and the final volume was adjusted to 2 mL with ethanol. The negative control was formulated by mixing 1.6 mL of the ABTS solution with 0.4 mL of ethanol. All samples were then incubated for 6 minutes at room temperature (25 ± 2 °C) in darkness, after which absorbance measurements were taken at 734 nm. The spectrophotometer was zeroed with ethanol (99.5%). The scavenging percentage of the sample and EC_{50} values were calculated as described previously.

2.6. Extract composition

2.6.1. Phytochemical screening

A qualitative phytochemical analysis of the dichloromethane and ethanolic extracts of *Baccharis dracunculifolia* was performed under ambient conditions, using standard procedures with minor adjustments, to identify the presence of phytochemical compounds, including flavonoids, phenols/tannins, saponins, and terpenoids.

To detect the presence of flavonoids, 10 mg of the dried crude extract was mixed with 2 mL of 2% sodium hydroxide (NaOH), and any subsequent color change was observed. For the test to verify the presence of tannins and phenols, 10 mg of the crude extract was mixed with 2 mL of distilled water, filtered, and then treated with a few drops of 2% ferric chloride ($FeCl_3$) in methanol, with the resulting color change being observed. The presence of saponins was indicated if vigorous shaking of 5 mL of distilled water with 10 mg of the crude extract resulted in the formation of stable foam.

To identify the presence of terpenoids, 10 mg of the dried crude extract were dissolved in 2 mL of chloroform ($CHCl_3$), followed by the addition of 3 mL of concentrated sulfuric acid (H_2SO_4), resulting in the formation of a distinct layer.

2.6.2. Determination of the total polyphenol content

The total polyphenol content was quantified using the Folin-Ciocalteu reagent, according to the procedure of Bonoli.[15] In summary, 10 mg of samples were dissolved in 25 mL of absolute ethanol (0.40 mg mL^{-1}), and 1.6 mL of this solution was combined with 1.2 mL of deionized water and 0.2 mL of Folin–Ciocalteu reagent (Cromoline). The mixture underwent agitation for 1 minute, followed by the addition of 0.8 mL of sodium carbonate solution (20% w/v). After agitation for 30 seconds, 0.2 mL of water was added, and the mixture was then incubated for 2 hours. The absorbance of the reaction mixture was measured at 725 nm against a deionized water blank using a spectrophotometer (UV-VIS Genesys 10S). Gallic acid (GA) served as the standard. A standard calibration plot was constructed at 725 nm using known concentrations of GA (2.26-22.6 μg mL^{-1}; $r^2 = 0.9984$; $y = 0.1151x + 0.0065$). Determinations of the extracts and the calibration curve were carried out with three replicates, and the results expressed as equivalents of gallic acid (mg GAE/g Alecrim-do-Campo).

2.6.3. Determination of total flavonoid contents

Total flavonoids were measured as described.[16] A 1 mL aliquot of extracts solution (0.40 mg/mL in absolute ethanol) was mixed with 1 mL of 2% $AlCl_3$. After incubation at room temperature for 10 minutes, the absorbance of the reaction mixture was measured at 420 nm against a blank consisting of extracts and ethanol using a spectrophotometer UV-VIS (Genesys 10S). Total flavonoids were quantified using a standard calibration curve of quercetin (QE) (2.22-22.2 μg mL1; $r^2 = 0.9993$; $y = 0.0518x - 0.0563$). The experiment was performed in triplicate and the results were expressed as mg of quercetin equivalents per g of sample (mg QEE/g Alecrim-do-Campo).

2.6.4. Gas Chromatography-Mass Spectrometry (GC/MS)

The extract was further analyzed to identify volatile compounds using GC/MS. The analysis was performed using a GC column (30 m x 250 μm x 0.25 μm particle size) with helium as the carrier gas (1.4 mL min^{-1}). A 1.0 μL sample of the dichloromethane extract of

Baccharis dracunculifolia (1.0 mg mL^{-1}) was injected in splitless mode. The injector and detector temperatures were set at 290 °C. The column temperature, initially at 100 °C (1 minute), was ramped up to 200 °C (5°C min^{-1}), followed by an increase of 10 °C min^{-1} until 290 °C, maintaining the isothermal condition for 10 min. Compound identification was based on relative retention time and comparison of mass spectra with NIST/2.0 library data.

2.7. Photoprotective formulation

The extracts exhibiting the highest SPF were incorporated into a photoprotective formulation using Pemulen TR-1, without the addition of UVA-UVB filters. Initially, the dichloromethane extract was dissolved in a solution of ethanol (99.5%) and propylene glycol in a 1:1 ratio and then added to the Pemulen TR-1 gel. The mixture was subjected to constant stirring until all components were fully homogenized. The final formulation consisted of 1% extract, 10% solvent, 10% propylene glycol, and Pemulen TR-1 gel to complete 100%. After weighing, the formulation was diluted in a mixture of dichloromethane and propylene glycol, also in a 1:1 ratio, achieving a final concentration of 0.04 g mL^{-1}. Negative control solutions were prepared using only the Pemulen TR-1 gel.

2.7.1. Nanoemulsion by phase onversion

From the ethanolic extract, a nanoemulsion was prepared by phase inversion method, consisting of an aqueous phase and an oil phase. The oil phase was composed of canola vegetable oil (5%), Ultramona RH400 (3%) and Brij S2 (2%) as non-ionic surfactants, DMSO (2%) and ethanolic extract (2%, first dissolved in DMSO). The aqueous phase consisted of ultrapure water (86%).[17] To obtain the nanoemulsion, the aqueous phase was heated to 75 °C and then added to the oil phase at the same temperature, while stirring at 1500 rpm for 3 minutes. The mixture was then stirring until cooled. After this process, the nanoemulsion was left to rest for 24 hours to assess its stability. Data related to Zeta potential, Polydispersity Index, and hydrodynamic particle size were analyzed 7, 14, 21, and 28 days after the development of the nanoemulsion. The normal distribution of the presented data was assessed using the Shapiro-Wilk test. Differences between 1, 7, 14, 21, and 28 days after the development of the nanoemulsion were evaluated using the t-test for zeta potential, hydrodynamic size, and polydispersity index. A significance threshold of $p < 0.05$ was considered to determine statistically significant differences. The GraphPad Prism 8.0.1 software was used for the analysis.

3. Results and Discussion

Based on the SPF data presented in the mentioned tables, it is observed that only the extracts prepared with dichloromethane and 95% ethanol exhibited photoprotective activity. This is evidenced by the appropriate SPF values (SPF > 6), with higher concentrations resulting in higher SPF values. The extract with the highest SPF value, as shown in Table 2, was the dichloromethane extract at a concentration of 0.1 mg mL^{-1}.

The phytochemical tests revealed positive results for the presence of flavonoids, terpenoids and phenols/tannins in both the dichloromethane and the 95% ethanolic extract of *Baccharis dracunculifolia*, as summarized in Table 3. The presence of flavonoids was indicated by a vivid yellow color, while a positive result for terpenoids was characterized by the formation of a brown color at the interface. The remaining tests yielded negative results, indicating no reaction.

Table 2. SPF values measured in different extracts and concentrations with standard deviation

Concentration (mg mL^{-1})	Hexane Extract	Dichloromethane Extract	Ethanolic Extract
0.02	0.496 ± 0.001	3.125 ± 0.195	3.525 ± 0.003
0.03	0.631 ± 0.231	4.725 ± 0.010	4.948 ± 0.007
0.05	1.257 ± 0.311	7.974 ± 0.011	8.387 ± 0.001
0.07	1.728 ± 0.508	12.773 ± 0.110	11.420 ± 0.002
0.1	3.423 ± 0.034	16.446 ± 0.145	16.090± 0.074

Table 3. Presence of phytochemical classes in the dichloromethane and ethanolic extracts of *Baccharis dracunculifolia*

Phytochemical Classes	Dichloromethane Extract	Ethanolic Extract
Flavonoids	(+)	(+)
Saponins	(-)	(-)
Phenols/ Tannins	(+)	(+)
Terpenoids	(+)	(+)

The stable organic radical DPPH$^{\bullet}$ has been widely used in antioxidant activity studies of single compounds[16], plant extracts[18], and foods[18] etc. The method is based on the reduction of alcoholic DPPH$^{\bullet}$ solutions at 517 nm in the presence of a hydrogen donating antioxidant (AH), resulting in the formation of the non-radical form DPPH-H, as described by Equation 3.

$$DPPH^{\bullet} + AH \rightarrow DPPH\text{-}H + A^{\bullet} \quad (3)$$

The remaining DPPH$^{\bullet}$, measured after a certain time, corresponds inversely to the radical scavenging activity of the antioxidant. The free radical scavenging capacity of plant extracts was also studied using the ABTS radical cation decolorization assay[1918]. The ABTS*$^{+}$ was produced by reacting the ABTS stock solution with potassium persulfate (2.45 mmol L^{-1}, final concentration) and allowing it to stand in the dark at room temperature for 12-16 hours. This reaction is based on the reduction of ABTS*$^{+}$ radicals by antioxidants of the plant extracts tested. The reaction's mechanism involves the electron-donating ability and results in the decolorization of the radical.

Naturally occurring phenolic substances, including carotenoids, flavonoids and hydrolysable tannins, are among the most significant plant-derived antioxidants. A correlation between antioxidant capacity and phenolic compounds revealed that ethanolic extract of Alecrim-do-Campo exhibited the highest levels of phenolic compounds, which coincided with intense antioxidant activity, as presented in Table 4.

Phenolic compounds are the most important antioxidants of plant materials and their antioxidant activity is based on their ability to donate hydrogen atoms to free radicals.[19] Some authors has shown a correlation between total phenolic content and antioxidant activity, as measured by different methods. However, some studies have found no such relationship[20]. The low correlations might be explained that total antioxidant activity is not due to only one contributor, the presence of non-phenolic antioxidants (vitamin C, vitamin E and carotenoids) having accountable antioxidant activity.

GC-MS analysis (Table S1) revealed that the most abundant compound is benzenepropanoic acid, a compound belonging to the class of phenylpropanoids. This phytochemical class has been previously identified in *Baccharis dracunculifolia*, confirming its presence in this species.

In this study, the dichloromethane extract showed a higher SPF. To further analyze its composition, GC/MS was employed, a technique suitable for volatile compounds. However, this method has limitations, as it may not detect non-volatile compounds, including flavonoids, even at high temperatures. Flavonoids were not detected in the GC/MS technique, but through the determination of total flavonoid contents technique applied in this work it was possible to perceive that the dichloromethane extract has a certain amount of flavonoids. These flavonoids, in combination with other constituents, may contribute to the enhanced SPF observed in this extract.

The SPF values of the Pemulen TR-1 Gel without filter and the SPF value of the dichloromethane and ethanolic extract in the formulations with Pemulen TR-1 Gel are represented in Table 5. The increase in SPF value in the formulation of the extract with Pemulen Gel can be explained by a synergy that occurs between the extract and the gel during incorporation, thus promoting an increase in the photoprotective activity of the formulation.

Flavonoids have shown significant absorption in ultraviolet A (UVA), ultraviolet B (UVB) region, due to their chemical structure, which features conjugated double bonds. This property makes them suitable as ingredients in cosmetics formulations for skin protection. A systematic review by Alencar Filho *et al.* (2016) explored the various mechanisms underlying the photoprotective potential of flavonoids.[21]

The Zeta potential is a critical parameter for evaluating the stability of nanoemulsions. Particles with highly positive or negative Zeta potential values repel each other, reducing aggregation and improving stability[22]. In this study, differences in Zeta potential were observed once different studies indicated differences between the periods, revealing

Table 4. Total phenolics, flavonoids and antioxidant activity of dichloromethane and ethanolic extract of Alecrim-do-Campo

Extract	Total Phenolics (mg GAE/g plant)	Total Flavonoids (mg QEE)	IC_{50} ABTS (mg mL^{-1})	IC_{50} DPPH (mg mL^{-1})
Dichloromethane	1.46	1.33	25.67	137.62
ethanolic	6.30	2.17	17.66	54.43

Table 5. The SPF values measured between the gel and the incorporated extract

	FPS Value	Standard Deviation
Pemulen TR-1 Gel	0.281730	0.003397
Formulation at the concentration of 0.04 g mL^{-1} - Dichloromethane	5.062400	0.488874
Pemulen TR-1 Gel	0.235360	0.009082
Formulation at the concentration of 0.04 g mL^{-1}- ethanol (99.5%)	25.481000	0.828385

a greater tendency for stability up to 7 days. Despite these differences, the nanoemulsion was stable throughout the evaluation, and although a reduction was noted, no differences in size or PDI were observed up to 28 days, suggesting that it remains stable despite the increased load.

Nanoemulsions typically have droplet sizes in the nanometer range (50-500 nm). [22] In this study, the average droplet sizes are around 100 nm for NE across all analyzed periods, as determined by photon correlation spectroscopy. Nanoemulsion systems can exhibit either monodisperse or polydisperse particle distributions. A polydispersity index (PDI) below 0.3 is considered favorable, indicating a monodisperse system. The formulations demonstrated a monodisperse profile, supported by their nanometer-scale size distribution, as shown in Figure 1.

No statistical difference was observed for PDI values in NE. However, NE showed minimal changes in particle size and PDI across the evaluation periods, indicating a high degree of stability. Overall, NE exhibited stability over the evaluated periods, supported by Zeta potential, particle size and PDI. No phase separation or other signs of instability were observed. However, further long-term stability studies are necessary to establish optimal storage conditions and ensure the viability of these formulations for commercial applications.

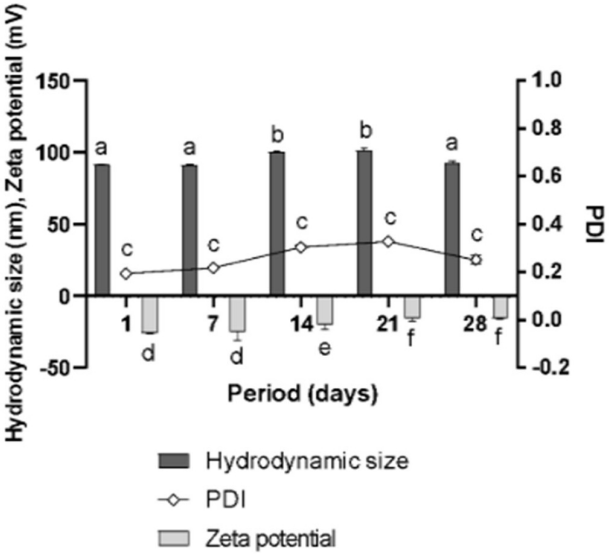

Figure 1. Nanoemulsion: Hydrodinamic size, Zetapotential and Polydispersity index (PDI). (*Note: Different lowercase letters indicate statistical differences over time)*

Nanoemulsions formulated from nonionic surfactants commonly acquire a negative surface charge. This phenomenon was observed by Sousa *et al.* (2024) in their study of similar nanoemulsions.[22] The acquisition of a negative charge at the oil/water interface, even in the absence of charged surfactants, can be explained by the preferential adsorption of hydroxyl ions (OH-), originating from the autoionization of water.[23]

4. Conclusions

Baccharis dracunculifolia emerges as a promising candidate for integrating natural sunscreens into topical formulations, aiming to mitigate skin damage caused by UV radiation. This assertion is supported by the conducted tests, which revealed satisfactory levels of SPF. The outcomes of this research underscore the relevance and potential use of *Baccharis dracunculifolia* extracts in the design of sunscreens, especially when combined with Pemulen TR-1 Gel, where remarkable SPF values were observed. The results suggested that the phenolic compounds contributed significantly to the antioxidant capacity of studied extracts. In addition, the developed nanoemulsion has emerged as a promising candidate for evaluating its photoprotective cosmetic potential.

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