

DEVELOPMENT AND CHARACTERIZATION OF OIL-IN-WATER PHOTOPROTECTIVE COSMETIC FORMULATIONS CONTAINING *VERONICA* (PLANTAGINACEAE) EXTRACTS

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Abstract

The present study aimed to develop and characterize cosmetic formulations containing selected *Veronica* L. (*Plantaginaceae*) extracts and to evaluate their physicochemical, rheological, occlusive, antioxidant, and *in vitro* photoprotective properties. Oil-in-water emulsions were prepared using an optimized emulsifying system and supplemented with extracts obtained from three *Veronica* species using different extraction solvents, in the absence or presence of 5% ethylhexyl triazone and 10% ethylhexyl methoxycinnamate. The formulations were evaluated for physicochemical characteristics, stability, apparent viscosity, spreadability, occlusive capacity, cupric reducing antioxidant capacity (CUPRAC), and *in vitro* sun protection factor (SPF). All formulations exhibited satisfactory physicochemical stability, homogeneous appearance, and pH values suitable for topical application. The extracts influenced the rheological properties and occlusive capacity without compromising formulation stability or spreadability. Formulations containing UV filters maintained satisfactory *in vitro* SPF values, although no increase in spectrophotometrically determined SPF was observed after extract incorporation. UV-containing formulations exhibited significantly higher CUPRAC antioxidant activity. Hierarchical clustering confirmed distinct formulation profiles based on their physicochemical, photoprotective, and antioxidant characteristics, demonstrating the suitability of *Veronica* extracts for photoprotective cosmetic applications.

Rezumat

Prezentul studiu a avut ca scop dezvoltarea și caracterizarea unor formulări cosmetice care conțin extracte selectate din *Veronica* L. (*Plantaginaceae*), precum și evaluarea proprietăților lor fizico-chimice, reologice, ocluzive, antioxidante și fotoprotectoare *in vitro*. Emulsiile de tip ulei în apă au fost preparate utilizând un sistem emulgator optimizat și îmbogățite cu extracte obținute din trei specii de *Veronica*, prin utilizarea unor solvenți de extracție diferiți, în absența sau în prezența a 5% etilhexil triazonă și 10% etilhexil metoxicinamat. Formulările au fost evaluate din punct de vedere al caracteristicilor fizico-chimice, stabilității, vâscozității aparente, întinderii, capacității ocluzive, capacității antioxidante prin metoda CUPRAC și factorului de protecție solară (SPF) *in vitro*. Toate formulările au prezentat o stabilitate fizico-chimică satisfăcătoare, un aspect omogen și valori ale pH-ului compatibile cu aplicarea topică. Extractele au influențat proprietățile reologice și capacitatea ocluzivă fără a compromite stabilitatea sau întinderea formulărilor. Formulările care conțin filtre UV au menținut valori satisfăcătoare ale SPF-ului *in vitro*, deși incorporarea extractelor nu a determinat o creștere a SPF-ului determinat spectrofotometric. Aceste formulări au prezentat, de asemenea, o activitate antioxidantă semnificativ mai ridicată, determinată prin metoda CUPRAC. Analiza prin grupare ierarhică (heatmap) a evidențiat profiluri distincte ale formulărilor pe baza caracteristicilor fizico-chimice, fotoprotectoare și antioxidante, confirmând potențialul extractelor de *Veronica* pentru dezvoltarea unor formulări cosmetice fotoprotectoare.

Keywords: *Veronica* sp., photoprotection, sunscreen, cosmetic formulation, SPF, antioxidant capacity

Introduction

Solar ultraviolet (UV) radiation is one of the main environmental factors responsible for acute and chronic skin damage (1). Prolonged exposure to UV radiation induces erythema, photoaging, oxidative stress, immunosuppression and DNA damage, contributing to the development of various skin disorders, including photocarcinogenesis (2-4). Although the use of sunscreen products remains the most effective

strategy for preventing UV-induced skin damage, increasing concerns regarding the safety, environmental impact and photostability of certain synthetic UV filters have stimulated the search for complementary photoprotective ingredients of natural origin (5-8). The effectiveness of sunscreen products depends not only on the UV filters employed but also on formulation-related factors, including the emulsifying system, emollient composition, film-forming properties

and product spreadability on the skin surface (6). Consequently, considerable attention has recently been directed toward plant-derived bioactive compounds capable of enhancing the overall efficacy of sunscreen formulations while providing additional biological benefits beyond UV absorption alone.

Among natural photoprotective agents, plant extracts rich in phenolic compounds have attracted particular interest because of their multifunctional biological activity (9-13). Phenolic acids, flavonoids and related phytochemicals possess remarkable antioxidant, anti-inflammatory and free radical scavenging properties that may reduce the oxidative stress generated by UV exposure (14-16). In addition to their intrinsic UV-absorbing capacity, these compounds may contribute to skin photoprotection by limiting reactive oxygen species formation, preserving endogenous antioxidant defenses and reducing inflammatory responses associated with solar radiation. Therefore, botanical extracts have increasingly been investigated as complementary ingredients capable of supporting conventional sunscreen systems rather than replacing organic or inorganic UV filters (2, 3).

The incorporation of plant extracts into topical formulations, however, represents a considerable technological challenge. Besides the biological activity of the extracts, the final performance of a cosmetic product depends on formulation composition, emulsifying systems, rheological properties, physical stability and compatibility between the extract and the vehicle (17, 18). Botanical extracts may modify the viscosity, spreadability and overall stability of emulsions owing to the complex mixture of phenolic compounds, carbohydrates and other high-molecular-weight constituents they contain (19). Consequently, the successful development of photoprotective formulations requires careful optimization of the cosmetic base to ensure both appropriate technological performance and adequate incorporation of plant-derived bioactive compounds (17, 18, 20).

Species belonging to the genus *Veronica* L. (*Plantaginaceae*) represent a promising source of biologically active phytochemicals. These plants are widely distributed throughout Europe and Asia and have long been used in traditional medicine for the treatment of inflammatory and skin-related disorders. Phytochemical investigations have demonstrated that *Veronica* species contain considerable amounts of phenolic acids, flavonoids, iridoid glycosides and other secondary metabolites associated with antioxidant, antimicrobial and anti-inflammatory activities. Previous studies on *Veronica officinalis* L. and related species have confirmed their high phenolic content and significant antioxidant potential, supporting their possible application as functional cosmetic ingredients (21, 22). Despite these promising biological properties, information regarding the incorporation

of *Veronica* extracts into cosmetic emulsions and their influence on formulation performance and photoprotective efficacy remains limited.

The *in vitro* evaluation of sunscreen efficacy is commonly performed using spectrophotometric methods, among which the Mansur method remains one of the most widely applied laboratory approaches because of its simplicity, reproducibility and rapid execution (23). Nevertheless, spectrophotometrically determined SPF reflects only the UV absorption capacity of the tested formulation and does not account for biological mechanisms involved in skin photoprotection, such as antioxidant or anti-inflammatory effects (24, 25). Consequently, botanical extracts that provide complementary protection through mechanisms other than direct UV absorption may contribute only marginally to *in vitro* SPF values despite potentially enhancing the overall biological photoprotection of sunscreen formulations (14, 16, 26). Therefore, the aim of the present study was to develop and optimize oil-in-water cosmetic formulations containing selected *Veronica* extracts with and without organic UV filters and to evaluate their physicochemical, rheological, occlusive, antioxidant, and photoprotective characteristics. Particular emphasis was placed on formulation development, physical stability, viscosity, spreadability and *in vitro* SPF determination in order to assess the compatibility of *Veronica* extracts with a photoprotective cosmetic vehicle and to establish their suitability for further development as multifunctional cosmetic ingredients.

Materials and Methods

Materials

The aerial parts of *Veronica officinalis* L., *Veronica hederifolia* L. and *Veronica prostrata* L. were collected during the flowering period from spontaneous flora of Romania. Voucher specimens were deposited in the Herbarium of the Faculty of Pharmacy, "Carol Davila" University of Medicine and Pharmacy, Bucharest, Romania (*V. hederifolia* - 23-502, *V. prostrata* - 23-503 and *V. officinalis* - 23-504). The three species were selected based on their content in phenolic compounds, and their reported antioxidant properties (21, 22). The extraction procedure was identical for all samples and is only briefly summarized here, as the present study focuses on the development and characterization of cosmetic formulations rather than extract preparation. The plant materials were extracted by reflux using distilled water, 50% (v/v) ethanol, or 96% (v/v) ethanol. After filtration, the extracts were concentrated under reduced pressure, freeze-dried, and stored in airtight amber glass containers protected from light until use. Based on their phytochemical characteristics, five lyophilized extracts were selected for cosmetic formulation

development: *V. officinalis* aqueous extract (VO-Aq) and 96% ethanolic extract (VO-E96), *V. hederifolia* 96% ethanolic extract (VH-E96), and *V. prostrata* 50% (VP-E50) and 96% ethanolic extracts (VP-E96).

The raw materials used during formulation development, together with their INCI names, functions and suppliers, are summarized in Table I. The cosmetic base of the final optimized formulation was prepared

using purified water, glycerin, phenoxyethanol, disodium EDTA, xanthan gum, caprylic/ capric triglyceride, shea butter, sodium citrate and the Heliofeel® emulsifying system, composed of glyceryl stearate citrate, polyglyceryl-3 stearate and hydrogenated lecithin. Ethylhexyl triazone and ethylhexyl methoxycinnamate were used as organic UVB filters at concentrations of 5% and 10%, respectively.

Table I

Raw materials used for the preparation of the cosmetic formulations

INCI	Role	Supplier
Aqua	Vehicle	In-house
Glycerin	Humectant	Oleochem a.s. (Strekov, Slovakia)
Phenoxyethanol	Preservative	THOR Personal Care S.A.S. (Compiègne, France)
Disodium EDTA	Chelating agent	Esperis S.p.A (Milan, Italy)
Xanthan Gum	Rheology modifier	Jianlong Biotechnology Co., Ltd (Inner Mongolia, China)
Caprylic/ Capric Triglyceride	Emollient	Industrial Quimica Lasem (Barcelona, Spain)
Coco-Caprylate/ Caprate	Emollient	Industria Chimica Panzeri Srl (Orio al Serio, Italy)
<i>Butyrospermum Parkii</i> Butter	Structuring agent	Interfat (Barcelona, Spain)
Cetearyl Alcohol	Structuring agent	Peter Cremer Central Europe s.r.o (Ústí nad Labem, Czech Republic)
Glyceryl Stearate Citrate, Polyglyceryl-3 Stearate, Hydrogenated Lecithin (Heliofeel® 22 MB)	Emulsifier	Lucas Meyer Cosmetics S.A.S. (Massy, France)
Glyceryl Stearate, Cetearyl Alcohol, Sodium Stearoyl Lactylate (Plantasens Emulsifier HP 30)	Emulsifier	Clariant Ibérica Production, S.A. (Tarragona, Spain)
Ethylhexyl Triazone	UVB filter	Yidu Huayang Chemical Co. Ltd (Hubei Province – China)
Ethylhexyl Methoxycinnamate	UVB filter	Salicylates and Chemicals Pvt Ltd. (Mumbai, India)
Sodium Citrate	pH adjuster	Art Chemicals (Bucharest, Romania)

Rational design of cosmetic formulations

The cosmetic formulations were rationally designed to provide a simple and stable vehicle suitable for the incorporation of *Veronica* extracts while minimizing the potential interference of excipients with the spectrophotometric determination of the photoprotective activity. Therefore, the number of excipients was intentionally limited to those required to ensure appropriate stability, sensory properties and technological performance.

A lightweight oil-in-water emulsion with serum-like texture was selected as the formulation platform due to its favourable sensory characteristics, rapid skin absorption and suitability for topical delivery of plant-derived bioactive compounds. Emollients were selected according to their ability to solubilize organic UV filters and support their homogeneous distribution within the oil phase (27). The emulsifying systems, together with the structuring agent, were selected based on their ability to provide stable O/W emulsions with good spreadability and appropriate rheological properties while maintaining the desired lightweight, serum-like texture.

Two UVB filters, ethylhexyl triazone and ethylhexyl methoxycinnamate were selected based on their high UVB absorption efficiency and their ability to produce clear ethanolic solutions suitable for spectrophotometric *in vitro* SPF determination according to the Mansur method. Their physicochemical properties also enabled complete incorporation into the oil

phase and homogeneous distribution within the cosmetic vehicle while minimizing analytical interference during SPF evaluation (5, 28).

Preparation of cosmetic formulations

The cosmetic formulations were prepared using a conventional oil-in-water (O/W) emulsification process. The aqueous phase consisted of purified water, disodium EDTA and glycerin. Xanthan gum was first dispersed in glycerin before being incorporated into the aqueous phase. In parallel, the oil phase, containing the emollients, shea butter as the structuring agent and the Heliofeel® emulsifying system, was prepared separately. Both phases were heated to 70°C and subsequently emulsified using an IKA high-shear homogenizer operating at 12,000 rpm for 3 - 4 min.

Following emulsification, the formulations were allowed to cool. The formulations were designed to achieve a slightly acidic pH (5.4 - 5.6), corresponding to the physiological skin pH and ensuring skin compatibility and formulation stability. At 25°C, the pH was adjusted to the target value using a sodium citrate solution.

The plant extracts, as heat-sensitive ingredients, were incorporated only after cooling. Prior to incorporation, the dried extracts were dispersed in a 1:1 (v/v) mixture of purified water and glycerin to obtain homogeneous dispersions.

For the photoprotective formulations, ethylhexyl triazone (5%) and ethylhexyl methoxycinnamate (10%)

were incorporated into the oil phase prior to emulsification to ensure adequate solubilization and homogeneous distribution throughout the formulation. The UV filters replaced an equivalent proportion of the emollient phase, while the composition of the aqueous phase remained unchanged. The formulations were stored at room temperature for 24 h before physicochemical, rheological, and photoprotective characterization.

Physicochemical characterization

The developed formulations were subjected to physicochemical evaluation immediately after preparation and during storage. Organoleptic properties, including appearance, color, odor and homogeneity, were assessed by visual inspection.

The pH values were determined at room temperature ($25 \pm 2^\circ\text{C}$) using a calibrated digital pH meter (Hanna Instruments, USA) following calibration with standard buffer solutions (pH 4.00 and 7.00).

Physical stability was initially evaluated by centrifugation at 3500 rpm for 30 min (Centurion Scientific Ltd., UK), on a previously heated sample at 40°C , to detect possible phase separation. Accelerated stability was assessed using a freeze–thaw test consisting of six consecutive cycles, each including storage at $-5 \pm 2^\circ\text{C}$ for 24 h followed by storage at $25 \pm 2^\circ\text{C}$ for 24 h. After each cycle, the formulations were visually inspected for changes in appearance, color, odor, homogeneity and phase separation.

Storage stability was evaluated by storing the formulations at $35 \pm 2^\circ\text{C}$ for six months. During the storage period, samples were periodically examined for changes in organoleptic characteristics, pH and physical stability.

Rheological characterization

The rheological properties of the developed cosmetic formulations were evaluated using a B-ONE Plus rotational viscometer (Lamy Rheology Instruments, France) equipped with a T-F 96 measuring system. Measurements were performed at room temperature over a rotational speed range of 50 - 250 rpm. Each measurement was maintained for 20 s, and the apparent dynamic viscosity, shear stress and torque were automatically recorded by the instrument software.

The rheological behaviour of the formulations was assessed by monitoring the changes in apparent dynamic viscosity (cStokes) as a function of the applied rotational speed (rpm). The obtained flow profiles were subsequently used to compare the influence of the incorporated *Veronica* extracts on the rheological characteristics of the cosmetic formulations.

The spreadability of the developed formulations was evaluated using the parallel-plate method. Briefly, 1 g of each formulation was placed between two glass plates, with the upper plate weighing 278 g. Additional loads of 50, 100, 200, 300, 400 and 500 g were

sequentially applied. After each load was applied, the formulation was allowed to equilibrate for 1 min before measuring the spread radius. The spread area was subsequently calculated using the equation $S = \pi r^2$, where S is the spread area and r is the measured radius.

Occlusive capacity evaluation

The occlusive capacity of the developed formulations was evaluated using an *in vitro* gravimetric water evaporation method (29,30). Topical formulations containing lipophilic ingredients can reduce transepidermal water loss by forming a semi-occlusive film on the skin surface. In sunscreen formulations, an appropriate occlusive effect may also improve product persistence on the skin, while excessive occlusion may negatively affect skin comfort, particularly in facial applications.

Plastic containers (75 mL capacity, 3.6 cm diameter) were filled with 70 mL of distilled water and accurately weighed. The containers were covered with laboratory filter paper, and 0.1 g of each formulation was evenly spread over the entire surface of the filter paper using a spatula. A control sample consisting of filter paper without formulation was prepared under identical conditions.

The containers were maintained for 48 h under controlled environmental conditions ($25 \pm 2^\circ\text{C}$, 35 - 40% relative humidity). At the end of the incubation period, the containers were reweighed, and the amount of evaporated water was determined gravimetrically.

The occlusive capacity (OC) was calculated according to the following equation:

$$\text{OC (\%)} = \frac{M_c - M_s}{M_c} \times 100$$

where OC is the occlusive capacity (%), M_c is the amount of water lost from the control sample, and M_s is the amount of water lost from the formulation-treated sample. According to this approach, an OC value of 0% indicates no reduction in water evaporation compared with the control, whereas 100% represents complete prevention of water loss (29).

Each formulation was tested in triplicate, and the results are expressed as mean $\pm$ standard deviation (SD).

In vitro SPF determination

The *in vitro* sun protection factor (SPF) of the developed formulations was determined spectrophotometrically according to the method described by Mansur *et al.* (23). The absorbance of the samples was measured using a Jasco V-530 UV–Vis spectrophotometer (Jasco, Tokyo, Japan) over the wavelength range of 290–320 nm at 5 nm intervals.

Prior to analysis, the cosmetic formulations were appropriately diluted in 96% ethanol to obtain clear and homogeneous solutions suitable for spectrophotometric evaluation. The SPF values were calculated using the Mansur equation, which correlates

the absorbance values with the erythral effect spectrum and the solar intensity at each wavelength:

$$\text{SPF}_{\text{in vitro}} = CF \times \sum_{290}^{320} EE(\lambda) I(\lambda) A(\lambda)$$

where CF is the correction factor (10), $EE(\lambda)$ is the erythral effect spectrum, $I(\lambda)$ is the solar intensity spectrum and $Abs(\lambda)$ is the absorbance measured at each wavelength. The calculated SPF values were used to compare the photoprotective performance of the formulations.

The optimized formulation without plant extract was used as the reference formulation, and the effect of the incorporation of different *Veronica* extracts on the *in vitro* SPF was comparatively evaluated.

CUPRAC antioxidant activity

The cupric ion reducing antioxidant capacity (CUPRAC) of the cream formulations was determined according to the method described by Çelik *et al.* (32), with minor modifications. An accurately weighed amount (approximately 70 mg) of each cream formulation was dispersed in 1.0 mL of ethanol by vortex mixing. Subsequently, 200 µL of the resulting suspension were diluted with 600 µL of ethanol, and 5 µL of the diluted solution were transferred into a 96-well microplate. The samples were mixed with 50 µL of 10 mM copper(II) chloride solution, 50 µL of 7.5 mM neocuproine solution, and 50 µL of 1 M ammonium acetate buffer (pH 7.0). After incubation for 30 min at room temperature, the absorbance was measured at 450 nm using a Multiskan FC Microplate Photometer (Thermo Fisher Scientific, Vantaa, Finland). Gallic acid (Scharlab S.L., Barcelona, Spain) was used as the reference standard for the calibration curve over the concentration range of 2.5 - 30 µg/mL. The antioxidant capacity was expressed as milligrams of gallic acid equivalents per gram of cream (mg GAE/g cream). All measurements were performed in triplicate, and the results are presented as mean ± standard deviation.

Statistical analysis

The viscosity, spreadability, occlusive capacity, and CUPRAC antioxidant activity of the cream formulations were determined in triplicate, and the results are expressed as mean ± standard deviation (SD). Data processing and descriptive statistical analysis were performed using Microsoft Excel 2021 (Microsoft Corporation, USA). Statistical analysis of the CUPRAC assay was performed using GraphPad Prism (GraphPad Software, San Diego, CA, USA), applying one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test, with statistical significance accepted at $P < 0.05$. Graphical representations were prepared using Microsoft Excel 2021 and GraphPad Prism. To facilitate the multivariate comparison of the investigated formulations, a hierarchical clustering heatmap was generated using ClustVis (33,34). Five formulation descriptors were included in the analysis: *in vitro* SPF, spread area index (AUC/500, cm²), mean apparent

viscosity, occlusive capacity, and CUPRAC antioxidant activity. Prior to hierarchical clustering, all variables were standardized using z-score normalization to eliminate the influence of differences in measurement units and value ranges. Hierarchical clustering of both formulations and variables was performed using correlation distance and average linkage. The resulting heatmap was used to visualize similarities among formulations and relationships among the investigated physicochemical, photoprotective, and antioxidant characteristics.

Results and Discussion

Formulation development and physicochemical characterization

The formulation development process resulted in a lightweight oil-in-water (O/W) emulsion with a serum-like texture (17). Two emulsifying systems (Plantasens HP 30 and Heliofeel®) were evaluated separately. Although both systems produced stable emulsions, the Heliofeel®-based formulations showed superior homogeneity, sensory properties and rheological performance and were therefore selected for subsequent studies.

Following optimization, two reference formulations were established: a blank formulation and a photoprotective formulation containing 5% ethylhexyl triazone and 10% ethylhexyl methoxycinnamate. Subsequently, five *Veronica* extracts were incorporated into each base, resulting in a total of twelve formulations. Their composition is presented in Tables II and III.

The *Veronica* extracts used in the study were VO-Aq, VO-E96, VH-E96, VP-E50 and VP-E96. Each extract was incorporated into both the blank and the UV filter-containing formulations. This design enabled direct comparison between formulations containing the same extract in the absence and presence of UV filters, as well as between the different *Veronica* extracts.

All formulations exhibited a homogeneous appearance, with no evidence of phase separation or visible instability. The incorporation of the extracts modified the color of the emulsions depending on the plant species and extraction solvent, an effect that may be related to differences in their phenolic composition and other naturally occurring colored constituents. Similar observations have been reported for O/W emulsions containing botanical extracts, where the incorporation of plant-derived ingredients did not adversely affect the homogeneity or physical appearance of the formulations (18, 35-37).

The pH values ranged from 5.5 to 5.6, remaining within the physiological pH range of healthy skin. No relevant changes in pH were observed following extract incorporation or accelerated stability testing. These values are consistent with those reported for

topical O/W emulsions intended for facial application and remain within the physiological skin pH range,

which is generally considered optimal for skin compatibility (18, 37).

Table II

Composition of the optimized cosmetic base formulations (% w/w)

INCI	Blank formulation	Photoprotective formulation
Aqua	66.5	66.5
Glycerin	3	3
Phenoxyethanol	1	1
Disodium EDTA	0.1	0.1
Xanthan Gum	0.3	0.3
Caprylic/Capric Triglyceride	24	9
Butyrospermum Parkii Butter	2	2
Heliofeel® (Glyceryl Stearate Citrate, Polyglyceryl-3 Stearate, Hydrogenated Lecithin)	3	3
Ethylhexyl Triazone	–	5
Ethylhexyl Methoxycinnamate	–	10
Sodium Citrate	0.1	0.1
Total	100	100

Table III

Composition of the formulations containing *Veronica* extracts (% w/w)

Component	Amount (% w/w)
Cosmetic base	88
Aqua	5
Glycerin	5
<i>Veronica</i> extract	2
Total	100

None of the formulations showed phase separation following centrifugation or freeze–thaw cycling. Similarly, storage at 35°C for six months did not affect the physical stability of the emulsions. Comparable physical stability has been reported for well-designed O/W emulsions containing botanical ingredients, where appropriate emulsifier selection and polymeric stabilizers effectively prevented phase separation during accelerated stability testing (20, 28). No differences in physical stability were observed between the blank, photoprotective and extract-containing formulations, indicating that neither the incorporation of UV filters nor that of *Veronica* extracts adversely affected emulsion stability. A slight increase in color intensity was observed in some extract-containing formulations during storage, which may be attributed to the gradual oxidation of phenolic constituents naturally present in the plant extracts (36).

Rheological and spreadability characterization

The rheological behaviour of the formulations was evaluated to investigate the influence of *Veronica* extracts and UV filters on the viscosity and spreadability of the optimized cosmetic base (19). The apparent viscosity profiles are presented in Figures 1A and 1B, while the spreadability profiles are shown in Figures 2A and 2B.

The apparent dynamic viscosity profiles of the formulations without and with UV filters are presented in Figure 1A and Figure 1B, respectively. All formulations exhibited viscosity values characteristic of

semisolid O/W emulsions suitable for topical application (18, 19, 28). In both rheograms viscosity decreases with increasing rotational speed, confirming non-Newtonian pseudoplastic behaviour. This flow pattern is desirable for topical emulsions, as it facilitates spreading under shear while maintaining sufficient consistency at rest to support formulation stability (19, 28, 37).

Compared with the blank formulation, the incorporation of *Veronica* extracts modified the viscosity of the cosmetic bases to different extents, depending on the plant species, extraction solvent and presence of UV filters. Among the formulations without UV filters (Figure 1A), the aqueous extract of *V. officinalis* (VO-Aq) exhibited the highest overall mean apparent viscosity (7899.6 cSt), whereas the ethanolic extract of the same species (VO-E96) showed the lowest mean apparent viscosity (2881.7 cSt) and the most pronounced fluidising effect. The formulations containing VH-E96, VP-E50 and VP-E96 displayed profiles broadly comparable to that of the blank, suggesting good compatibility between these extracts and the emulsion matrix (20).

The addition of UV filters produced more pronounced and formulation-dependent changes in viscosity (Figure 1B). VP-E96 showed the strongest structuring effect, while VO-Aq caused the greatest reduction in viscosity. Nevertheless, all formulations retained their characteristic pseudoplastic behaviour, indicating that the emulsifying system could accommodate both the plant extracts and UV filters without

disrupting the overall rheological profile. These findings suggest that the rheological effects of the extracts are governed by specific interactions among

their constituents, the UV filters and the components of the emulsifying system (17, 35, 36).

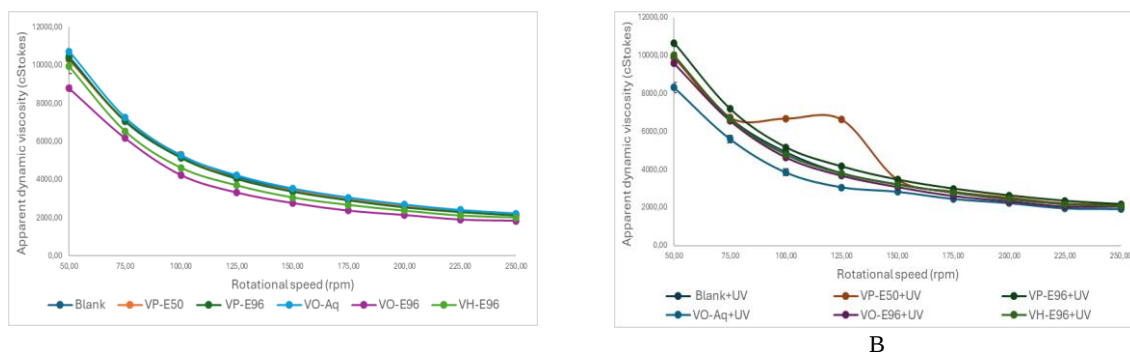


Figure 1.

Apparent viscosity–rotational speed profiles of the experimental formulations. The sigmoidal fitted curves illustrate the progressive reduction in viscosity with increasing shear rate, characteristic of pseudoplastic systems: (A) formulations without UV filters; (B) formulations containing UV filters. Values are presented as mean $\pm$ SD (n=3)

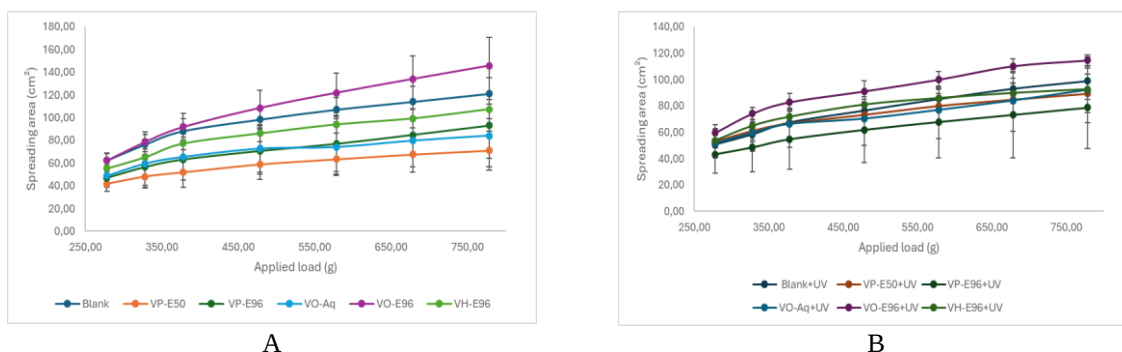


Figure 2.

Spread area of the developed formulations as a function of applied load: (A) formulations without UV filters; (B) formulations containing UV filters. Values are presented as mean $\pm$ SD (n=3)

Among the formulations without UV filters (Figure 2A), VO-E96 showed the highest spreadability, followed by the blank, whereas VP-E50 exhibited the lowest values. VP-E96 and VO-Aq also showed lower spreadability than the blank, particularly at higher loads.

The incorporation of UV filters produced formulation-dependent changes (Figure 2B). VO-E96+UV remained the most readily spreadable formulation, while VP-E96+UV displayed the lowest values. Compared with the corresponding formulations without UV filters, spreadability generally decreased for Blank, VO-E96, VH-E96, and VP-E96, but increased moderately for VP-E50 and VO-Aq. However, the overlap of several error bars indicates that statistical significance should be confirmed by inferential analysis.

The observed differences are likely related to interactions among extract constituents, UV filters, xanthan gum network and the dispersed emulsion structure (20, 37). Nevertheless, all formulations retained

adequate load-dependent spreadability, an important property for forming a continuous and uniform film on the skin (18, 38, 39).

The rheological and spreadability evaluations demonstrated that the developed emulsions showed physico-mechanical properties suitable for topical application, and the Heliofeel®-based system maintained satisfactory technological performance despite the incorporation of plant extracts and UV filters.

Occlusive capacity

The occlusive capacity of topical formulations is an important parameter influencing skin hydration by limiting water evaporation from the skin surface (29, 30). In sunscreen formulations, moderate occlusion may also contribute to improved film persistence and water resistance, whereas excessive occlusion may compromise skin comfort, particularly in facial products.

The occlusive capacity of the developed formulations is presented in Figure 3. Overall, all formulations exhibited low to moderate occlusive capacity,

with values ranging from approximately 12% to 36%. Such values are consistent with lightweight emulsion systems intended for daily facial

application, providing a balance between reducing water loss and maintaining acceptable sensory properties.

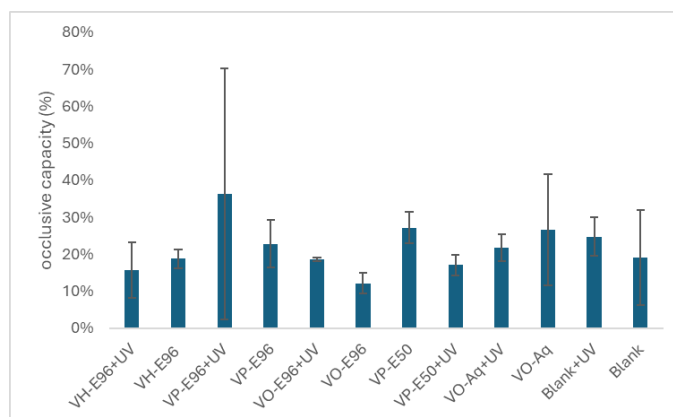


Figure 3.

Occlusive capacity of the developed photoprotective formulations. Values are presented as mean $\pm$ SD (n = 3)

Among the tested formulations, VP-E96+UV exhibited the highest mean occlusive capacity (approximately 36%), although this formulation also showed the largest variability between replicates. Elevated occlusive values were also observed for VP-E50 and VO-Aq+UV, whereas VO-E96 displayed the lowest occlusive effect (approximately 12%).

The incorporation of UV filters generally resulted in a slight increase in occlusive capacity compared with the corresponding blank formulations, most likely due to the increased lipophilic content and enhanced film-forming properties of the oil phase (29–31). However, this effect was formulation-dependent and did not follow a consistent trend across all extract-containing emulsions.

Overall, the incorporation of the investigated *Veronica* extracts did not markedly alter the occlusive properties of the emulsions, indicating that these botanical ingredients can be incorporated without substantially affecting this functional characteristic. This finding is particularly relevant for facial photoprotective products, where excessive occlusion is generally undesirable due to its potential to impair skin comfort and cosmetic acceptability. The moderate occlusive capacity observed for all formulations is consistent with the formulation strategy adopted in this study, which aimed to develop lightweight serum-like emulsions rather than highly occlusive creams. Such formulations are expected to provide an appropriate balance between limiting water evaporation, maintaining skin hydration, and ensuring pleasant sensory properties while supporting the formation of a uniform sunscreen film on the skin surface.

In vitro photoprotective evaluation

The *in vitro* SPF values of the formulations without and with UV filters are presented in Figure 4. As expected, the blank formulation exhibited negligible

intrinsic photoprotective activity (SPF 0.44). Compared with the blank formulation, the incorporation of *Veronica* extracts into the formulations without UV filters produced only limited and extract-dependent variations in SPF, with values ranging from 0.15 to 1.17 (3, 40–42). These results indicate that, at the concentration used in the cosmetic formulations, the extracts alone did not provide a substantial photoprotective activity under the experimental conditions.

Compared with the blank formulation (SPF 0.44), the incorporation of 5% ethylhexyl triazone and 10% ethylhexyl methoxycinnamate increased the SPF of the reference formulation from 0.44 to 25.82. The addition of *Veronica* extracts to the UV filter-containing formulations did not result in a further increase of the measured SPF values, which ranged from 18.34 to 25.39, depending on the incorporated extract. Among the tested formulations, VO-E96+UV showed the highest SPF (25.39), that was comparable to the blank formulation containing UV filters, whereas VO-Aq resulted in the lowest SPF value (18.34). None of the extract-containing formulations exceeded the SPF of the corresponding blank formulation containing UV filters. Overall, these findings indicate that the effect of *Veronica* extracts on SPF depended on the extract type but remained limited under the experimental conditions employed, with none of the tested extracts enhancing the photoprotective performance of the optimized UV filter system.

The absence of a marked SPF-enhancing effect should be interpreted considering the analytical conditions used for spectrophotometric determination (2, 6, 39, 43). Although the cosmetic formulations contained 2% dry extract, the dilution procedure required for the Mansur assay resulted in a final extract concentration of approximately 0.004 mg/mL during absorbance measurements.

This concentration is approximately 31-fold lower than the lowest concentration previously investigated for the isolated extracts (0.125 mg/mL) and 500-fold lower than the concentration (2 mg/mL) used for preliminary extract screening. Consequently,

the photoprotective activity observed for isolated extracts cannot be directly translated to the final cosmetic formulations, where substantially lower extract concentrations are present during spectrophotometric analysis (24).

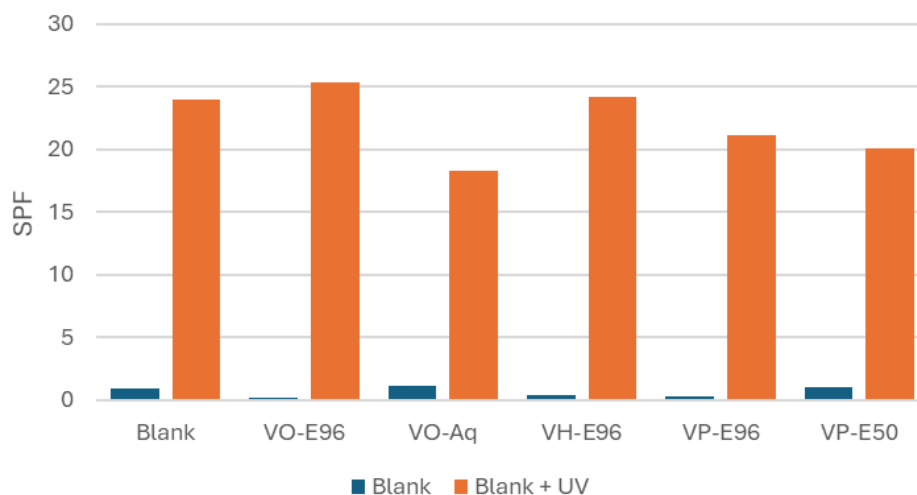


Figure 4.

In vitro sun protection factor (SPF) values of the developed cream formulations with and without UV filters

In addition to the dilution effect, the apparent photoprotective performance of the formulations may also be influenced by interactions between plant-derived constituents, the emulsion matrix and the incorporated UV filters (6, 44, 45). Similar observations have been reported for botanical cosmetic formulations, where the intrinsic UV-absorbing capacity of plant extracts was only partially reflected in the SPF values of the final products because of formulation-related factors, including active concentration, dispersion within the vehicle and light scattering phenomena (6, 20, 43, 46).

It should also be considered that the spectrophotometric Mansur method evaluates photoprotection exclusively based on the UV absorption properties of the tested formulations and therefore does not account for biological mechanisms involved in skin photoprotection (25, 47). Recent advances in standardized *in vitro* sunscreen testing, including the ISO 23675:2024 double-plate method, have improved the reproducibility and predictive value of laboratory SPF measurements; however, these methods still primarily assess the optical UV-filtering performance of formulations and cannot fully reproduce the complex biological responses measured during *in vivo* SPF evaluation (47). Several studies have shown that botanical extracts rich in antioxidant compounds may improve the overall photoprotective efficacy of sunscreen formulations *in vivo* by reducing UV-induced oxidative stress, inflammation and erythema, despite producing little or no increase in

spectrophotometrically determined SPF values (14-16, 26, 48-51). Therefore, the absence of a significant increase in spectrophotometrically determined SPF should not necessarily be interpreted as the absence of a photoprotective contribution, since antioxidant-mediated mechanisms are reflected only during *in vivo* evaluation, where SPF is determined according to the erythral response of human skin rather than solely by UV absorbance (16, 25, 52).

CUPRAC antioxidant activity

The antioxidant capacity of the cream formulations, determined by the CUPRAC assay and expressed as mg gallic acid equivalents per gram of cream (mg Eq GA/g), is presented in Figure 5. The formulations containing UV filters exhibited higher CUPRAC values than their corresponding formulations without UV filters, indicating a greater overall reducing capacity of these formulations. Among all samples, VO-E96+UV showed the highest reducing capacity (36.84 ± 6.32 mg Eq GA/g), followed by VH-E96+UV (25.76 ± 1.45 mg Eq GA/g), VP-E50+UV (24.72 ± 2.66 mg Eq GA/g), VP-E96+UV (23.67 ± 3.32 mg Eq GA/g) and VO-Aq+UV (23.66 ± 4.30 mg Eq GA/g). The corresponding formulations without UV filters exhibited considerably lower antioxidant activity, ranging from 9.17 ± 0.70 to 12.49 ± 1.32 mg Eq GA/g. The blank formulation also demonstrated a marked increase in antioxidant capacity following UV incorporation, from 10.63 ± 1.60 to 20.83 ± 1.34 mg Eq GA/g.

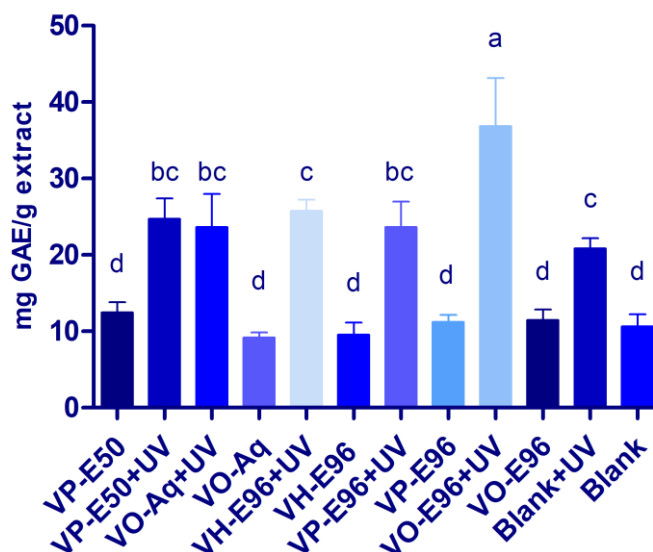


Figure 5.

CUPRAC antioxidant activity of the developed cream formulations expressed as mg gallic acid equivalents per gram of cream (mg GAE/g). Data are presented as mean $\pm$ SD ($n = 3$). Different lowercase letters indicate statistically significant differences among formulations according to one-way ANOVA followed by Tukey's multiple comparison test ($p < 0.05$)

One-way ANOVA showed statistically significant differences among the twelve formulations ($F = 30.82$, $p < 0.0001$, $R^2 = 0.9339$). Post hoc Tukey's multiple comparison test confirmed that the UV-containing formulations generally possessed significantly greater antioxidant capacity than their respective non-UV formulations, with the most pronounced difference observed for VO-E96+UV, which exhibited significantly higher antioxidant activity than all non-UV formulations ($p < 0.001$). No statistically significant differences were observed among several non-UV formulations, indicating comparable baseline reducing capacities.

To obtain an integrated overview of the physicochemical, photoprotective and antioxidant characteristics of the developed formulations, hierarchical clustering analysis was performed using five descriptors, namely the spreadability index (AUC/500), mean apparent viscosity, occlusive capacity, *in vitro* SPF and CUPRAC antioxidant activity (Figure 6). The heatmap revealed two main clusters of variables. *In vitro* SPF and CUPRAC antioxidant activity were grouped within the same branch, suggesting

that formulations exhibiting greater antioxidant capacity generally displayed higher photoprotective performance. In contrast, spreadability and mean apparent viscosity formed a second cluster, reflecting the close relationship between these rheological properties. Occlusive capacity occupied an intermediate position between the two branches, indicating a moderate association with both the physicochemical and biological characteristics of the formulations. Hierarchical clustering of the formulations further discriminated samples according to their overall performance profile. Formulations characterized by higher SPF and CUPRAC values clustered separately from those exhibiting lower photoprotective and antioxidant activity, whereas differences in rheological behaviour and spreadability contributed to the secondary grouping pattern. Overall, the hierarchical clustering analysis demonstrated that the combined evaluation of rheological, occlusive, antioxidant and photoprotective parameters provides an effective approach for distinguishing the developed cosmetic formulations and identifying similarities in their overall functional profiles.

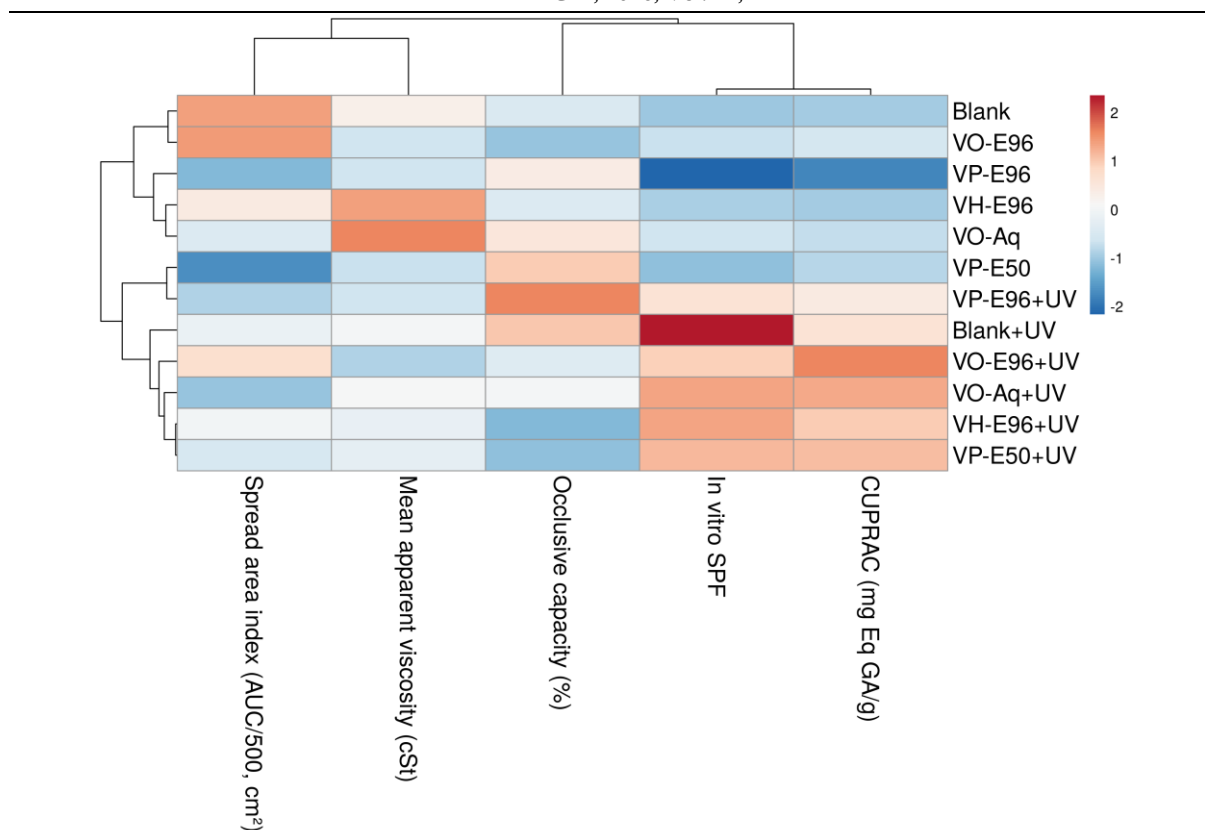


Figure 6.

Hierarchical clustering heatmap illustrating the relationships among the developed formulations according to their physicochemical, photoprotective, and antioxidant characteristics. Clustering was performed using standardized (z-score normalized) values of spreadability index (AUC/500), mean apparent viscosity, occlusive capacity, *in vitro* SPF, and CUPRAC antioxidant activity, applying correlation distance and average linkage. Warmer colors indicate relatively higher values, whereas cooler colors indicate relatively lower values for each investigated parameter

Our findings demonstrate that the incorporation of *Veronica* extracts did not compromise the photoprotective performance or technological quality of the optimized sunscreen formulations. Although no increase in spectrophotometrically determined SPF was observed under the experimental conditions employed, the formulations exhibited satisfactory physicochemical stability, rheological behavior, spreadability, and occlusive capacity, while the UV-containing formulations showed significantly enhanced antioxidant activity as determined by the CUPRAC assay. The antioxidant-rich phytochemical composition of the *Veronica* extracts may therefore provide complementary biological photoprotection that cannot be assessed by *in vitro* UV absorbance measurements alone (6, 53). Hierarchical clustering analysis further confirmed distinct formulation profiles based on the investigated physicochemical, photoprotective, and antioxidant characteristics, highlighting the influence of extract type and UV filter incorporation on the overall performance of the formulations. Therefore, further *in vivo* investigations are warranted to clarify the contribution of antioxidant-mediated mechanisms to the overall

photoprotective efficacy of the developed formulations. Similar observations have been reported for antioxidant-enriched sunscreen formulations, where improvements in biological photoprotection were not necessarily accompanied by proportional increases in spectrophotometrically determined SPF values (25, 26, 47, 49, 54).

Conclusions

The present study successfully developed and characterized oil-in-water cosmetic formulations containing *Veronica* extracts and organic UV filters. The optimized Heliofeel®-based emulsifying system provided stable formulations with suitable physicochemical properties, satisfactory rheological behavior, appropriate occlusive capacity, and appropriate spreadability for topical application. The incorporation of the plant extracts influenced the viscosity profiles to varying degrees without compromising formulation stability or application performance. The developed photoprotective formulations maintained satisfactory *in vitro* SPF values following the incorporation of *Veronica* extracts. Although no

increase in spectrophotometrically determined SPF was observed under the experimental conditions employed, the results demonstrated that the selected botanical extracts were compatible with the cosmetic vehicle and the UV filter system. Moreover, formulations containing UV filters exhibited significantly higher antioxidant activity, as demonstrated by the CUPRAC assay, while maintaining satisfactory physicochemical and photoprotective performance. The present work provides a rational formulation strategy for the incorporation of *Veronica sp.* extracts into photoprotective cosmetic formulations while preserving both formulation performance and the photoprotective efficacy of the selected UV filter system.

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Availability of data and materials

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Ethics approval and consent to participate

Not applicable.

AI use disclosure

During the preparation of this manuscript, the authors used OpenAI to improve the clarity, grammar and linguistic quality of the manuscript and to assist with language editing. All scientific content, interpretation of results and final decisions remained entirely the responsibility of the authors.

Conflict of interest

The authors declare no conflict of interest.

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