Journal of Drug Delivery Science and Technology 114 (2025) 107544

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Grape pomace and quercetin: a synergistic approach using PG-PEVs for Psoriasis treatment

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ARTICLE INFO

Keywords:
Side-stream
Phospholipid vesicles
Antioxidant activity
Inflammation
Nanotechnology
HaCaT cells
In vivo

ABSTRACT

Psoriasis is a chronic inflammatory skin disorder that affects millions worldwide and poses significant therapeutic challenges due to the adverse effects of conventional treatments. In this

study we explored a sustainable approach based on Cannonau grape pomace extract, a winemaking side-stream rich in antioxidant polyphenols, combined with quercetin, to develop effective treatments for psoriasis. The extract was obtained using an eco-friendly extraction process and incorporated into phospholipid vesicles containing propylene glycol (PG-PEVs), which exhibited favourable physicochemical features: small size (~77–172nm), homogeneously dispersed (PDI ~0.29), highly negative zeta potential (~−45 mV), high entrapment efficiency (≥82 %), and long-term stability over 18 months. Biological evaluations revealed the high biocompatibility of the formulations (≥80 %), and their ability to protect keratinocytes from oxidative stress and hyperproliferation, hallmarks of psoriasis.

In a psoriasis-like murine model, PG-PEVs significantly reduced inflammation, neutrophil infiltration, and epidermal hyperplasia, as confirmed by decreased MPO levels (from ~353 to ~179 ng/mL) and improved skin architecture observed through histological analysis, which outperformed the effects of the natural chemicals in dispersion. These findings highlight the therapeutic potential of Cannonau pomace extract-loaded PG-PEVs as a promising, sustainable dermatological strategy. Moreover, this study supports the valorisation of agricultural side-streams into high-value cosmeceuticals, aligning with circular economy principles.

1. Introduction

Psoriasis is a chronic, multifactorial inflammatory skin disorder that affects approximately 2–4 % of the global population [1]. It manifests as hyperproliferative epidermal lesions, accompanied by erythema, scaling, and thickening of the skin [2]. The underlying pathophysiology of psoriasis involves a complex interplay of genetic, immunological, and environmental factors, leading to the dysregulated interactions between the innate and adaptive components of the immune system, abnormal keratinocyte differentiation and heightened infiltration of immune cells, particularly neutrophils and T cells [2]. While conventional therapies, such as corticosteroids, biologics, and immunosuppressants, provide

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https://doi.org/10.1016/j.jddst.2025.107544
Received 3 July 2025; Received in revised form 6 September 2025; Accepted 17 September 2025
Available online 17 September 2025

Table 1
Composition of vesicular formulations.

	S75 mg/mL	Extract mg/mL	Quercetin mg/mL	H2O %	PG %
Cannonau PG-PEVs	200	30		80	20
Quercetin PG-PEVs	200		5	80	20
CQ PG-PEVs	200	30	5	80	20

Table 2
Mean diameter (MD), polydispersity index (PDI), zeta potential (ZP) and entrapment efficiency (E) of the vesicular formulations. Mean values ± standard deviations are reported (n = 6). For each group (MD, ZP and E) the same symbol (*,§, °) indicates values that are not statistically different ($p > 0.05$ among values with same symbol and $p < 0.05$ *versus* values with different symbol).

	MD (nm)	PDI	ZP (mV)	E (%)
Empty PG-PEVs	* 96 ± 10	0.28	*,§ −41 ± 4	–
Cannonau PG-PEVs	§ 161 ± 8	0.26	*,§ −46 ± 5	*,§ 102 ± 2
Quercetin PG-PEVs	* 77 ± 12	0.38	§ −36 ± 7	° 82 ± 7
CQ PG-PEVs	§ 172 ± 12	0.25	*,§ −46 ± 6	§,# 104 ± 1

Table 3
Antioxidant activity (AA) and Trolox Equivalents (TE) of the extract and/or quercetin in dispersion or loaded in vesicles. Mean values ± standard deviations are reported (n = 6). For each group (AA, and TE) the same symbol (*,§, °,#) indicates values that are not statistically different ($p > 0.05$ among values with same symbol and $p < 0.05$ *versus* values with different symbol).

	AA (%)	TE (mg/mL)
Cannonau dispersion	70 ± 0.5	1.41 ± 0.01
Quercetin dispersion	*,§ 76 ± 1	*,§ 1.54 ± 0.01
CQ dispersion	*,§ 76 ± 2	*,§ 1.54 ± 0.04
Cannonau PG-PEVs	°80 ± 1	# 1.62 ± 0.01
Quercetin PG-PEVs	* 77 ± 1	*, ° 1.57 ± 0.03
CQ PG-PEVs	* 76 ± 0.2	*,§ 1.55 ± 0.004

symptomatic relief, their prolonged use often results in significant adverse effects and diminished patient compliance [3,4]. For this reason, patient and researcher attention has been catalysed into growing interest in alternative, safer treatments that harness natural chemicals for their therapeutic properties [5–7].

Thanks to their strong antioxidant and anti-inflammatory activities, polyphenols, a class of plant-derived secondary metabolites, have gained considerable attention [5,8–10]. These compounds, found abundantly in several plants and fruits, are capable of neutralizing oxidative stress, modulating inflammatory pathways, and promoting cellular protection [11,12]. Among polyphenols, those derived from grape pomace, a side-stream of the winemaking process, are particularly promising. Grape pomace is rich in anthocyanins, flavan-3-ols, and other phenolic compounds, making it a promising candidate for valorisation into high-value cosmeceutical and nutraceutical applications [13]. Cannonau wine, a traditional red wine from Sardinia, is known for its high polyphenol content, and the use of its grape pomace produced during the vine-making process may provide a sustainable and effective resource for the development of health-promoting products [14–16]. To the best of our knowledge, Cannonau pomace has not been previously investigated in topical nanocarrier systems for psoriasis, and its distinctive polyphenolic profile may offer therapeutic advantages. Additionally, quercetin is one of the most abundant flavonoids found in citrus, green leafy vegetables as well as olive oil, red grapes, red wine and berries [17]. Due to its ability to inhibit the dysregulated inflammatory pathways, quercetin exhibited a variety of beneficial properties, such as anti-cancer, anti-inflammatory, and antioxidant, being effective against several chronic diseases (e.g., arthritis, dermatitis, hepatitis, psoriasis) [18]. Chen et al. demonstrated that the pre-treatment with different doses of quercetin (30, 60 and 120 mg/kg) was able to exert beneficial effects in imiquimod-induced psoriasis-like mice model, probably improving antioxidant and anti-inflammatory status and inhibiting the activation of the non-canonical NF-κB signalling [19].

Despite their remarkable bioactivities, the clinical application of phenolic compounds is often hampered by their low bioavailability, poor chemical stability, and limited ability to penetrate the skin barrier [20]. To overcome these limitations, lipid-based nanocarriers, such as liposomes and liposome-like systems, have been developed. Thanks to the particular composition of their phospholipidic bilayer, liposomes are able to encapsulate both hydrophilic and hydrophobic compounds. Moreover, they offer several advantages, including enhanced stability of encapsulated compounds, improved skin permeability, controlled release, and reduced systemic toxicity, making them the ideal tool for topical applications in dermatology [21,22].

In their work Liu et al. obtained quercetin-loaded liposomes and evaluated the preventive and therapeutic effects against dermal eczema-induced mice. They observed that compared with untreated mice, mice treated with quercetin-loaded liposomes demonstrated a statistically significant reduction in dermatopathological symptoms [23].

In a previous work, Manca et al. obtained an extract rich in polyphenols from Cannonau grape pomace that was incorporated into liposome and liposome-like vesicles including penetration enhancer containing vesicles (PEVs). The obtained vesicles were able to promote the proliferation of keratinocytes and fibroblasts, and, thanks to their antioxidant activity, provided a cytoprotective effect against induced oxidative stress damage [24].

Considering previous studies, in the present work, we explored the therapeutic potential of Cannonau grape pomace extract, enriched with quercetin, to potentially enhance the overall therapeutic effect through complementary or synergistic mechanisms. Quercetin was co-loaded with Cannonau pomace extract to enrich the formulation with a well-characterised flavonoid known for its strong antioxidant and anti-inflammatory effects. Although quercetin is naturally present in the extract, its supplementation was intended to enhance the biochemical profile and potentially provide additive or complementary activity in modulating psoriasis-associated pathways. The natural chemicals were incorporated into vesicular formulations, obtained using Lipoid S75, a natural phospholipid, along with propylene glycol and water to create stable lamellar vesicles, so called PG-PEVs. This nanocarrier design differs from conventional liposomes and from other PEVs employing alternative co-solvents, as propylene glycol confers both penetration-enhancing and rheological properties specifically optimized in this work, representing an advanced vesicular system characterised by enhanced deformability and improved encapsulation efficiency due to the intercalation of propylene glycol within the phospholipid bilayers. This incorporation not only modulates the bilayer fluidity and mechanical properties but also significantly enhances the penetration capability through biological barriers by disrupting lipid organization in the stratum corneum, thereby facilitating transdermal delivery of bioactive compounds [25,26]. The main physico-chemical and technological characteristics (size, zeta potential, polydispersity index, and entrapment efficiency) of the obtained vesicles, along with their stability over time were measured. Moreover, the impact of extract and/or quercetin on vesicles assembly was observed by using small-angle X-ray scattering, together with their influence on the rheological properties of the formulations.

The biological efficacy of these formulations was assessed through a series of *in vitro* and *in vivo* experiments. Human keratinocytes (HaCaT cells) were used to evaluate the biocompatibility and protective effects of the formulations against oxidative stress and hyperproliferation induced by hydrogen peroxide and 12-O-tetradecanoylphorbol 13-acetate (TPA), respectively. Furthermore, the *in vivo* efficacy of the formulations was investigated in a murine model of epidermal hyperplasia, a condition that mimics the key pathological features of psoriasis.

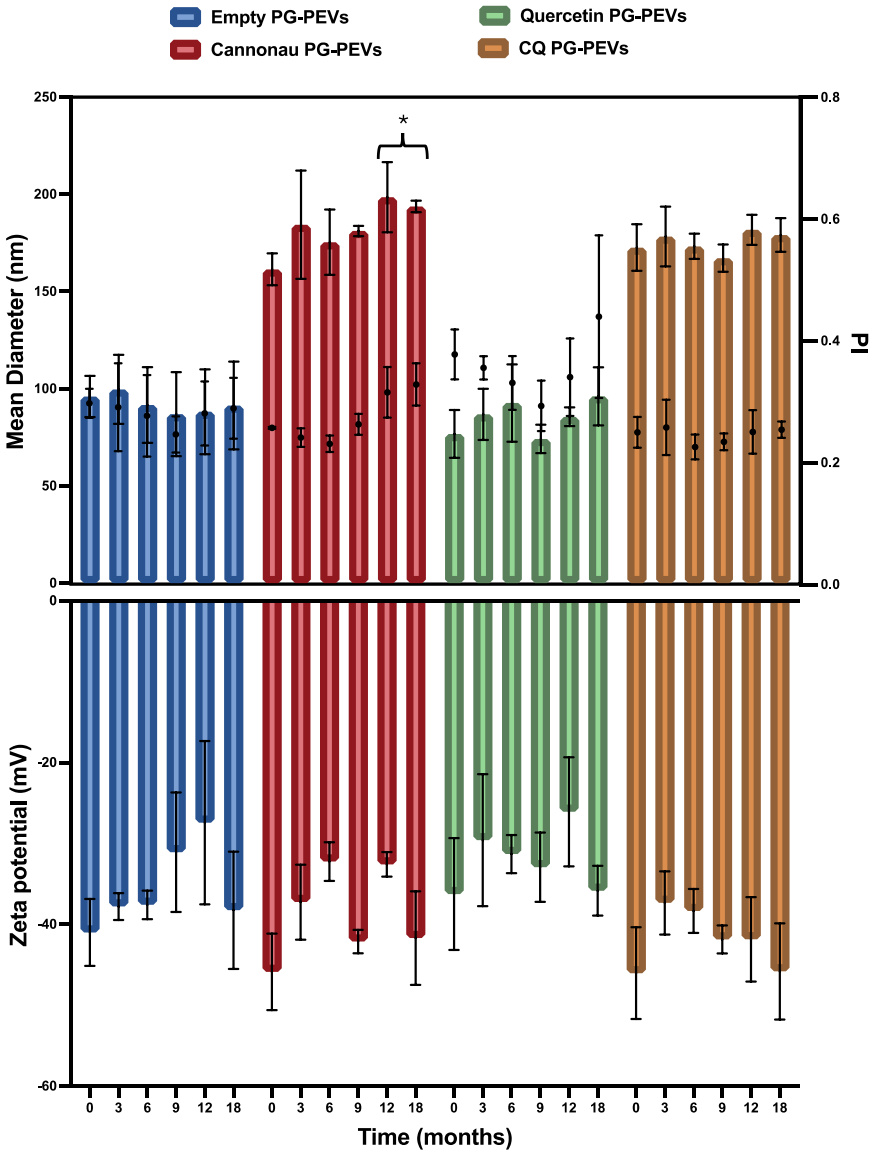

Fig. 1. Mean diameter (nm), polydispersity index (PDI) and zeta potential (mV) of vesicles stored at 4 ± 1 °C for 18 months. Mean values (bars) ± standard deviations (error bars) are reported (n ≥ 3). Symbol * indicates values statistically different from that of the same formulation measured the first day (time 0), ($p < 0.05$).

Histological analysis and biochemical assays were performed to quantify the extent of inflammation and neutrophil infiltration, providing insights into the therapeutic potential of the formulations.

2. Materials and methods

2.1. Materials

Grape pomace from Cannonau red wine were provided by a local winery (Argiolas SPA). Lipoid S75 (S75) was purchased from Lipoid (Ludwigshafen, Germany). Propylene glycol was purchased from Galeno (Potenza, Italy). Quercetin, ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trolox and all other reagents of analytical grade were purchased from Sigma-Aldrich (Milan, Italy). Reagents and plastics for cell culture were purchased from Life Technologies Europe (Monza, Italy).

2.2. Extraction process

Grape pomace from Cannonau red wine were provided by a local winery (Cantine Argiolas) and harvested in southern Sardinia (Italy) in September-October 2020. Samples were dried and stored under vacuum at −20 °C until use. Dried grape pomace have been grinded to obtain a powder with small particles, to increase the surface contact between the raw material and the extraction solvent. The resulting grape pomace powder was kept under vacuum, at room temperature and darkness until further extraction. The extraction was performed using a solid–liquid extraction method, as previously described [27]. Briefly, aliquots of 100 g (L/S = 10) of grinded grape pomace were suspended in 1000 mL of hydroalcoholic solution, ethanol:water (70:30 v/v). The suspension was left under constant stirring, at room temperature (25 °C) and darkness for 48 h. At the end of the extraction process, the extractive dispersion was centrifuged (15 min, 4000 rpm) to remove the coarse fractions. The extract solution was then put into a rotavapor (Rotavapor RII, BÜCHI Labortechnik AG, Flawil, Switzerland) to evaporate and recover all the

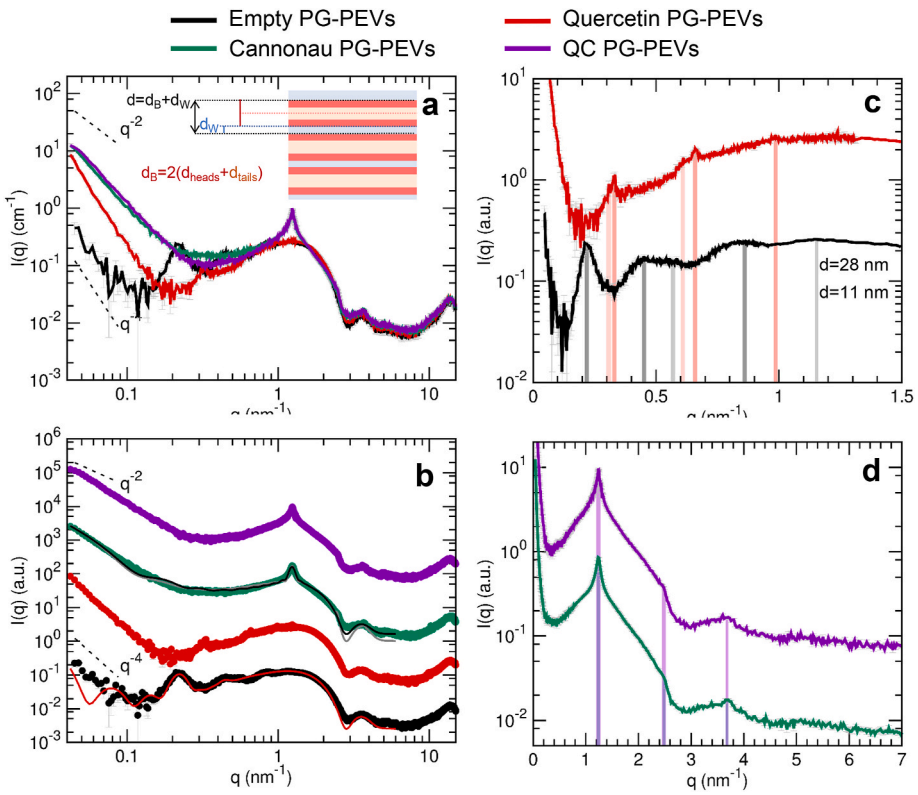

Fig. 2. SAXS profiles of Empty PG-PEVs (black), Cannonau PG-PEVs (green), Quercetin PG-PEVs (red) and CQ PG-PEVs (violet) reported on absolute scale with double logarithmic axes (a), with intensities shifted by an arbitrary factor (b), and on linear q axis to highlight diffraction peaks (c, d). In panel b) the red solid line compared to the Empty PG-PEVs data represents a bilayer model with $d_{heads} = 1.2$, $d_{tails} = 1.08$, $SLD_{heads} = 10.6 \cdot 10^{-4}$ nm^{-2}, $SLD_{tails} = 8.0 \cdot 10^{-4}$ nm^{-2}, $N_{layers} = 4$, d = 27.9 nm, Caille parameter = 0.37; the black solid line compared to the Cannonau PG-PEVs data represents the sum of 60 % unilamellar vesicles and 40 % multilamellar vesicles with $N_{layers} = 10$, d = 5.1 nm, Caille parameter = 0.35. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

ethanol. Then the final aqueous solution of the extract was freeze-dried and stored at −20 °C, under vacuum and darkness until use.

2.3. Vesicles preparation and characterisation

S75 (200 mg/mL), Cannonau grape pomace extract (30 mg/mL) and/or quercetin (5 mg/mL) were weighed in a glass vial and hydrated with 2 mL of a propylene glycol:water (20:80) mixture to obtain Cannonau PG-PEVs, quercetin PG-PEVs and Cannonau and quercetin (CQ PG-PEVs), respectively (Table 1). The dispersions were sonicated (10 + 15 cycles, 5 s on and 2 s off, 13 μm of probe amplitude, allowing cooling between each sonication), using a Soniprep 150 ultrasonic disintegrator (MSE Crowley, London, UK), to obtain homogeneous systems with small particle size. Empty vesicles were also prepared and used as reference. The composition was defined based on a pre-formulation study (data not shown) aimed at identifying the optimal ratio of components to promote efficient vesicle formation, narrow size distribution, high encapsulation efficiency, and good stability, while ensuring biocompatibility and long-term physical integrity during storage. The average diameter, polydispersity index, and zeta potential of empty (control) and natural chemicals loaded vesicles were measured by means of dynamic light scattering technique, using a Zetasizer Ultra (Malvern Instruments, Worcestershire, UK).

The entrapment efficiency (E%) of Cannonau pomace extract, quercetin, and their combination in PG-PEVs was determined using an indirect method based on the measurement of total antioxidant capacity. Briefly, the suspensions were purified from the unloaded phytochemicals, dialysing the samples (1 mL) (Spectra/Por® membranes: 12–14 kDa MW cut-off, 3 nm pore size; Spectrum Laboratories Inc., DG Breda, Netherlands) against water (1 L) for 2 h, refreshing the water each hour. Samples were collected before and after dialysis, and their antioxidant activity was assessed using the DPPH assay (see paragraph 2.4) and the entrapment efficiency was calculated according to the following equation:

$$Entrapment\ Efficiency\ \% = \left(\frac{TE_{dialysed}}{TE_{non-dialysed}}\right) \times 100$$

where, $TE_{dialysed}$ indicates the Trolox Equivalents of the sample before dialysis, while $TE_{non\text{-}dialysed}$ indicates the Trolox Equivalents of the sample after dialysis. All the experiments were performed in triplicate.

This method has been previously validated and provides a global estimation of the bioactive fraction retained in the vesicles, which is particularly appropriate for complex phytocomplexes containing multiple polyphenolic constituents with overlapping antioxidant activities, where single-compound quantification would underestimate the true extent of encapsulation [7,28].

The stability of the vesicles was preliminarily assessed for 18 months under refrigerated conditions (4 ± 1 °C, dark) to provide an initial indication prior to full ICH Q1A(R2)-compliant studies, including accelerated and intermediate storage.

2.4. DPPH assay

The ability of the formulations to scavenge free radicals was measured according to the 2,2-diphenyl-1-picrylhydrazyl colorimetric assay. Briefly, 10 μL of Cannonau pomace extract and/or quercetin in dispersion or loaded in vesicles were added to 990 μL of 2,2-diphenyl-1-picrylhydrazyl methanolic solution (40 μg/mL). After 30 min of incubation in darkness at room temperature, the absorbance was read

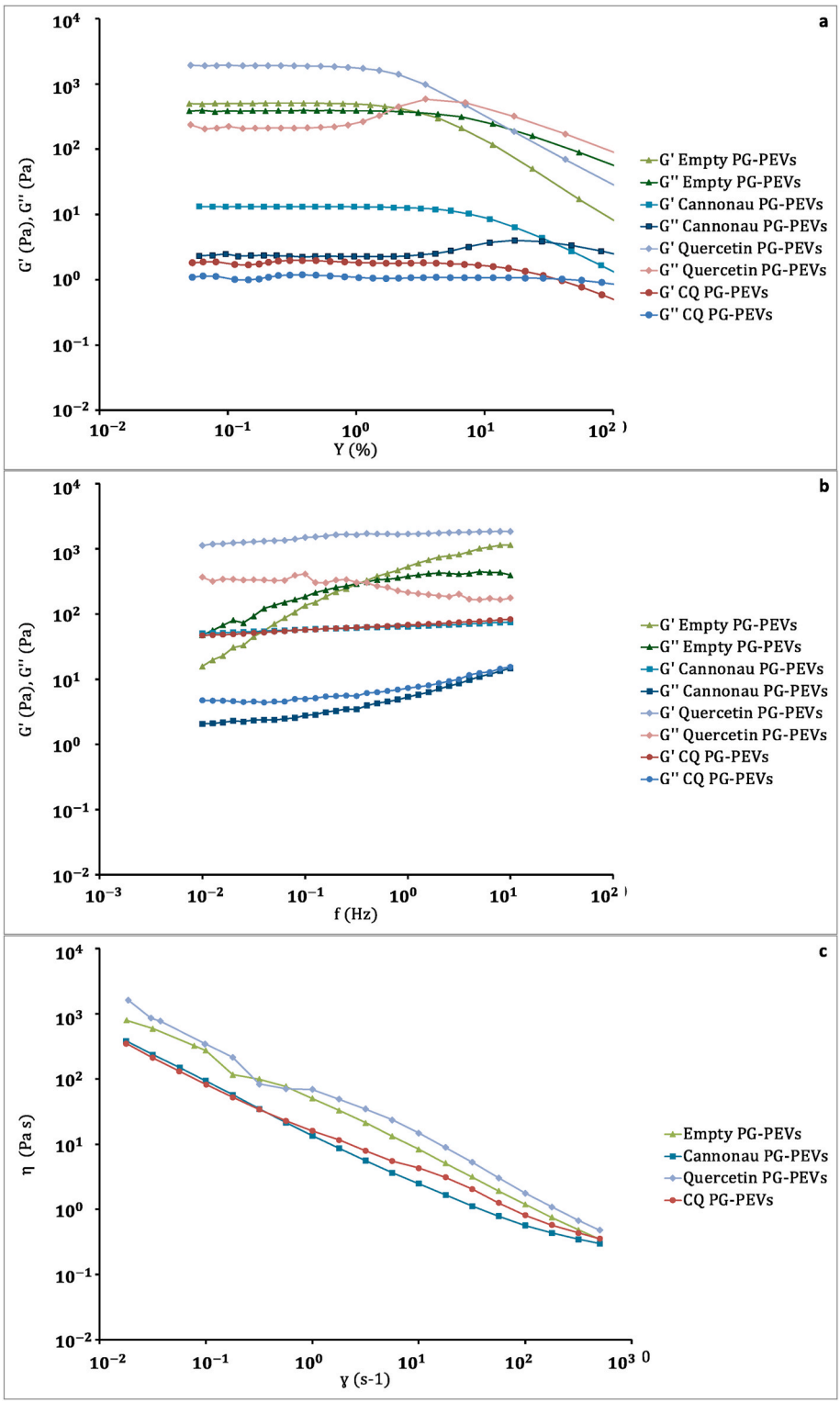

Fig. 3. Rheological characterisation of the vesicular formulations. (a) amplitude sweep tests, (b) frequency sweep curves, and (c) flow curves for empty PG-PEVs, Cannonau PG-PEVs, quercetin PG-PEVs and CQ PG-PEVs.

against a blank at 517 nm. The percentage of antioxidant activity (AA) was calculated according to the following equation:

$$Antioxiodant\ Activity\ \% = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100$$

where, A_{blank} indicates the absorbance of the control (DPPH solution), while A_{sample} is the absorbance of the formulation.

To quantify the antioxidant activity, a calibration curve was built using Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (0–2.0 mg/mL) as reference and expressed as mg/mL of Trolox Equivalents [29].

To account for the intrinsic colour of the Cannonau grape pomace extract, appropriate blank controls containing the extract without assay reagents were included in all colorimetric assays. No significant

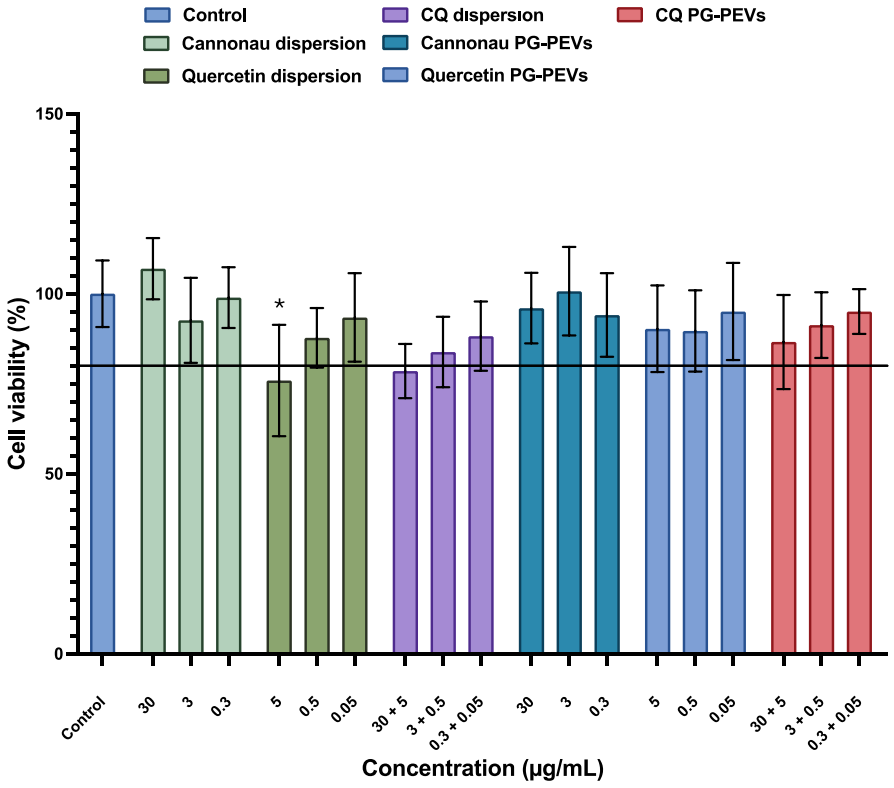

Fig. 4. Viability of HaCaT cells treated with the extract and/or quercetin in dispersion or loaded in vesicles. Mean values (bars) ± standard deviations are reported. Symbol * indicate values statistically different from the control, ($p < 0.05$). The horizontal bar on the graph indicates 80 % cell viability. Formulations resulting in viability ≤80 % were considered slightly toxic [38]. All tested formulations were highly biocompatible, as the viability of cells treated with the extract and/or quercetin in dispersion or encapsulated in PG-PEVs was ≥80 %. The only exception was represented by the highest concentration of the dispersion of quercetin alone (5 μg/mL) or combined with the extract (30 + 5 μg/mL), where keratinocyte viability is below 80 % ($p < 0.05$ *versus* control) suggesting the positive influence of vesicles in modulating the toxicity of the natural chemicals.

interference from the extract colour was observed under the tested conditions, ensuring the accuracy of the measurements. All the experiments were performed in triplicate.

2.5. Small-angle X-ray scattering (SAXs) analysis of formulations

SAXS measurements of empty and extract and/or quercetin-loaded PG-PEVs were performed at SAXSLab Sapienza with a Xeuss 2.0 Q-Xoom system (Xenocs SAS, Grenoble, France), equipped with a micro-focus Genix 3D X-ray source with Cu anode ($\lambda = 0.1542$ nm) and a two-dimensional Pilatus3 R 300K detector, which can be placed at a variable distance from the sample (Dectris Ltd., Baden, Switzerland). The beam size through the two-pinhole collimation system equipped with "scatterles" slits was 0.5 mm × 0.5 mm. Calibration of the scattering vector q range ($q = 4\pi\sin\theta/\lambda$ with 2θ the scattering angle and λ the photon wavelength), was performed using silver behenate. Measurements were performed with three sample-to-detector distances, resulting in an overall studied q region of 0.04 nm–1 < q < 17 nm-1. The samples were put into vacuum-tight quartz capillary cells and measured in the sample chamber at decreased pressure (~0.2 mbar) in a thermalized holder (25 °C). The two-dimensional scattering patterns collected over a 5- or 6-h period were subtracted for "dark" counts before being masked, azimuthally averaged, and normalized for transmitted beam intensity, exposure time, and subtended solid angle per pixel using SOLEIL's FOXTROT software. The one-dimensional intensity vs. q profiles was then subtracted for the data of the empty capillary and of the solvent measured in the same cell and divided by the capillary thickness calibrated using water scattering to obtain intensity in absolute scale units (cm^{-1}). The three angular ranges were merged using the SAXSUtilities tool [30]. The theoretical scattering profiles of stacked bilayers with head-tail-tail-head sharp electron density profiles and Caille structure factor were calculated with SasView 6.0 using the lamellar hg stack caille model and compared to the experimental data. The model involves the following parameters: d_{heads}, SLD_{heads} (thickness and scattering length density of the hydrophilic layer in contact with the water background), d_{tails}, SLD_{tails} (half thickness and scattering length density of the hydrophobic layer composed by lipid tails), N_{layers}, (number of bilayers correlated by the multilamellar structure factor), d (stacking distance between two repeating bilayers in the multilamellar structure), Caille parameter (related to bending fluctuations).

2.6. Rheology of formulations

Rheological measurements were carried out at 25 ± 1 °C, using a Kinexus rotational rheometer (Malvern Instruments, Worcestershire, United Kingdom) equipped with data acquisition and elaboration software rSpace; a cone-plate geometry (CP1/60) was used.

The linear viscoelastic region (LVR) was determined according to a dynamic amplitude sweep study, with shear stress increasing from 0.001 to 100 Pa at a constant frequency of 1 Hz. Oscillation frequency sweep tests were performed over a frequency range of 0.001–10.0 Hz, with a constant amplitude of 1 % strain to remain within the LVR. The viscous modulus (G′), and elastic modulus (G″) were measured. Flow sweep tests were performed with the shear rate ranging from 0.0001 s to 1–500 s−1, and viscosity flow curves were obtained as a function of shear rate.

2.7. Biocompatibility of formulations against keratinocytes

Human keratinocytes (HaCaT) were grown as monolayer in 75 cm^2 flasks, incubated at 37 °C with 100 % humidity and 5 % of carbon

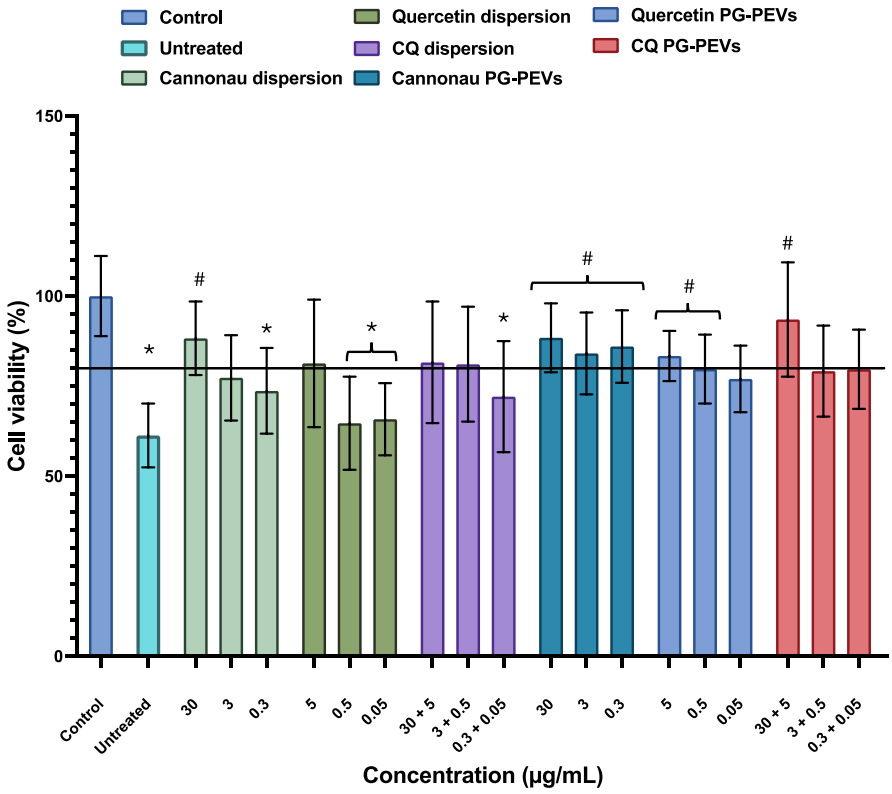

Fig. 5. Viability of HaCaT cells stressed for 4 h with hydrogen peroxide and untreated or treated with the extract and/or quercetin in dispersion or loaded in vesicles. Mean values ± standard deviations (error bars) are reported. Symbol (*) indicates values statistically different from the control ($p < 0.05$). Symbol (#) indicates values statistically different from cells stressed with hydrogen peroxide and untreated ($p < 0.05$). The horizontal bar on the graph indicates 80 % cell viability.

dioxide. Dulbecco's Modified Eagle Medium (DMEM) with high glucose, containing L-glutamine, supplemented with 10 % foetal bovine serum, 1 % penicillin/streptomycin and 0.1 % fungizone was used as growth medium. The cells were seeded into 96-well plates at a density of 5×10^4 cells/well. After 24 h of incubation, cells were treated for 48 h with Cannonau pomace extract in propylene glycol and water mixture or loaded in vesicles. Samples were diluted with DMEM to reach different concentrations of Cannonau pomace extract and quercetin (30, 3, and 0.3 μg/mL and 5, 0.5, and 0.05 μg/mL respectively). The tested concentrations were selected based on preliminary cytotoxicity screening on HaCaT cells (data not shown) and on literature values reported for similar polyphenol-based vesicular formulations [28]. After 48 h MTT [3 (4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide] (100 μL, 0.5 mg/mL final concentration) was added to each well and the cells were incubated again for 3 h. The formed formazan crystals were dissolved with dimethyl sulfoxide (100 μL/well), and the absorbance was measured at 570 nm using a microplate reader (Synergy 4 Reader, BioTek Instruments, AHSI S.p.A, Bernareggio, Italy).

To account for the intrinsic colour of the Cannonau grape pomace extract, appropriate blank controls containing the extract without assay reagents were included in all colorimetric assays. No significant interference from the extract colour was observed under the tested conditions, ensuring the accuracy of the measurements.

2.8. Evaluation of the protective effect of formulations against cell damage caused by oxidative stress

The *in vitro* protective effect of the extract-loaded vesicles against cell damages caused by oxidative stress induced using hydrogen peroxide was evaluated. The cells were seeded into 96-well plates at a density of 5×10^4 cells/well. After 24 h of incubation, cells were treated with hydrogen peroxide (1:50000 final dilution) and simultaneously with the samples properly diluted as above (see paragraph 2.7). The cells stressed with hydrogen peroxide only, were used as negative control, while untreated cells (100 % of viability) were used as positive control. After 4 h of incubation, the cells were washed with PBS, and their viability was determined by the MTT assay as above (see paragraph 2.7).

2.9. Evaluation of the ability of formulations to counteract cell proliferation caused by 12-O-tetradecanoylphorbol 13-acetate

The ability of the extract-loaded vesicles against cell proliferation induced using 12-O-tetradecanoylphorbol 13-acetate (TPA) was evaluated. 12-O-tetradecanoylphorbol 13-acetate is a carcinogenic agent able to stimulate cell proliferation [31]. Cells were seeded into 96-well plates at a density of 5×10^4 cells/well. After 24 h of incubation, cells were treated with 12-O-tetradecanoylphorbol 13-acetate (1 μg/mL) and simultaneously with the samples properly diluted with DMEM to reach different concentrations of Cannonau pomace extract and quercetin (30 and 3 μg/mL and 5 and 0.5 μg/mL respectively). The cells stressed with 12-O-tetradecanoylphorbol 13-acetate only, were used as negative control, while untreated cells (100 % of viability) were used as positive control. After 1 h of incubation, the cells were washed with PBS, and their viability was determined by the MTT assay as described above (see paragraph 2.7).

2.10. Evaluation of the protective effect of vesicles against skin damage in vivo

CD-1 female mice, 5–6 weeks old and 25–35 g of weight were supplied by Envigo laboratories (Barcelona, Spain). Before performing the experiments, mice were acclimatized for ~ 7 days. All the experiments were carried out according to the European regulations concerning the handling and use of experimental animals, and the protocols were

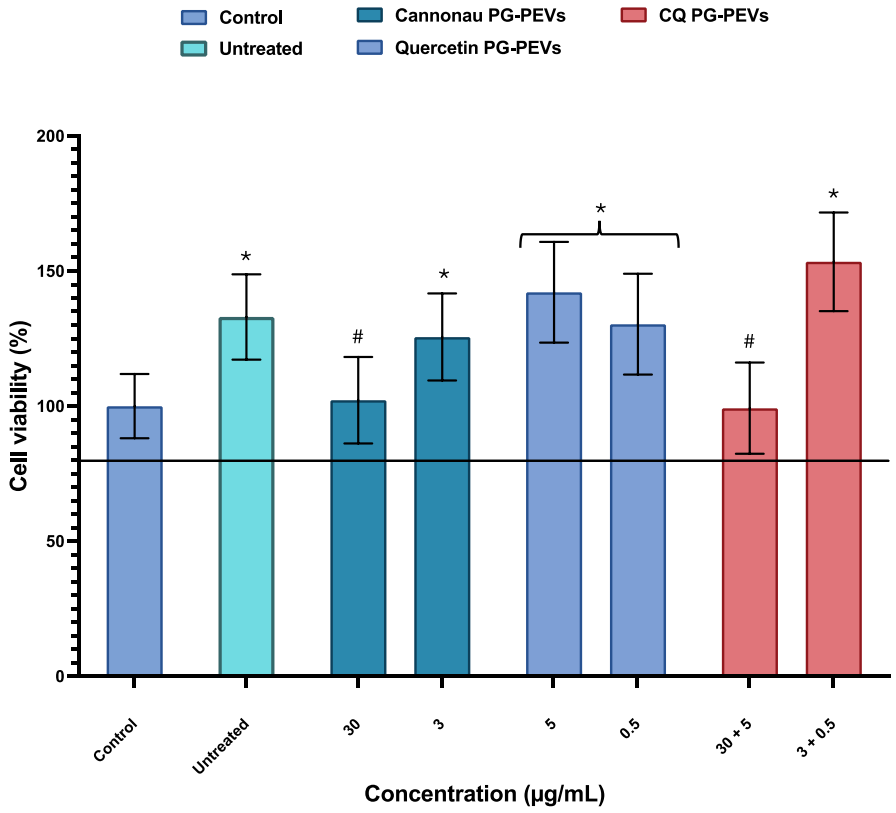

Fig. 6. Viability of HaCaT cells stressed for 1 h with by 12-O-tetradecanoylphorbol 13-acetate and untreated or treated with the extract and/or quercetin loaded in vesicles. Mean values ± standard deviations (error bars) are reported. Symbol (*) indicate values statistically different from the control ($p < 0.05$). Symbol (#) indicates values statistically different from cells stressed with by 12-O-tetradecanoylphorbol 13-acetate and untreated ($p < 0.05$).

approved by the Institutional Animal Care and Use Committee of the University of Valencia (2021/VSC/PEA/0178). CD-1 female mice (n = 4) were divided in different groups: untreated animals (positive control), animals treated with 12-O-tetradecanoylphorbol 13-acetate and saline solution (negative control), and animals treated simultaneously with both 12-O-tetradecanoylphorbol 13-acetate and the natural chemicals in dispersion or loaded into vesicles. One day before the experiments, the back skin of mice was shaved, obtaining a shaved area of ~ 2 cm^2. During the first day, skin damages were induced by applying 20 µL of 12-O-tetradecanoylphorbol 13-acetate dissolved in acetone (243 µM) on the shaved area. After 3 h, samples (200 µL) were gently applied over the TPA-treated area allowing the complete absorption [1,32]. The procedure was repeated for additional 2 days. At the end of experiment, mice were sacrificed by cervical dislocation, and samples of the treated skin area were excised and immediately stored at −80 °C.

To determine the neutrophil infiltration into the skin, myeloperoxidase (MPO) activity was measured as previously described [1]. Briefly, skin samples were homogenized and centrifuged, the supernatant was diluted 1:10 with sodium phosphate buffer (pH 5.4). Then, 10 µL of the diluted supernatant was incubated with 20 µL of sodium phosphate buffer (pH 5.4), 200 µL of phosphate buffer (pH 7.4), 40 µL of 0.052 % hydrogen peroxide and 20 µL of 3,30,5,50-tetramethylbenzidine dihydrochloride (18 mM). To stop the reaction, 50 µL of H_2SO_4 (2 N) was added. MPO activity, expressed as ng/mL, was determined from the linear portion of a standard curve by reading the absorbance at 450 nm.

2.11. Histological examination

Skin biopsies (see paragraph 2.10) were excised from the treated mice dorsal region, after 72 h of treatment (on day 4) and maintained in formaldehyde (10 % v/v) for microscopic studies. Tissue samples were processed routinely and embedded in paraffin wax. Longitudinal sections (5 µm) were stained with haematoxylin and eosin. Microscopic assessment by light microscope (DMD 108 Digital Micro-Imaging Device, Leica, Wetzlar, Germany) was performed blind on coded slices.

Quantitative analysis was performed using Fiji/ImageJ software. Epidermal thickness was measured in five randomly selected fields per section by drawing perpendicular lines from the basal membrane to the outer edge of the stratum corneum, and the mean ± standard deviation (SD) was calculated for each group. Inflammatory cell infiltration was evaluated semi-quantitatively according to a previously established scoring system: 0 = absent, 1 = mild, 2 = moderate, 3 = severe. Two blinded observers performed the scoring independently, and discrepancies were resolved by consensus.

2.12. Data analysis and statistics

Statistical analyses were carried out with Prism software (GraphPad, USA), and data were analysed by one-way (factor: treatment) analysis of variance (ANOVA) followed by either Tukey's or T-Student post-hoc test, when applicable. Results are expressed as mean ± SD and were considered statistically significant if $p < 0.05$.

3. Results

3.1. Extraction and characterisation of Cannonau pomace extract

The Cannonau grape pomace extract used for this study was the same obtained in a previous work [27]. Anthocyanins and Flavan 3-ols were the most abundant phenolic compounds, 6.12 mg/g of extract and 11.16 mg/g of extract respectively, with Malvidin-3-O-(p-coumaroyl) glucoside and (+)-Catechin being the most representative ones, 2.26

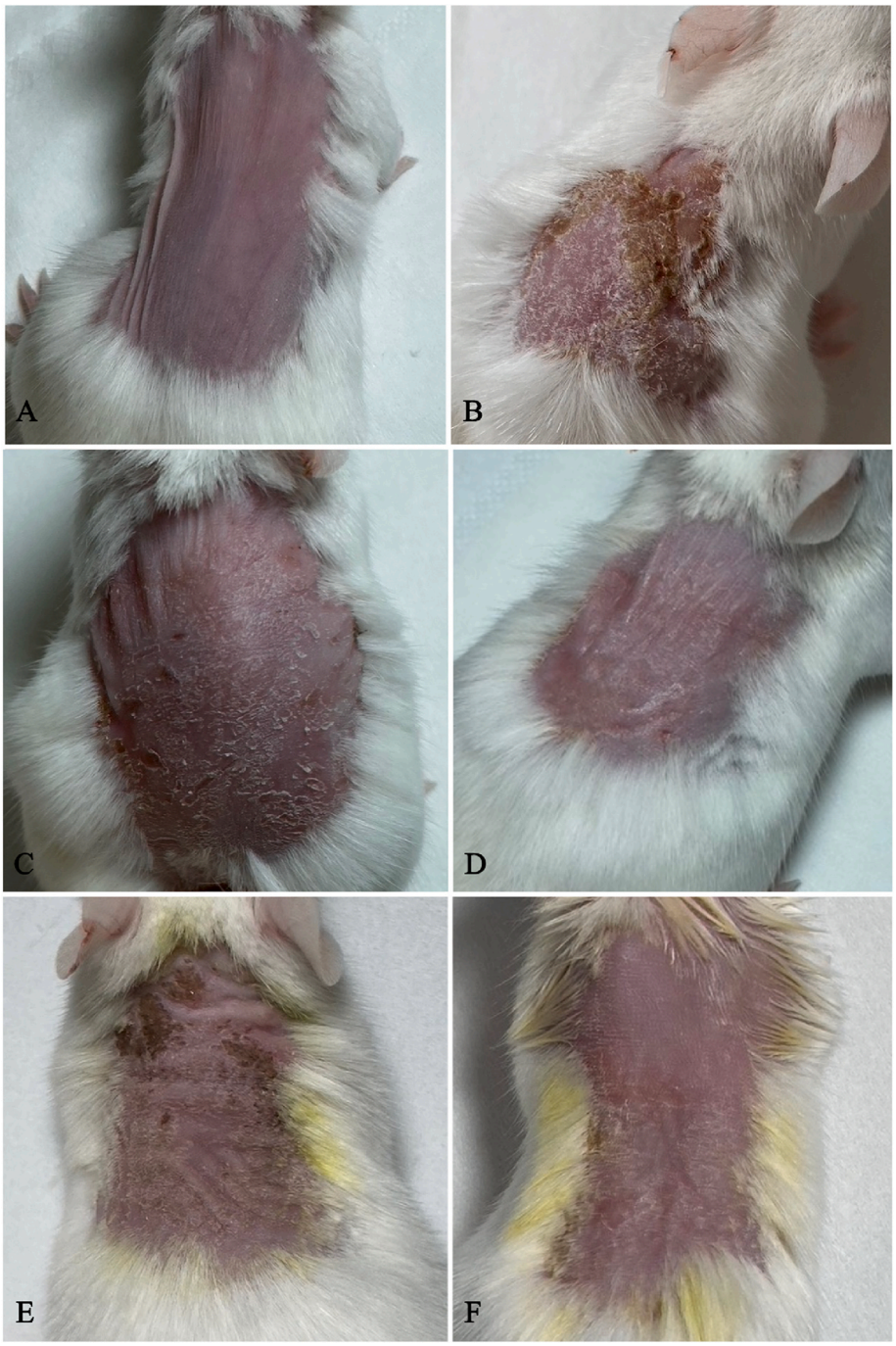

Fig. 7. Representative figure of healthy skin (A), skin damaged with 12-O-tetradecanoylphorbol 13-acetate and treated with: saline solution (B), extract in dispersion (C), or loaded in PG-PEVs (D), extract and quercetin in dispersion (E) or co-loaded in PG-PEVs (F).

mg/g of extract and 2.98 mg/g of extract, respectively. The Total Phenolic Content of Cannonau pomace extract, determined performing the Folin-Ciocalteu colorimetric assay, was 185.1 ± 10.4 mg of gallic acid equivalents (GAE)/g of dry extract [27].

3.2. Preparation and characterisation of vesicles

Cannonau grape pomace extract and quercetin were incorporated into PG-PEVs prepared with 200 mg/mL phospholipid (S75), 30 mg/mL extract, and/or 5 mg/mL quercetin and with 20 % propylene glycol (PG) in the hydration medium. Additionally, empty vesicles were prepared as control to assess the effect of the loading of the extract and/or quercetin on the phospholipid bilayer assembling (Table 1). The preparation method yielded homogeneous dispersions of PG-PEVs, confirming the spontaneous self-assembly of phospholipids into vesicles upon hydration in the presence of propylene glycol. The average diameter, polydispersity index, zeta potential, and encapsulation efficiency of the vesicles were measured and compared (Table 2).

The incorporation of Cannonau grape pomace extract induced a significant increase in vesicles size, regardless of the presence of quercetin, from ~96 nm for empty PG-PEVs to ~161, and ~172 nm for Cannonau PG-PEVs and CQ PG-PEVs, respectively ($p < 0.05$ *versus* empty PG-PEVs). In contrast, the loading of quercetin alone did not affect the average diameter of vesicles, ~77 nm ($p > 0.05$ *versus* empty PG-PEVs). The polydispersity index was almost the same across all formulations (~0.27). The only exception were Quercetin PG-PEVs, for

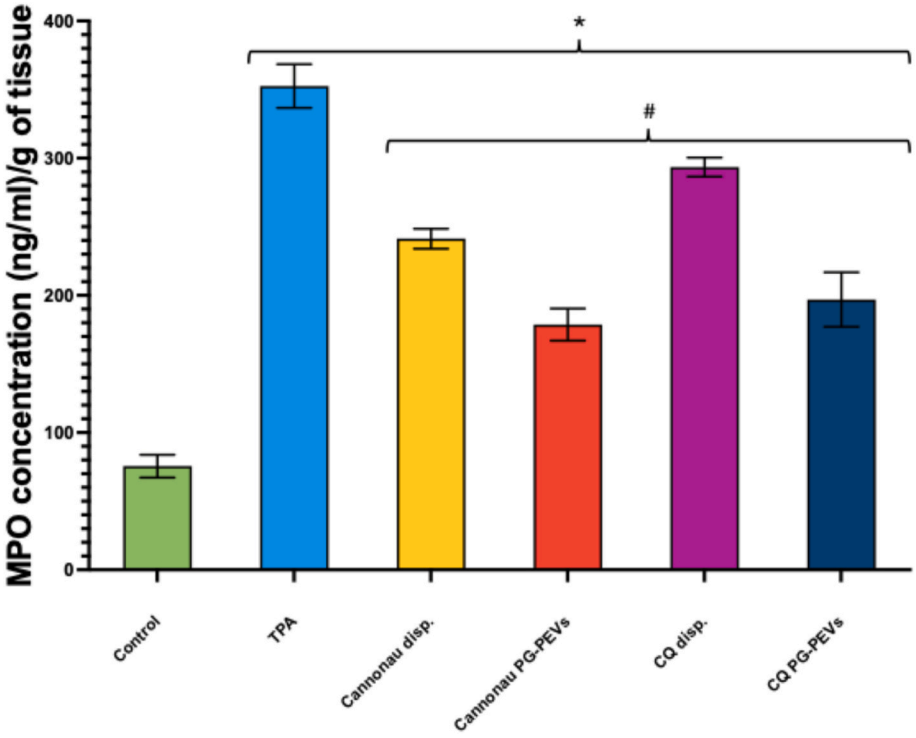

Fig. 8. Myeloperoxidase (MPO) concentration in mice untreated (control), exposed to 12-O-tetradecanoylphorbol 13-acetate and treated with saline (TPA) or treated with the extract in dispersion (Cannonau disp.), or loaded in PG-PEVs (Cannonau PG-PEVs), or with the extract and quercetin in dispersion (CQ disp.), or loaded in PG-PEVs (CQ PG-PEVs). Mean values ± standard deviations are reported. Symbol * indicates values statistically different from control ($p < 0.05$). Symbol # indicates values statistically different from 12-O-tetradecanoylphorbol 13-acetate ($p < 0.05$).

which the polydispersity index was ~0.38, indicating a less homogeneous system compared to other vesicular formulations, probably due to the presence of a small amount of non-entrapped drug (~18 %) in the dispersions, as previously reported by Manca et al. [33]. All formulations exhibited a negative surface charge, approximately −45 mV, and were capable of incorporating high amounts of natural chemicals. The entrapment efficiency was ~100 % for all vesicles, except for Quercetin PG-PEVs, where it was slightly lower, (~82 %, Table 2) confirming the presence of free quercetin in the intervesicle medium responsible of the reduced homogeneity of the system. Additionally, macroscopically, all PG-PEVs formulations appeared translucent and slightly opalescent with a fluid-like consistency, indicating a homogeneous dispersion suitable for topical application.

3.3. Evaluation of the antioxidant activity

The antioxidant activity of the extract and/or quercetin in dispersion or loaded in vesicles was measured evaluating their free radical scavenging activity by means of the DPPH colorimetric test (Table 3).

The extract in dispersion exhibited a high antioxidant activity (~70 %). Quercetin in dispersion, alone or in association with the extract, had a slightly higher antioxidant activity, ~76 % suggesting a slight synergic effect due to the combination of the natural chemicals ($p < 0.05$ against Cannonau dispersion). The incorporation of the extract and quercetin into vesicular systems did not alter their antioxidant activity, while the incorporation of the extract alone into PG-PEVs determined an increase in its antioxidant activity up to ~80 % ($p < 0.05$ against Cannonau dispersion) probably due to the combination of the phytocomplex and phospholipids, as previously reported [34]. This approach, previously applied successfully in our earlier work on polyphenolic phytocomplexes, provides a reliable estimate of the proportion of antioxidant compounds retained within the vesicles. Slight variations above 100 % may result from experimental fluctuations and from the intrinsic contribution of phospholipids to the DPPH response [34]. Overall results confirm that incorporating the extract and/or quercetin into vesicles does not affect the antioxidant properties of the natural chemicals.

3.4. Stability of the formulations on storage

Stability of vesicles over time was evaluated storing them at 4 ± 1 °C in the dark for 18 months and measuring at scheduled times their size, polydispersity index and zeta potential (Fig. 1).

The average diameter of empty PG-PEVs remained relatively constant over time, with slight variations ($p > 0.05$ *versus* time 0). The average diameter of Cannonau PG-PEVs did not increase significantly during the first 6 months ($p > 0.05$ *versus* time 0), at 12 months it slightly increased to approximately 200 nm ($p < 0.05$ *versus* time zero) remaining relatively stable for the remaining time of storage. Quercetin PG-PEVs had a smaller average diameter, below 100 nm, with minimal changes over time ($p > 0.05$ *versus* time 0). Similarly, the mean diameter of CQ PG-PEVs remained relatively stable within 9 months, after that time the diameter slightly increased at 12 and 18 months of storage ($p < 0.05$ *versus* time 0).

The polydispersity index ranged between 0.1 and 0.6. Empty vesicles and PG-PEVs co-loading extract and quercetin, exhibited greater stability throughout the entire period. The polydispersity index of Cannonau PG-PEVs remained relatively stable up to 9 months, with a slight increase observed from the twelfth month onward, likely due to aggregation phenomena, which also contributed to the observed increase in vesicle size, ultimately affecting the homogeneity of the system. Quercetin PG-PEVs were characterised by an increasing polydispersity index over time, suggesting a decrease in particle homogeneity.

Finally, the zeta potential of all vesicular formulations was highly negative, between −30 mV and −40 mV, and remained nearly constant over the entire period, suggesting high electrostatic stability of all the obtained vesicles.

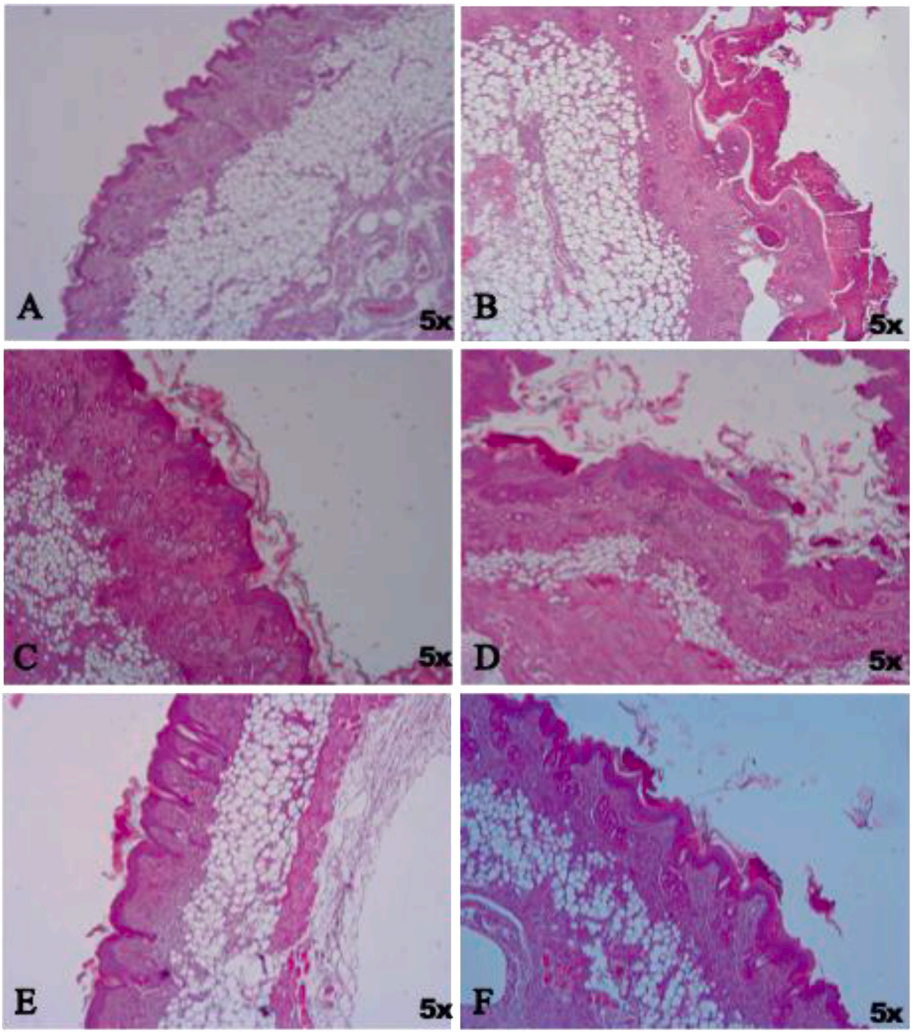

Fig. 9. Representative images of the histological observation of healthy skin (A), skin damaged with 12-O-tetradecanoylphorbol 13-acetate and treated with: saline solution (B), extract in dispersion (C), or loaded in PG-PEVs (D), extract and quercetin in dispersion (E) or co-loaded in PG-PEVs (F). In TPA-damaged skin treated with saline solution (Fig. 9B), multiple pustules were observed across the epidermis, along with significant exocytosis. Additionally, signs of epidermal necrosis and moderate panniculitis were present. Hyperkeratosis and severe inflammatory infiltrates in both the superficial and deep dermis were also noted. Consistent with the myeloperoxidase activity results, inflammatory infiltrates, moderate panniculitis, mild to moderate myositis, and absence of exocytosis were observed in the skin treated with Cannonau pomace extract containing PG-PEVs. While, pustules, hyperkeratosis, inflammatory infiltrates, moderate panniculitis in the deep hypodermis, and absence of myositis were observed in skin treated with extract and quercetin co-loaded in PG-PEVs (Fig. 9D and F). Skin samples treated with the extract in dispersion, alone or in association with quercetin, (Fig. 9C and E) exhibited more severe damage compared to the PG-PEVs-treated groups but less damage than TPA-damaged and untreated skin.

3.5. *Small angle X-ray scattering of formulations*

The nanoscale structure of empty and extract and/or quercetin-loaded PG-PEVs was evaluated by SAXS measurements (Fig. 2).

The SAXS data collected for all the formulations presented the characteristic oscillations related to the lipid bilayer structure, which has an overall thickness of 4.6 nm (Fig. 2a,b). The data collected for empty PG-PEVs presented additional broad peaks in the lowest q range, suggesting packing of the bilayers in ordered domains with characteristic spacings much larger than a single bilayer, implying a rather thick water inter-layer and notable swelling. The first most intense peak observed in the absence of the extract and/or quercetin suggest a lamellar spacing of 28 nm, whereas in the presence of quercetin such peaks appear less pronounced and are shifted to larger q, suggesting a spacing of 19 nm (Fig. 2c). The samples also show an initial slope close to that expected for large particles interfaces (q^{-4}, Porod's law), suggesting that the formulation involves large structural domains above the size of individual vesicles.

On the other hand, extract-loaded PG-PEVs were characterised by an initial slope closer to that expected for locally flat bilayer geometries (q^{-2}). The extract loaded formulations, on top of the oscillatory form factor characteristic of the bilayer thickness, show a sharp diffraction peak corresponding to a lamellar spacing of only 5.1 nm, suggesting the presence of a population of vesicles with a notable degree of multi-lamellarity of the walls. These observations suggest that the grape pomace extract mainly contains hydrophilic and ionizable compounds that do not insert into the hydrophobic portion of the lipid bilayer but strongly affect the inter-bilayer correlations, causing the formulation to change from a highly swollen liquid crystalline phase to collapsed multilamellar vesicles with much lower inter-lamellar hydration.

3.6. *Rheology of formulations*

The amplitude sweep test allows the evaluation of the viscoelastic behaviour of the formulations as a function of the applied strain (Fig. 3A). At lower strains, the formulations exhibited constant elastic

Table 4
Epidermal thickness and inflammatory cell infiltration of healthy skin (A), skin damaged with 12-O-tetradecanoylphorbol 13-acetate and treated with: saline solution (B), Cannonau in dispersion (C), or loaded in PG-PEVs (D), Cannonau and quercetin in dispersion (E) or co-loaded in PG-PEVs (F). Mean values ± standard deviations are reported (n = 5). The same symbol (*,§,+) indicates values that are not statistically different ($p > 0.05$ among values with same symbol and $p < 0.05$ *versus* values with different symbol).

		Epidermal thickness (μm)	Inflammatory cell infiltration
A	Healthy skin	47 ± 13	0.2 ± 0.4
B	TPA-damaged skin	260 ± 65	2.6 ± 0.5
C	Cannonau dispersion	* 141 ± 17	1.8 ± 0.8
D	Cannonau PG-PEVs	* 163 ± 30	2.0 ± 0.7
E	Cannonau and quercetin dispersion	§ 76 ± 13	+0.6 ± 0.5
F	CQ-PG-PEVs	§ 72 ± 19	+0.6 ± 0.5

Epidermal thickness was significantly increased in TPA-damaged skin treated with saline solution compared to healthy control, reflecting the marked hyperplasia induced by the irritant. The treatment with the extract in dispersion, alone or in association with quercetin, determined a significant reduction of thickness if compared to the TPA-damaged group ($p < 0.05$). Moreover, the treatment with extract-loaded PG-PEVs further reduced epidermal thickening, irrespective of the presence of quercetin, approaching values observed in healthy skin.
Similarly, inflammatory cell infiltration was markedly increased in TPA-damaged skin treated with saline solution (2.6 ± 0.5) compared to healthy skin (0.2 ± 0.4). The extract in dispersion and in PG-PEVs (Cannonau PG-PEVs) partially reduced infiltration (1.8 ± 0.8 and 2.0 ± 0.7, respectively). The combination of extract and quercetin, both in dispersion and in PG-PEVs (CQ-PG-PEVs), displayed the strongest anti-inflammatory effect, with values approaching those of healthy skin (0.6 ± 0.5).

(G′) and viscous (G‘‘) moduli, the so called linear viscoelastic region (LVR). Beyond this region, structural breakdown occurs, reflected by a drop in the moduli.

The frequency sweep test highlighted a significant difference between empty and natural chemicals loaded vesicles (Fig. 3B). Empty PG-PEVs curves exhibited G’’ values closer and even exceeding G′, suggesting a more viscous or less structured behaviour. On the contrary, the incorporation of Cannonau pomace extract and/or quercetin led to the formation of a predominantly gel-like behaviour with a higher structural stability, being the storage modulus (G′) values consistently higher than loss modulus (G’’) [35,36].

Moreover, the incorporation of the extract and/or quercetin into PG-PEVs did not alter the shear-thinning behaviour of the system as the viscosity of all samples decreased as the shear rate increased (Fig. 3C). In particular, empty and quercetin-loaded PG-PEVs presented slightly higher initial viscosities, indicating a stronger initial resistance to flow. On the contrary, Cannonau pomace extract-loaded PG-PEVs were characterised by slightly lower initial viscosities and steeper declines, suggesting weaker internal structures, irrespective of the presence of quercetin within the formulation. In agreement with these findings, the formulations also appeared macroscopically as homogeneous, opalescent dispersions with a soft gel-like consistency (Supplementary Fig. 1), confirming their suitability for skin application.

3.7. Evaluation of the biocompatibility of the vesicles

The biocompatibility of the extract and/or quercetin, either in aqueous dispersion or encapsulated in the vesicles, was evaluated using human keratinocytes (HaCaT), as these cells are significantly involved in the pathophysiological processes of psoriasis [1,37]. HaCaT cells were incubated for 48 h with samples at different dilutions, resulting in different concentrations of Cannonau pomace extract and quercetin, 30, 3, and 0.3 μg/mL for the extract and 5, 0.5, and 0.05 μg/mL for quercetin (Fig. 4).

3.8. Evaluation of the protective effect of the formulations against cell damage caused by oxidative stress

To assess the protective effect of the formulations against oxidative stress, keratinocytes were exposed to hydrogen peroxide, and their viability was measured after simultaneous treatment with the formulations at different concentrations (Fig. 5).

Oxidative stress induced by hydrogen peroxide led to a reduction in cell viability to ~61 % ($p < 0.05$ *versus* control). Simultaneous treatment with the highest concentration of the dispersion of Cannonau pomace extract (30 μg/mL) resulted in an increase in cell viability to ~88 % ($p < 0.05$ *versus* stressed and untreated cells, and $p > 0.05$ *versus* control). A similar trend was observed with the quercetin dispersion either alone (5 μg/mL) or combined with Cannonau pomace extract at two different concentrations (30 + 5 μg/mL and 3 + 0.5 μg/mL) as viability was slightly ≥80 %, but there was no significant difference if compared to stressed and untreated cells ($p > 0.05$). Treatment with Cannonau pomace extract containing PG-PEVs also improved cell viability (≥85 %), regardless of concentration tested ($p < 0.05$ *versus* stressed and untreated cells and $p > 0.05$ *versus* control). Similarly, quercetin or its combination with Cannonau pomace extract loaded PEVs at the higher concentration (5 and 30 + 5 μg/mL) improved cell viability ($p < 0.05$ *versus* stressed and untreated cells and $p > 0.05$ *versus* control), and notably, CQ PEVs nearly restored cell viability to ~93 % ($p > 0.05$ *versus* control).

3.9. Evaluation of the ability of formulations to counteract cell proliferation caused by 12-O-tetradecanoylphorbol 13-acetate

To evaluate the ability of the formulations to counteract cell hyperproliferation, keratinocytes were exposed to 12-O-tetradecanoylphorbol 13-acetate, and their viability was measured after simultaneous treatment with the formulations at the two higher concentrations (Fig. 6).

The horizontal bar on the graph indicates 80 % cell viability (Fig. 6). Treatment with 12-O-tetradecanoylphorbol 13-acetate led to an increase in cell viability to ~133 % ($p < 0.05$ *versus* control), thus confirming their ability to stimulate hyperproliferation of HaCaT cells [39]. Simultaneous treatment with the highest concentration of PG-PEVs containing Cannonau pomace extract alone (30 μg/mL) or combined with quercetin (30 + 5 μg/mL) resulted in a reduction of cell proliferation as cell viability was similar to that of control, ~100 % ($p > 0.05$ *versus* control and $p < 0.05$ *versus* stressed and untreated cells).

3.10. Evaluation of the protective effect of vesicles against skin damage in vivo

Given the promising results obtained with previous *in vitro* studies, Cannonau PG-PEVs and CQ PG-PEVs were subsequently tested *in vivo* on a murine skin model of epidermal hyperplasia [1] (Fig. 7).

Skin damaged by 12-O-tetradecanoylphorbol 13-acetate and treated with saline solution (positive control) appeared desquamated and covered with necrotic tissue (Fig. 7B). The treatment of the damaged skin with Cannonau pomace extract alone or in combination with quercetin containing PG-PEVs significantly reduced the damage induced by TPA compared to the same natural chemicals in dispersion (Fig. 7C,D, E and F). In the healthy tissue (not damaged with TPA) the concentration of myeloperoxidase was ~74 (ng/mL)/g of tissue while in tissue damaged with TPA and treated only with saline it was ~353 (ng/mL)/g of tissue (Fig. 8).

Treatment with Cannonau pomace extract and with its combination with quercetin in dispersion, determined a reduction in the expression of myeloperoxidase (~241 and ~294 (ng/mL)/g of tissue, respectively). In fact, observing Fig. 7C and E, a clear improvement can be noted compared to the tissue damaged with TPA (Fig. 7B), with thickening, desquamation and necrosis significantly reduced. Furthermore,

treatment with Cannonau pomace extract containing PG-PEVs significantly reduced the effect of TPA, and the concentration of myeloperoxidase decreased to ~179 (ng/mL)/g, leading to an almost complete healing of the skin, which appeared less inflamed, less thickened and with practically imperceptible areas of necrosis (Fig. 7D). Finally, treatment with extract and quercetin co-loaded in PG-PEVs also reduced the concentration of myeloperoxidase, ~197 (ng/mL)/g of tissue, even if the skin appeared with a slightly higher presence of thickening and desquamation (Fig. 7F).

3.11. Histological examination

The histological examination of skin biopsies (Fig. 9) broadly confirmed the observations obtained from both macroscopic analysis and myeloperoxidase activity assessment.

Quantitative analysis supported these qualitative observations (Table 4).

4. Discussion

This study builds upon previous works demonstrating the antioxidant and anti-inflammatory properties of polyphenolic compounds, emphasizing their enhanced efficacy when encapsulated within lipid-based nanocarriers [8,40,41]. To the best of our knowledge, it is the first study to formulate Cannonau grape pomace extract, characterised by a unique polyphenolic profile, into propylene glycol–containing PEVs, a nanocarrier system specifically optimized for topical delivery in psoriasis. This approach combines a locally sourced and underutilised agricultural side-stream with a tailored vesicular design, setting it apart from previous studies using conventional liposomes or other grape-derived extracts [8,40,41]. The Cannonau pomace extract was formulated in innovative liposome-like vesicles so called PG-PEVs, ad hoc formulated for topical administration with the addition of propylene glycol, a penetration enhancer widely used in topical formulations [25]. During hydration and sonication, the amphiphilic phospholipids spontaneously self-assembled into closed bilayer vesicles, with the hydrophilic constituents of the extract preferentially located in the aqueous core and the lipophilic components intercalated within the lipid bilayer [42,43]. Propylene glycol acts as a co-solvent, enhancing the solubilisation of poorly water-soluble compounds, increasing bilayer flexibility, and improving the skin penetration potential of the final formulation [25,26]. Such structural features are of particular relevance for dermal delivery, as they facilitate vesicle penetration through the stratum corneum and promote the intracellular bioavailability of encapsulated compounds.

The vesicular formulations developed in this study successfully addressed key challenges associated with the clinical application of polyphenolic compounds, such as their low bioavailability, poor chemical stability, and limited skin penetration [44,45]. The incorporation of the extract (30 mg/mL) into PG-PEVs determined a significant increase of the mean diameter of vesicles, ~167 nm irrespective of the presence of quercetin (Table 2; $p < 0.05$ *versus* empty vesicles). This result may suggest the effective intercalation of the natural chemicals contained in the Cannonau pomace extract within the bilayer, especially catechins, the most abundant phenolic group present in the Cannonau pomace extract obtained in this work (11.16 mg/g of dry extract) [25]. In fact, it was previously demonstrated that catechins are able to interact with lipid bilayers and locate on the surface region of it, thus modifying their assembling and improving the curvature radius [16,46,47].

These results are in agreement with those previously found by Manca et al., as the incorporation of high amounts of Cannonau seed extract (40 mg/mL) into liposomes and liposome-like vesicles, specifically tailored for oral delivery, induced a strong increase of the vesicles size from ~100 nm for empty vesicles to ~151 nm, for extract-loaded vesicles, respectively ($p < 0.05$ *versus* empty vesicles) [16]. On the other hand, the addition of quercetin alone (5 mg/mL) did not affect vesicles size, as the mean diameter was ~77 nm (Table 2; $p > 0.05$ *versus* empty vesicles).

The formulations obtained in this study were able to load high amounts of both Cannonau pomace extract and quercetin, or their combination, as the entrapment efficiency was ~102, ~82 and ~104 %, respectively. Similar results were obtained by Gibis et al. following the incorporation of grape seed extract into liposomes and chitosan coated liposomes as entrapment efficiency was ~88 and ~100 %, respectively (Table 2) [47]. Regarding quercetin-loaded PG-PEVs, similar results were obtained by Liu et al., as they also were able to load high amounts of quercetin into liposomes, being the entrapment efficiency ~90 % [48].

The stability evaluation performed in this study, although extended to 18 months, was conducted under refrigerated conditions only, as part of preliminary formulation screening. The main physico-chemical properties of the vesicles remained consistent over an extended storage period of 18 months, with the only exception for quercetin-loaded PG-PEVs, which exhibited greater instability in terms of homogeneity of the system, as the polydispersity index increased over time (Fig. 1).

SAXS analysis (Fig. 2) revealed the multilamellar vesicle architectures, rearranging from a highly swollen and long-range ordered phase to tighter stacking upon extract incorporation, giving a mixed unilamellar-multilamellar system capable of efficient bioactive encapsulation and delivery.

The rheological behaviour is crucial for ensuring proper flow, particularly in the context of pharmaceutical technology. In topical products, it plays an essential role in determining the efficacy of formulations as well as their capacity to deliver sustained action [49]. Several properties can influence the viscosity of the final formulation (e.g., internal spaces between lipid vesicles, vesicles shape, type and concentration of active ingredients and excipients). The present research aimed to understand if and to what extent the incorporation of Cannonau pomace extract and/or quercetin into PG-PEVs could affect the rheological properties of the formulations. Rheological studies (Fig. 3) further support their suitability for topical applications, as they revealed that the incorporation of both Cannonau pomace extract and/or quercetin into PG-PEVs determined the switch of the formulations from the typical rheological behaviour of solution (empty PG-PEVs) to a more gel-like formulations, suggesting a substantial structuring effect probably due to the presence of polyphenols [50]. Moreover, viscosity values plotted against the shear rate clearly indicate a shear-thinning system, a favourable property for effective topical formulations (Fig. 3) [36]. These findings have been also confirmed by SAXS measurement, as the incorporation of Cannonau pomace extract led the formation of a more deformable system due to the change of multilamellarity from long to short radius. This rheological/structural feature is closely connected with spreadability, which is considered one of the most important properties for topical applications [51].

These findings underscore the stability and robustness of the obtained vesicular systems, which are crucial for their practical application in dermatology.

Biological evaluations demonstrated that the liposomal formulations were highly biocompatible, as evidenced by the high viability of keratinocytes treated with the formulations, even at relatively high concentrations of the natural chemicals (Fig. 4). The selected concentrations were supported by preliminary *in vitro* toxicity screening and align with those reported for similar antioxidant-loaded vesicular systems, ensuring both safety and relevance for psoriasis-related models [7,28]. Furthermore, the formulations exhibited significant protective effects against oxidative stress and hyperproliferation, two hallmark features of psoriasis [52,53]. Treatment with Cannonau pomace extract and quercetin co-loaded in PG-PEVs nearly restored keratinocyte viability under oxidative stress (Fig. 5) and normalized cell proliferation under TPA-induced hyperproliferative conditions (Fig. 6). These results are consistent with a previous study in which betamethasone dipropionate, and calcipotriol-loaded solid lipid nanoparticles were able to abrupt

HaCaT cells growth [54].

The *in vivo* experiments provided further validation of the therapeutic potential of these formulations. Treatment with PG-PEVs significantly reduced 12-O-tetradecanoylphorbol 13-acetate-induced epidermal hyperplasia, inflammation, and neutrophil infiltration in a murine model of skin damage (Fig. 7). Notably, the co-loading of Cannonau pomace extract and quercetin into PG-PEVs demonstrated a comparable protective effect to those containing only the extract, albeit with slightly higher residual thickening and desquamation. This suggests that, while the combination of bioactives may offer added value, the extract alone is sufficiently effective when delivered via PG-PEVs. The co-loading of quercetin was intended to complement the intrinsic polyphenolic content of Cannonau pomace extract, enriching the formulation with a well-documented flavonoid that may provide additive or context-specific anti-inflammatory benefits. The lack of significant enhancement with the addition of quercetin may be attributed to the high intrinsic polyphenol content of the Cannonau pomace extract, which already contains quercetin and other phenolic compounds (e.g., Malvidin-3-O-(p-coumaroyl)glucoside and (+)-Catechin), potentially leading to overlapping antioxidant and anti-inflammatory mechanisms. Nevertheless, the strategic inclusion of quercetin enriches the biochemical profile of the formulation, potentially offering complementary mechanisms of action that might be beneficial in more complex or varied pathological contexts. This underscores the importance of designing versatile, multi-component vesicular systems capable of adapting to diverse therapeutic needs rather than relying solely on single-agent efficacy [55]. Additionally, competitive interactions during vesicle encapsulation or cellular uptake may have limited the incremental benefit of quercetin. Similar outcomes have been reported in previous studies, which demonstrated that mixtures of structurally related antioxidants do not always yield synergistic effects and may instead display non-additive or even antagonistic behaviours depending on concentration, solvent environment, molecular ratios, and experimental conditions [56]. Such variability has been extensively documented in binary antioxidant systems. For instance, Olszowy-Tomczyk et al. (2023) demonstrated that binary mixtures of flavonoids (e.g., quercetin with myricetin or kaempferol) often yield antagonistic rather than additive outcomes, largely influenced by mutual relation of components, concentration, and assay method; the non-additive behaviour has been linked to intramolecular hydrogen-bond formation that dampens radical scavenging efficiency [57]. In this context, the high intrinsic polyphenolic load of Cannonau pomace extract, already containing quercetin and catechin, may contribute to biological redundancy, thereby attenuating the apparent advantage of quercetin supplementation. Overall, these insights highlight the importance of considering phytochemical composition, mechanistic overlap, and interaction dynamics when designing vesicular antioxidant formulations, particularly for intracellular delivery and therapeutic applications.

The histological analysis (Fig. 8) confirmed these findings, showing reduced hyperkeratosis, inflammatory infiltrates, and necrosis in skin treated with vesicular formulations compared to dispersions of the natural chemicals. These results agree with those previously found by several authors for the treatment of psoriasis with natural chemicals loaded vesicles (i.e., quercetin, curcumin, thymoquinone) [58–60]. For example, Zhang et al. developed a novel liposome-in-gel formulation, in which the quercetin-loading liposomes were surface-modified with and stabilized by cyclodextrin, for the treatment of imiquimod-induced psoriatic plaque in BALB/c mice. *In vivo* results showed that the loading of quercetin into vesicles improved the treatment efficacy of psoriatic plaque compared to free quercetin. In particular, it alleviated the symptoms of skin thickening and downregulated proinflammatory cytokines, including TNF-α, IL-17A, and IL-1β, suggesting that the obtained formulations could be a stable carrier for topical quercetin therapy with good potential in psoriasis treatment [60].

In addition to their therapeutic potential, through the valorisation of Cannonau grape pomace, a winemaking side-stream, the formulations obtained in this work align with sustainability goals. This approach not only reduces waste but also contributes to the development of eco-friendly cosmeceuticals, meeting the growing consumer demand for natural and responsible products. Combined with the comprehensive physicochemical, *in vitro,* and *in vivo* evaluation, these findings provide a solid foundation for translating such formulations into clinically relevant dermatological therapies and for extending the concept to other agricultural side-streams.

5. Conclusion

This study highlights the potential of Cannonau grape pomace extract, enriched with quercetin, as a sustainable and effective therapeutic approach for managing psoriasis, a chronic inflammatory skin disorder with complex pathophysiology. The valorisation of agri-food side-streams, such as grape pomace, aligns with circular economy principles by transforming a traditionally considered useless into a high-value products. The polyphenol-rich extract was successfully incorporated into PG-PEVs specifically tailored for topical application, exhibiting favourable physico-chemical properties including high encapsulation efficiency, long-term stability, and rheological profiles suitable for skin application.

Both Cannonau pomace extract-loaded PG-PEVs, with or without quercetin, effectively delivered bioactives to the target site and exerted protective effects against oxidative stress, inflammation, and keratinocyte hyperproliferation in *in vitro* and *in vivo* psoriasis models. Notably, the extract alone was sufficient to achieve significant therapeutic benefits, while quercetin co-loading did not provide statistically significant additional effects under the tested conditions. Nevertheless, quercetin may offer supplementary mechanistic advantages, potentially enhancing the formulation versatility and robustness in different pathological contexts.

The gel-like consistency of the formulations supports their practical use as topical treatments, ensuring ease of application, good skin adhesion, and prolonged therapeutic action.

Future research should further explore the molecular mechanisms underlying the bioactivity of these natural compounds and assess potential synergistic effects in more complex or chronic models of skin inflammation. The use of biocompatible excipients and eco-friendly extraction methods supports scalability and regulatory compliance, facilitating translation to clinical use.

Overall, the formulations developed in this work represent a promising and sustainable option for psoriasis management and potentially other inflammatory skin disorders, meeting the growing demand for effective, eco-conscious cosmeceuticals.

CRediT authorship contribution statement

Matteo Perra: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Rita Abi Rached:** Investigation, Formal analysis, Data curation. **Laura Fancello:** Investigation, Formal analysis, Data curation. **Amparo Nacher:** Validation, Supervision, Methodology, Formal analysis. **Octavio Diez-Sales:** Validation, Supervision, Methodology, Formal analysis. **Amparo Ruiz-Sauri:** Validation, Methodology, Formal analysis. **Carmen Duque-Soto:** Investigation, Formal analysis, Data curation. **Jesus Lozano-Sanchez:** Validation, Methodology, Formal analysis. **Carmen Escobedo-Lucea:** Validation, Methodology, Formal analysis. **Pietro Matricardi:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Alessandra Del Giudice:** Writing – review & editing, Validation, Methodology, Formal analysis, Data curation. **Ines Castangia:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Maria Manconi:** Writing – review & editing, Validation, Supervision, Resources, Data curation. **Maria Letizia Manca:** Writing – review & editing, Validation, Supervision,

Resources, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This publication has been produced with the financial assistance of Fondazione di Sardegna 2021, "Valorisation of agri-food side-streams to manufacture sustainable medical devices, nutraceutical and cosmeceutical products, feeds and fertilizers boosting health and quality of life (Waste2Health)" and Italian PNRR Program, specifically the Project "Ecosystem of Innovation for Next Generation Sardinia, Spoke n. 9, Environment protection and valorisation". The Sapienza Research Infrastructure is acknowledged for the SAXS measurements at SAXSLab Sapienza, funded by the Large Equipment Project 2015-C26J15BX54. This work benefited from the use of the SasView application, originally developed under NSF award DMR-0520547. SasView contains code developed with funding from the European Union's Horizon 2020 research and innovation programme under the SINE2020 project, grant agreement No 654000. Carmen Escobedo Lucea (CEL) is funded by the Plan GenT Excellence Program of the Conselleria de Sanidad, with reference CDEI-02/20-A.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jddst.2025.107544.

Data availability

The data that has been used is confidential.

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