cosmetics

MDPI

Article

Pomegranate Peel Extract Microemulsions for Skin Delivery: Insights into Drug Release, Skin Permeation and UV Protection

Nataša Bubić Pajić [1,*], Teodora Trninić [1], Darija Knežević Ratković [1], Vesna Antunović [1], Katarina Šavikin [2], Jelena Živković [2] and Ranko Škrbić [3,4,5]

[1] Department of Pharmacy, Faculty of Medicine, University of Banja Luka, Save Mrkalja 14, 78000 Banja Luka, Bosnia and Herzegovina; teodora.trninic@student.med.unibl.org (T.T.); darija.knezevic.ratkovic@med.unibl.org (D.K.R.); vesna.antunovic@med.unibl.org (V.A.)
[2] Institute for Medicinal Plant Research "Dr Josif Pančić", Tadeuša Košćuška 1, 11000 Belgrade, Serbia; ksavikin@mocbilja.rs (K.Š.); jzivkovic@mocbilja.rs (J.Ž.)
[3] Department of Pharmacology, Toxicology and Clinical Pharmacology, Faculty of Medicine, University of Banja Luka, Save Mrkalja 14, 78000 Banja Luka, Bosnia and Herzegovina; ranko.skrbic@med.unibl.org
[4] Academy of Sciences and Arts of the Republic of Srpska, Bana dr Todora Lazarevića 1, 78000 Banja Luka, Bosnia and Herzegovina
[5] Department of Pathologic Physiology, I.M. Sechenov First Moscow State Medical University, Moscow 119435, Russia
* Correspondence: natasa.bubic.pajic@med.unibl.org

Abstract

Pomegranate peel, an abundant agro-industrial by-product, represents a sustainable source of bioactive polyphenols, particularly punicalagin, which has been associated with antioxidant and photoprotective potential. This study aimed to develop microemulsions (MEs) containing pomegranate peel extract for dermal delivery of punicalagin using biocompatible surfactant systems. Three MEs differing in surfactant–cosurfactant composition (ME-A, ME-P, and ME-E) were prepared. Each formulation solubilized 1% (*w*/*w*) of pomegranate peel extract and was evaluated regarding *in vitro* release behavior, skin permeation/retention, antioxidant activity, and *in vitro* sun protection factor (SPF). All investigated MEs provided sustained release of punicalagin (≈10–17% of the applied dose in 8 h). ME-A, based on an alkyl polyglucoside surfactant, showed a significantly higher cumulative release of punicalagin (60.4 μg/cm^2) compared with ME-E and ME-P. In skin penetration/permeation studies, ME-A also exhibited the highest numerical total delivery of punicalagin (≈48.2 μg/cm^2 after 24 h), although differences among formulations were not statistically significant. All formulations demonstrated high antioxidant activity in the DPPH assay and measurable *in vitro* photoprotective potential, with SPF values ranging from approximately 11 to 14. Overall, pomegranate peel extract-loaded MEs showed potential as dermal delivery systems capable of improving solubilization and modulating skin delivery of punicalagin. The combination of agro-waste-derived bioactives with biocompatible surfactants highlights the potential of these systems as sustainable approaches for skincare formulations.

Keywords: microemulsion; pomegranate peel extract; punicalagin; dermal delivery

Check for updates

Academic Editor: Luis Alfonso Trujillo-Cayado

Received: 28 April 2026
Revised: 22 May 2026
Accepted: 29 May 2026
Published: 1 June 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

Extracts derived from *Punica granatum* L. (pomegranate) demonstrate a broad range of biological activities, such as antimicrobial, antiviral, antioxidant, anticancer, anti-inflammatory, cardioprotective, and antidiabetic effects [1]. Pomegranate peel, a major

by-product of juice production, contains significantly higher concentrations of polyphenolic compounds than other parts of the fruit, which contribute to its pronounced antioxidant activity and notable pharmacological potential [2]. Among these compounds, punicalagin (a dominant ellagitannin) (Figure 1) is considered the principal bioactive constituent responsible for the biological activity of pomegranate peel extract. Pomegranate polyphenols have demonstrated antioxidant, anti-inflammatory, and photoprotective effects, including UV absorption capacity and reduction in UV-induced oxidative stress in experimental models [3]. In addition, some studies suggest their potential to protect keratinocytes from UV-induced damage and to modulate enzymes associated with skin aging processes [2–4]. Due to these properties, pomegranate peel polyphenols have attracted increasing interest for topical and cosmetic applications, particularly in formulations intended for skin protection against photoaging-related damage.

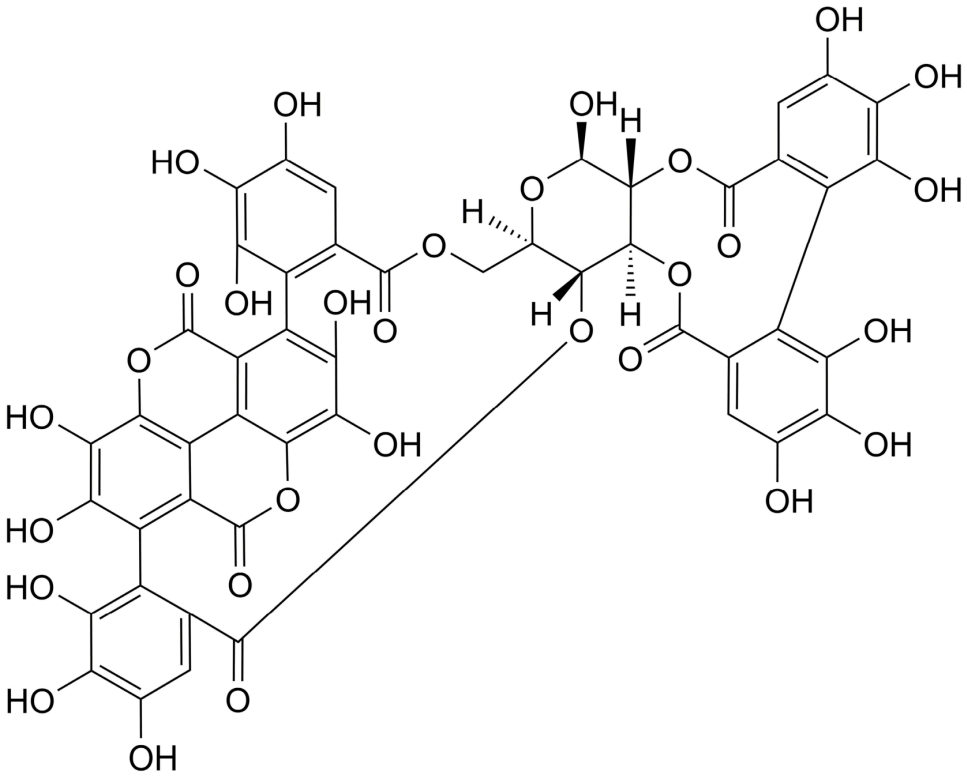


Figure 1. Chemical structure of punicalagin (created with ChemDraw Ultra 7.0).

However, despite their therapeutic potential, effective dermal delivery remains challenging due to the chemical diversity and physicochemical characteristics of their constituents [5]. The abundance of hydroxyl groups in polyphenolic structures makes them prone to degradation reactions such as oxidation and epimerization, which may compromise their stability and bioavailability [6,7]. In addition, many of these bioactives are hydrophilic, high-molecular-weight molecules with limited lipid solubility and poor penetration through the *stratum corneum*, resulting in low dermal bioavailability in conventional formulations. Punicalagin shares these limitations, being a high-molecular-weight (Mw ~1084.72 g/mol), highly polar compound (log P of approximately 2.36 [8]), with good solubility in polar solvents such as water and ethanol. These characteristics justify the development of delivery systems capable of improving solubilization, stability, and dermal delivery of structurally diverse polyphenols [5].

To overcome these limitations, advanced delivery systems, such as liposomes [9], solid lipid nanoparticles [10,11], and nanoemulsions [12,13], have been explored. Among

them, microemulsions (MEs) represent promising carriers for dermal application due to their ability to accommodate compounds with a broad polarity range and improve the solubilization of poorly permeable phytochemicals. MEs are thermodynamically stable, isotropic systems composed of oil, water, surfactant, and cosurfactant, characterized by nanometer-scale internal domains (<100 nm) capable of solubilizing both hydrophilic and lipophilic compounds. Their unique interfacial structure may enhance the solubility and stability of plant-derived actives while promoting skin permeation through increased drug thermodynamic activity and transient modification of the *stratum corneum* lipid organization. In addition, their oil and water phases may contribute to improved skin hydration. MEs are relatively simple to prepare and do not require high-energy equipment, representing a practical advantage over many advanced delivery systems [14–16].

However, the relatively high surfactant content of MEs may increase the risk of skin irritation, making careful excipient selection essential in dermal formulation development. In this context, surfactants with high skin tolerability, good biocompatibility, and preferably natural origin are particularly desirable. Based on these considerations, alkyl polyglucoside was selected as a biosurfactant due to its favorable biocompatibility, low irritation potential, and possible interaction with *stratum corneum* lipids, which may support skin hydration and penetration enhancement [17]. In addition, biocompatible ethoxylated surfactants, namely polysorbate 80 and glycereth-7-caprylate/caprate, were included to optimize the ME system, owing to their well-established solubilizing properties and compatibility with dermal formulations.

Despite the potential of MEs for the topical delivery of plant-based bioactives [14], their application in the dermal delivery of polyphenol-rich pomegranate peel extract remains insufficiently explored [18,19]. Previous studies have mainly focused on cosmetic evaluation [18] or non-dermal applications [19], while important aspects such as release behavior, skin penetration, and permeation mechanisms of the active polyphenols remain poorly understood.

Therefore, the aim of this study was to systematically investigate the potential of previously developed biocompatible MEs [20] for dermal delivery of punicalagin from pomegranate peel extract. An integrated *in vitro–ex vivo* approach was applied to evaluate the relationship between formulation composition and microstructure with punicalagin release kinetics, skin penetration/deposition, antioxidant activity, and photoprotective potential.

2. Materials and Methods

2.1. Pomegranate Peel Extract Preparation

For this study, we used the same pomegranate peel extract that was prepared and fully characterized in our previously published work [21]. A triple percolation method with 70% ethanol as the extraction solvent was used. The solvent was evaporated to dryness under vacuum to obtain the dry extract as a dry yellow-brown powder. The phenolic profile of the extract, known to be responsible for its biological activity, has been well characterized in our previously published HPLC analysis [21], which identified punicalagin (69.67 $\pm$ 0.72 mg/g) as the major constituent, followed by punicalin (30.41 $\pm$ 0.11 mg/g), ellagic acid (23.83 $\pm$ 0.07 mg/g), and gallic acid (10.46 $\pm$ 0.04 mg/g). Total tannins were determined spectrophotometrically according to the European Pharmacopoeia [22], and their content was 11.8%, which is consistent with the WHO monograph [23]. The final extract was stored under cool and dark conditions until further use, but no longer than three months after preparation, based on previous stability assessments.

2.2. Chemicals

Caprylyl/capryl glucoside (Plantacare® P810 UP) was kindly provided by BASF (Ludwigshafen, Germany). Ethoxylated fatty alcohol glycereth-7-caprylate/caprate (Emanon® EV-E) was donated by Kao Chemicals (Barbera del Vallès, Spain), while propylene glycol monocaprylate (Capryol™ 90) and highly purified diethylene glycol monoethyl ether (Transcutol® P) were gifts from Gattefossé (Lyon, France). Propylene glycol was purchased from Fagron (Zagreb, Croatia), and polysorbate 80 was obtained from Sigma-Aldrich (St. Louis, MO, USA). A reference standard of punicalagin (≥98%) was purchased from Sigma-Aldrich (St Louis, MO, USA). Double-distilled water was obtained using a Barnstead™ LabTower™ EDI Water Purification System (Thermo Scientific™, Waltham, MA, USA). All other chemicals were of pharmaceutical grade and used as received without purification.

2.3. Preparation of Formulations

Biocompatible MEs with a favorable preliminary safety profile, which were previously developed in our research as delivery systems for different drugs [20], were used as vehicles for the solubilization of pomegranate peel extract (Table 1). First, the mixture of surfactant and cosurfactant (1:1, *w*/*w*) was prepared using a magnetic stirrer for 15 min at 300 rpm (IKA® KS 260 basic shaker, Werke GmbH & Company KG, Staufen, Germany). Afterwards, the oil phase (Capryol™ 90) was added to this mixture and stirred at the same rate for another 15 min. Finally, the proper amount of double-distilled water was added precisely in a dropwise manner with gentle stirring for an additional 30 min at 300 rpm until transparent, homogenous, non-opalescent, and low-viscosity ME samples were obtained. The pomegranate peel extract was incorporated into the previously prepared MEs by magnetic stirring at room temperature. This approach was selected as a practical strategy to enable simultaneous solubilization of constituents with different polarities within the bicontinuous ME system. Thus, the extract was gradually added to the MEs under continuous stirring (300 rpm) until complete solubilization was achieved, resulting in a clear and homogeneous formulation. The concentration of the incorporated extract (1%, *w*/*w*) was selected based on its solubility limit. To ensure complete solubilization of the extract, the sample was subjected to ultrasonication for 15 min at room temperature using a Sonorex Digitec DT 102H (Bandelin, Berlin, Germany) operating at a fixed frequency of 35 kHz. The process was conducted for a total of 15 min (3 × 5 min), with intermittent cooling between cycles to prevent temperature increase and potential thermal degradation of the analyte.

Table 1. Qualitative and quantitative composition of the selected ME formulations (%, *w*/*w*) [20] as vehicles for incorporation of pomegranate peel extract at a concentration of 1% (*w*/*w*).

Function	Ingredients	Formulation Code		
		ME-A	ME-E	ME-P
Oil phase	Capryol™ 90	14.0	14.0	14.0
Surfactant	Plantacare® 810 UP	28.0	-	-
	Emanon® EV-E	-	28.0	-
	Polysorbate 80	-	-	28.0
Cosurfactant	Propylene glycol	28.0	-	-
	Transcutol® P	-	28.0	28.0
Water phase	Double-distilled water	30.0	30.0	30.0

2.4. Physicochemical Characterization of the Investigated Formulations

ME formulations loaded with pomegranate peel extract were evaluated for their visual appearance, pH, electrical conductivity, and viscosity. Droplet size was not determined,

as the studied bicontinuous MEs do not possess discrete droplets; hence, measurements by dynamic light scattering (DLS) or by photon correlation spectroscopy (PCS) would yield apparent or pseudo-size values that do not represent true physical diameters [24]. Zeta potential was not assessed, as it is generally unnecessary for thermodynamically stable MEs [16].

pH values were determined by direct immersion of the pH electrode into the samples using a pH meter (Model 3110, WTW, Weilheim, Germany). All measurements were performed in triplicate.

To gain insight into the influence of the incorporation of pomegranate peel extract on the structural characteristics of blank MEs, electrical conductivity was measured using a conductometer (Multi 9620 IDS, WTW, Weilheim, Germany).

Rheological measurements were performed over a shear rate range of 75–750 s^{-1} for placebo MEs [20], while formulations containing pomegranate peel extract were analyzed at 20 ± 1 °C over a reduced shear rate range (up to 250 s^{-1}) due to equipment limitations (DV-I PRIME viscometer, Brookfield Engineering Laboratories, Middlesboro, KY, USA). Viscosity values reported for comparison were taken at a shear rate of 100 s^{-1}, which is considered relevant for topical application.

In order to accurately quantify punicalagin in the ME formulations, an aliquot of 50 µL from each sample was diluted with ethanol to a final volume of 10 mL. This dilution ensured complete disruption of the ME structure and yielded clear solutions suitable for HPLC analysis. The punicalagin content was then determined using the HPLC method described in Section 2.9.

2.5. In Vitro Release Studies

Since it is the most abundant compound in pomegranate peel extract, punicalagin was selected as a marker to assess formulation *in vitro* release behavior, skin permeation, and retention performance. *In vitro* release of punicalagin was evaluated using Franz-type vertical diffusion cells (PermeGear, Hellertown, PA, USA) in compliance with the USP general monograph for semisolid drug products [25]. The receptor chambers (12 mL) were filled with a preheated (32 °C) mixture of ethanol and water (25/75, *v*/*v*), selected based on literature data demonstrating its suitability to maintain *sink* conditions for punicalagin [8].

The tested ME formulations (1 g) were evenly applied to cellulose dialysis membranes (pore size 2.4 nm; MWCO 12,000; Sigma-Aldrich, Saint Louis, MO, USA), which were previously activated by immersion in the receptor medium for 12 h to ensure proper hydration and equilibration. The membranes were carefully mounted between the donor and receptor compartments, with an effective diffusion area of 2.0 cm^2. To prevent evaporation, donor chambers were sealed with Parafilm™ M (Bemis, Neenah, WI, USA). The receptor solution was continuously stirred at 500 rpm and maintained at 32 °C throughout the experiment. Samples (600 µL) were withdrawn at predetermined intervals (2, 4, 6, and 8 h) and immediately replaced with fresh preheated receptor medium to maintain sink conditions. The collected samples were analyzed for punicalagin content using an HPLC method.

Punicalagin release results were expressed as the cumulative amount released per unit area (µg/cm^2) versus time, allowing calculation of the transmembrane flux. To compare release profiles, model-dependent kinetic analyses were applied, including zero-order, first-order, Higuchi, and Korsmeyer–Peppas models. The correlation coefficient (r^2) was used to determine the most appropriate release model.

For comparison with the developed ME systems, the carbomer-based hydrogel containing 25% (*w*/*w*) pomegranate peel extract was used as a conventional reference semisolid formulation. Due to the lower solubilization and penetration-enhancing capacity of the hydrogel vehicle, a higher extract concentration was required compared to the ME formu-

lations (1% w/w), which were specifically designed to improve extract solubilization and skin delivery efficiency.

2.6. Ex Vivo Penetration/Permeation Studies

Ex vivo penetration and permeation of punicalagin from the investigated ME formulations were evaluated using full-thickness porcine ear skin mounted on Franz diffusion cells. Fresh pig ears were obtained from a local abattoir immediately after slaughter, rinsed with cold tap water, dried, and stored at −20 °C for up to 30 days. On the day of the experiment, the skin was thawed at room temperature, and visible hair was removed using an electric clipper. Subcutaneous fat was carefully excised with a surgical blade, and 25 mm circular sections were prepared and inspected under magnification to ensure the absence of physical damage. Additionally, skin barrier integrity was assessed by measuring transepidermal water loss (TEWL) using a Tewameter® TM 210 (Courage + Khazaka, Köln, Germany). Skin samples exhibiting TEWL values higher than 15 g m^{-2} h^{-1} were considered unsuitable and were excluded from the *in vitro* penetration/permeation studies. Prior to mounting, the skin sections were rehydrated for 1 h in appropriate physiological conditions and then clamped between the donor and receptor chambers with the dermal side facing the receptor compartment. The ethanolic water 25/75 (v/v) was used exclusively as the receptor medium to maintain *sink* conditions [8]. The experimental setup and data analysis followed the same procedure as described for the *in vitro* release study, except that receptor medium samples were collected after 2, 4, 6, 8, and 24 h following formulation application. Upon completion of the experiment, the skin samples were analyzed for punicalagin content using HPLC. To remove residual formulation, the skin surface was gently wiped three times with PBS (pH 7.4). Each section was then cut into smaller pieces and transferred to glass tubes containing 4 mL of methanol [8,26]. The resulting mixtures were first shaken to facilitate initial disruption of the tissue matrix (IKA® Werke GmbH, Staufen, Germany). They were then sonicated to enhance the release of punicalagin from the *stratum corneum* and deeper skin layers. Sonication was performed in an ultrasonic bath (Sonorex RK 120H, Bandelin, Berlin, Germany, fixed-frequency system of 35 kHz) twice for 30 min, applied in 5 min intervals with intermittent cooling to prevent temperature increase and potential thermal degradation of the analyte. Samples were centrifuged at 3000 rpm for 30 min (MPW-56 centrifuge, MPW Med. Instruments, Warsaw, Poland) to remove particulate matter, and the supernatants were filtered through 0.45 µm membrane filters prior to quantitative analysis.

The hydrogel formulation previously described as the reference conventional semisolid system was also used for permeation and skin penetration studies.

2.7. In Vitro Antioxidant Activity Evaluation

The antioxidant capacity of MEs loaded with pomegranate peel extract was evaluated using the DPPH assay [27]. Briefly, 0.5 mL of sample ME (diluted in methanol) was mixed with 3 mL of freshly prepared DPPH solution in methanol (50 µg/mL). After 30 min of incubation in the dark at room temperature, the radical-scavenging activity (i.e., Abs) was measured spectrophotometrically at 515 nm. Methanolic solutions of the corresponding MEs served as blanks, while the DPPH solution alone was used as a negative control. The percentage of inhibition was calculated using the following equation:

$$Inhibition\ (\%) = 100 - \frac{Abs\ (test\ sample) - Abs\ (blank)}{Abs\ (control)} \times 100$$

Since the experimentally determined solubility of pomegranate peel extract in ethanol was limited to 0.07% (w/w), it was not possible to prepare an ethanolic solution containing

the same extract concentration as the ME formulations (1% *w*/*w*). Therefore, a 0.06% (*w*/*w*) ethanolic stock solution was selected to ensure complete solubilization of the extract. Prior to antioxidant activity and SPF determination, this stock solution was further diluted with methanol to obtain the same final extract concentration as that present in the corresponding diluted ME samples. This approach enabled a direct comparison of the antioxidant activity and SPF values based on an equivalent amount of pomegranate peel extract in all tested samples.

2.8. Determination of Sun Protection Factor (SPF)

An accurately weighed 1.0 g sample was sonicated in 50 mL of ethanol and diluted to 100 mL, then filtered. Afterwards, this solution was placed in 1 cm cuvettes, and UVB absorbance was recorded from 290 to 320 nm at 5 nm intervals in triplicate. SPF values were calculated using the Mansur equation [28]:

$$SPF = CF \times \sum_{290}^{320} EE(\lambda) \times I(\lambda) \times Abs(\lambda)$$

Here, CF represents the correction factor (10), EE(λ) denotes the erythemogenic effect of radiation at wavelength λ, and Abs(λ) corresponds to the spectrophotometric absorbance of the sample at that wavelength. The values of EE(λ) × I are constant and were determined by Sayre et al. [29], as presented in Table 2.

Table 2. Values of EE (λ) × I at different wavelengths.

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

For comparison, placebo MEs (without the extract) were prepared, and their SPF values were determined under the same experimental conditions as the active formulations. In addition, an ethanolic solution of pomegranate peel extract was prepared and used as a reference sample representing the free (non-encapsulated) extract. The solution was diluted under the same experimental conditions as the ME samples to ensure that the final extract concentration matched that of the correspondingly diluted extract-loaded ME samples, enabling direct comparison at equivalent extract concentrations.

Hence, for ME samples and the reference solution with the same extract concentration, SPF determination was performed using sequential ethanolic dilutions combined with brief sonication. This procedure was applied to ensure appropriate optical conditions for UV–Vis spectrophotometric analysis by minimizing potential light scattering effects associated with dispersed systems. All diluted samples were visually inspected and appeared as clear, transparent solutions prior to measurements, confirming their suitability for spectrophotometric analysis.

Additionally, the SPF of ethanolic solutions of pomegranate peel extract at increasing concentrations was also examined; however, measurements were performed only within the validated working range of the spectrophotometric method (10–200 μg/mL). Concentrations below 10 μg/mL produced absorbance values below the reliable detection limit, while concentrations above 200 μg/mL resulted in excessively high absorbance values,

exceeding the linear response range of the method; therefore, only data obtained within this range were considered for SPF determination.

2.9. HPLC Analysis

To quantify the amount of punicalagin released from the formulations, as well as diffused into and across the porcine ear skin, a validated HPLC method was employed [30]. Chromatographic separation was performed using an Agilent 1200 Series HPLC system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump (G1311A) and an autosampler (ALS G1329A). The analysis was carried out on a Zorbax Eclipse C18 column (150 × 4.6 mm, 5 µm particle size). The mobile phase consisted of glacial acetic acid (2%, *v*/*v*) and acetonitrile in a ratio of 80:20 (*v*/*v*), at a flow rate of 0.8 mL/min. The column temperature was maintained at 30 °C, with an injection volume of 10 µL. Detection was performed using a diode array detector (DAD) set at 280 nm.

2.10. Statistical Analysis

Where appropriate, data are presented as mean ± standard deviation (SD). Statistical comparisons were performed using Student's *t*-test or one-way analysis of variance (ANOVA), depending on the number of groups. Due to the small sample size (n = 3), formal normality tests have limited statistical power, and therefore, visual inspection and distribution characteristics were considered. A p-value < 0.05 was considered statistically significant. All analyses were conducted using GraphPad Prism version 8 for Windows (GraphPad Software Inc., La Jolla, CA, USA).

3. Results and Discussion

3.1. Preparation and Physicochemical Characterization of the Investigated Formulations

ME vehicles selected for solubilization of pomegranate peel extract were developed in our previous study, initially as carriers for the two poorly soluble drugs with different physicochemical properties (adapalene and sertaconazole nitrate) [20]. These ME systems were formulated using three surfactants with favorable safety and compatibility profiles: alkyl polyglucoside (caprylyl/capryl glucoside) (ME-A), glycereth-7-caprylate/caprate (ME-E), and polysorbate 80 (ME-P). Caprylyl/capryl glucoside belongs to the group of alkyl polyglucosides, a class of mild nonionic surfactants derived from renewable resources such as glucose and fatty alcohols, known for their biodegradability and low irritation potential. Glycereth-7-caprylate/caprate is a vegetable-based surfactant with low toxicity, while polysorbate 80 is widely used in pharmaceutical and cosmetic formulations. Such an approach integrates renewable or naturally derived excipients with bioactive compounds from agro-industrial waste, contributing to more sustainable dermal formulations. In particular, alkyl polyglucosides support the development of environmentally acceptable systems due to their renewable origin and favorable ecological profile.

As previously demonstrated [20], these formulations possess suitable characteristics for topical delivery and have shown acceptable preliminary *in vivo* safety profiles. The solubilization capacity of each formulation was evaluated to select an appropriate extract concentration for comparative studies. Initially, the pomegranate peel extract concentration was tested at 3% (*w*/*w*), with stepwise reductions of 0.5%. Precipitation was observed after 7 days in all formulations at 3% and 2.5%. At 2% and 1.5%, pomegranate peel extract precipitated in the ME-A sample, whereas no precipitation was detected in the other formulations. To ensure stability and comparability across all systems, the final concentration of 1% (*w*/*w*) was selected, as it remained stable in all formulations. The lower apparent solubilization capacity of ME-A is likely due to its higher water content, partly originating from the surfactant (Plantacare® 810 UP), resulting in 40.2% water and 17.8%

caprylyl/capryl glucoside. Although increased water content may favor solubilization of hydrophilic components, reduced surfactant fraction may limit solubilization of lipophilic constituents [6]. This highlights the importance of balancing hydrophilic and lipophilic domains in bicontinuous MEs to optimize overall extract solubilization.

Hence, all 1% (*w*/*w*) extract-loaded MEs were obtained as clear, homogeneous, transparent brownish liquids with a mild odor characteristic of the employed surfactants. No visible precipitates were observed, either macroscopically or under optical microscopy.

Electrical conductivity is governed by the ionic content and the proportion of the aqueous phase within the system, typically increasing with the addition of polar constituents or electrolytes. Earlier structural analyses of the placebo formulations confirmed a bicontinuous ME structure (ME-E and ME-P) or inverted bicontinuous inner microstructure (ME-A). Among them, the ME–A sample exhibited the highest conductivity, which can be attributed to the presence of residual electrolytes formed during the synthesis of alkyl polyglucosides, as well as the intrinsic water content of the surfactant Plantacare® 810 UP [20]. Upon incorporation of the pomegranate peel extract, a marked increase in electrical conductivity was observed (Table 3). This enhancement is likely associated with the presence of polyphenolic compounds acting as polar species, together with naturally occurring ions such as K^+, Na^+, and Ca^{2+} [31]. These findings suggest a potential structural reorganization of the system from a bicontinuous toward an inverted bicontinuous or a more oil-in-water–dominant ME, facilitated by a slight increase in the effective aqueous phase and improved ion mobility [32].

Table 3. Physicochemical properties of the investigated ME formulations (the values for blank samples were reported in our previous work [20]).

Formulation Code	Electrical Conductivity (Mean ± SD, *n* = 3)	pH (Mean ± SD, *n* = 3)	Viscosity η (mPas) (Mean ± SD, *n* = 3)	Entrapment Efficiency (% ± SD, *n* = 3)
ME-A blank	834.7 ± 1.3	8.77 ± 0.07	25.31 ± 1.10	/
ME-A	1065.0 ± 12.0 [a]	6.90 ± 0.02 [a]	27.70 ± 1.04	89.53 ± 2.12
ME-E-blank	62.5 ± 1.0	7.47 ± 0.06	18.84 ± 1.43	/
ME-E	124.6 ± 2.6 [a]	3.94 ± 0.02 [a]	21.60 ± 1.93	95.07 ± 3.68
ME-P-blank	48.5 ± 1.1	4.42 ± 0.03	48.79 ± 1.17	/
ME-P	102.4 ± 0.8 [a]	3.65 ± 0.005 [a]	48.89 ± 0.93	101.09 ± 3.87

[a] $p < 0.05$ in comparison with the corresponding blank sample.

In line with electrical conductivity behavior, pH values also changed after solubilization of the extract (Table 3). Taking into account a considerable quantity of acidic constituents in the extract, i.e., ellagic and gallic acid [21], a pH decrease could be anticipated. Nevertheless, the measured values for pomegranate peel-loaded samples remained adequate for topical application.

All investigated formulations exhibited Newtonian flow behavior, as indicated by the independence of viscosity from shear rate within the tested ranges. Incorporation of the pomegranate extract into the MEs insignificantly increased their viscosity values (η; Table 3), suggesting the absence of major microstructural alterations. This could be ascribed to possible solubilization of extract constituents with adequate polarity in the interfacial film, contributing to the higher η of bicontinuous systems. However, it seems that the interface of the ME-P formulation was less sensitive to the guest molecules of pomegranate peel extract.

Based on the observed viscosity behavior, it can be assumed that the microstructure of the extract-loaded formulations remained largely unchanged compared to the blank

vehicles, suggesting a preserved bicontinuous to inverted bicontinuous organization upon extract incorporation.

The actual punicalagin content in the ME formulations was determined to assess the efficiency of incorporation. The results showed high encapsulation (Table 3), with values ranging from 89.53 ± 2.12% to 101.09 ± 3.87% for ME-A and ME-P, respectively, indicating that the ME systems effectively retained the active compound.

3.2. In Vitro Release Studies

The *in vitro* release of punicalagin from the developed ME formulations containing 1% (*w*/*w*) pomegranate peel extract was evaluated and expressed as a cumulative amount released (μg/cm^2) over time (Figure 2a), as well as the percentage of the solubilized extract released (Figure 2b). Although pomegranate peel extract is a complex phytochemical mixture, this study focused on punicalagin as the analytical marker due to its abundance in the extract. However, this approach inherently limits interpretation, as other polyphenols (e.g., punicalin, ellagic acid, and gallic acid) may exhibit distinct release behaviors and permeation profiles. Therefore, the results should be considered representative of the dominant component of the extract rather than the entire phytochemical complex.

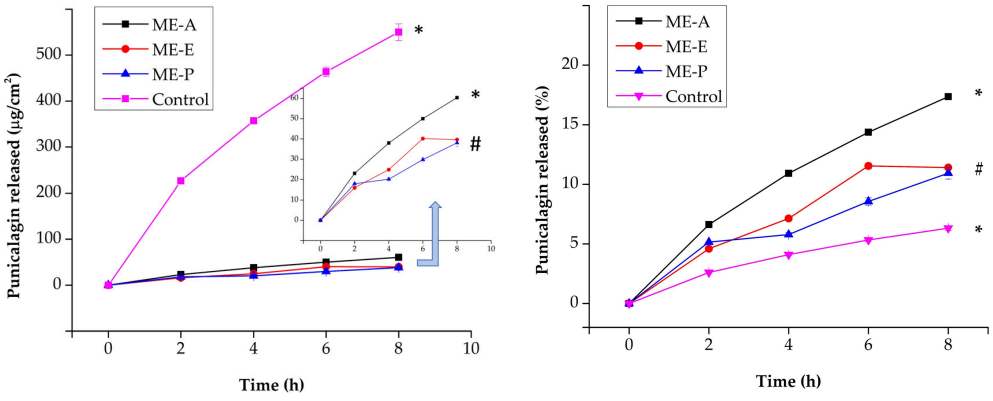


Figure 2. *In vitro* release of punicalagin from the investigated formulations was determined across the synthetic cellulose dialysis membrane, under *infinite* dose conditions (mean ± SD, *n* = 3), expressed (**a**) in μg/cm^2 (inset highlights differences among MEs); and (**b**) as a percentage of the initially applied punicalagin amount. * Statistically significant change ($p < 0.05$) at the end of the experiment compared to all other samples; # statistically significant change ($p < 0.05$) at the end of the experiment compared to ME-A and control (hydrogel containing 25% *w*/*w* pomegranate peel extract).

The release profiles revealed noticeable differences among formulations. The amounts of punicalagin released from the ME vehicles were generally low, reaching 10.96–17.36% of the applied dose after 8 h of the experiment (Table 4, Figure 2b) and remaining below 30% of the initial amount. This suggests that back diffusion of the drug into the donor phase was prevented, thereby satisfying the "30% rule" [33].

A significantly higher cumulative amount of punicalagin was released from ME-A (60.41 ± 0.41 μg/cm^2), which differed in both qualitative and quantitative composition from the other samples. In addition to using a different surfactant system, ME-A contained propylene glycol instead of Transcutol® P and a slightly higher aqueous phase content, along with a lower surfactant fraction. Increased water content can improve the partitioning of ionized phenolic compounds into the water phase of the ME, thereby facilitating their diffusion toward the release interface, while lower surfactant levels may reduce entrapment within interfacial domains, increasing drug availability for release. These compositional fac-

tors likely contributed to the observed differences in release behavior and are in agreement with literature reporting improved release from more hydrophilic MEs [34–36].

Table 4. *In vitro* release parameters of formulations loaded with 1.0% (*w*/*w*) of pomegranate peel extract obtained across the synthetic cellulose dialysis membrane (mean ± SD, *n* = 3).

Formulation Code	Transmembrane Flux (μg/cm^{2}h^{-1})	Q_{8h} (μg/cm^2)	Punicalagin Released (%)	Kinetic Model (r^2)
ME-A	7.38 ± 0.06 [a]	60.40 ± 0.41 [a]	17.36 ± 0.12 [a]	Korsmeyer–Peppas (0.9995)
ME-E	5.19 ± 0.03 [b]	39.68 ± 0.03 [b]	11.40 ± 0.01 [b]	Higuchi (0.9527)
ME-P	4.40 ± 0.23 [b]	38.06 ± 1.73 [b]	10.94 ± 0.50 [b]	Higuchi (0.9632)
Control	66.86 ± 1.97 [a]	549.95 ± 18.14 [a]	6.32 ± 0.21 [a]	Korsmeyer–Peppas (0.9996)

[a] $p < 0.05$ compared to all other samples; [b] $p < 0.05$ compared to ME-A and control.

The higher release from ME-A may also be partly related to pH effects. This formulation showed a higher pH, which can enhance the solubilization of punicalagin. With a pKa of ~6.6 [37], punicalagin becomes more ionized at higher pH, increasing its aqueous solubility and release. This is supported by Radan et al. [6], who reported pH-dependent release of pomegranate peel polyphenols in different simulated biological media.

The type of surfactant did not significantly influence the release profile, as comparable amounts of punicalagin were released from formulations ME-E and ME-P (p = 0.2085), which differed only in surfactant composition. The similarity may be related to the comparable hydrophilic–lipophilic balance (HLB) values (15–17) and the structural similarity of the ethoxylated surfactants used, resulting in formulations with comparable interfacial properties. However, this result is not in complete agreement with our previous study [38], where ME-P showed higher release of a lipophilic drug (sertaconazole nitrate). The difference may be attributed to the higher viscosity of ME-P, which could have influenced diffusion of the hydrophilic punicalagin, as well as possible differences in solubilization capacity, leading to altered thermodynamic activity of the compound.

When ME formulations were compared to the reference formulation (hydrogel containing 25% *w*/*w* pomegranate peel extract), ME systems exhibited a higher relative release (Figure 2, Table 4). Despite a higher cumulative amount released from the hydrogel (549.95 ± 18.14 μg/cm^2), the fraction released was lower, indicating diffusional limitations within the hydrophilic gel matrix, which can restrict the mobility of high-molecular-weight polyphenols [39]. However, these comparisons should be interpreted with caution due to the substantially different extract loading of the investigated formulations, which may also affect the thermodynamic activity and release behavior of the incorporated polyphenols.

The release kinetics further confirmed structural differences among the investigated formulations. ME-A and the reference hydrogel followed the Korsmeyer–Peppas model, with the diffusion exponent (n) value of 0.70, indicating anomalous (non-Fickian) transport governed by a combination of diffusion and matrix relaxation mechanisms [40]. Such behavior is expected for high-molecular-weight polyphenols, whose diffusion is partially restricted by the characteristics of the surrounding matrix. In the hydrogel, this limitation results from the hydrophilic crosslinked network, known to reduce the mobility of large solutes [39].

In contrast, ME-E and ME-P exhibited release profiles best described by the Higuchi model, indicating predominantly Fickian, diffusion-controlled kinetics [41]. However, the total amount of punicalagin released from these two formulations was lower, likely due to their reduced water content and more hydrophobic internal environment, which can limit solubility and diffusion of polar compounds. In such systems, polyphenols may be more strongly retained within interfacial regions, reducing their effective diffusion [42].

These observations are consistent with reports emphasizing the role of microstructure and interfacial properties in controlling release behavior [16,43].

According to the results of the *in vitro* release study, the ME-A formulation exhibited the highest punicalagin release, which may indicate potential differences in the drug's subsequent skin delivery [44]. To verify this, an *ex vivo* skin penetration/permeation study was subsequently performed.

3.3. Ex Vivo Penetration/Permeation Studies

The penetration/permeation study was performed with full-thickness porcine ear skin, selected due to its well-established similarity to human skin in terms of structure, morphology, and permeability, as well as its suitability for simulating *in vivo* behavior on human skin [45,46]. The results are presented in Figure 3. Dermal delivery of punicalagin was quantified as the amount of compound that penetrated per unit skin surface (μg/cm^2) after application of the ME formulations and compared with the control formulation.

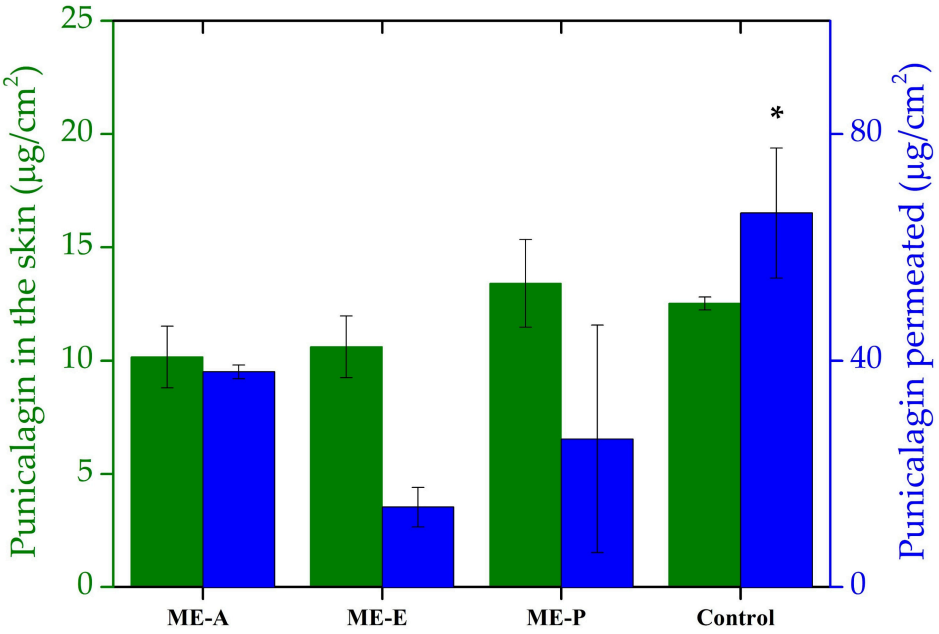


Figure 3. *Ex vivo* penetration/permeation of punicalagin from the tested formulations obtained using full-thickness porcine ear skin under *infinite* dose conditions (mean ± SD, $n = 3$). * Statistically significant change ($p < 0.05$) compared to ME-E and ME-P.

No statistically significant differences in skin retention or permeation were observed among the tested ME formulations. However, certain numerical trends could be identified. ME-P showed the highest values of skin retention, although this difference did not reach statistical significance. This observation contrasts with the *in vitro* release results (Section 3.2), implying that artificial membranes may not fully reflect the complexity of skin–formulation interactions, as previously reported in the literature [38].

Although definitive conclusions cannot be drawn, the observed trends may be partially related to formulation composition and the presence of excipients reported to affect skin transport. For example, polysorbate 80 and Transcutol® P in ME-P have previously been associated with increased fluidity of *stratum corneum* lipids and improved solubilization of hydrophilic compounds [47,48], which could potentially contribute to the somewhat higher skin retention observed for this formulation. In addition, the internal organization of MEs may influence drug distribution and transport behavior. Bicontinuous systems are characterized by a dynamic oil–water interface and low interfacial tension properties that have been associated with enhanced exchange of compounds between formulation domains

and the *stratum corneum* [49]. Nevertheless, given the lack of statistical significance among the investigated formulations, such potential effects should be interpreted with caution.

Differences in water content and pH among the formulations may also have contributed to the observed trends. ME-P and ME-E contained less water than ME-A, which could potentially influence skin transport behavior [34–36]. In addition, pH-related effects cannot be excluded due to the presence of multiple phenolic hydroxyl groups. Punicalagin shows pH-dependent ionization behavior characteristic of ellagitannins [37]. Changes in ionization may affect both aqueous solubility and partitioning behavior, which could influence release from the vehicle and subsequent interaction with the *stratum corneum*. Such factors may partly contribute to the differences observed between release and skin delivery.

Although permeation of punicalagin could be expected to be limited due to its high molecular weight and hydrophilicity, measurable amounts were detected, consistent with previous reports [50]. Among the tested formulations, ME-A showed the highest permeation values (38.04 ± 1.19 μg/cm^2), although without statistical significance. Therefore, any interpretation regarding the contribution of specific formulation components should be considered only tentative. Nevertheless, the presence of propylene glycol and alkyl polyglucosides, previously reported as excipients capable of influencing skin hydration and *stratum corneum* lipid organization [17,51,52], may have contributed to the observed permeation behavior. In addition, partitioning of punicalagin within the ME system could also play a role in modulating its transfer into the skin.

When considering the total amount of punicalagin (retained in the skin and permeated to the receptor phase), ME-A showed the highest numerical values (48.24 ± 2.52 μg/cm^2), followed by ME-P (39.96 ± 17.5 μg/cm^2) and ME-E (24.52 ± 5.32 μg/cm^2), indicating a trend toward greater overall transport. Similar tendencies have previously been reported for hydrophilic compounds such as caffeine delivered from MEs with different internal structures [35].

Compared with literature data reporting relatively low skin retention of punicalagin from conventional hydrophilic creams (0.3–2.8 μg/cm^2 after 24 h) [53], the investigated ME systems demonstrated higher deposition values, which may indicate improved dermal delivery potential. In the present study, incorporation of pomegranate peel extract into MEs resulted in skin deposition levels comparable to those obtained with a control gel containing a substantially higher concentration of the extract. However, direct comparison between these systems is limited by the substantially different extract concentrations used in the formulations, which may also influence the driving force for release and skin transport of punicalagin. Although the control gel exhibited higher permeation values, the difference compared to ME-A was not statistically significant. The observed behavior may partly reflect the higher extract concentration and corresponding thermodynamic activity of punicalagin in the gel.

From an application perspective, retention of active compounds within the skin layers is generally preferred for topical anti-aging and photoprotective effects, whereas extensive systemic permeation is usually not intended. In this context, the absence of detectable punicalagin in the receptor phase during the first 8 h of the experiment for the investigated ME formulations may be relevant. Considering that topical products are typically applied for limited periods (e.g., 6–8 h), these findings may suggest the potential for predominantly localized activity with limited systemic exposure under typical conditions of use.

Overall, the results highlight the importance of evaluating both skin retention and permeation when assessing dermal delivery systems. Focusing solely on permeation may underestimate the fraction of the active retained within the skin, which can serve as a reservoir for sustained local activity.

3.4. In Vitro Antioxidant Activity Evaluation

The antioxidant capacity of the ME formulations was assessed using the DPPH assay. ME-A demonstrated the strongest radical-scavenging activity (47.68 ± 0.18%), while ME-E (36.30 ± 0.90%) and ME-P (35.58 ± 0.21%) showed moderately lower but comparable values (Table 5). It should be emphasized that MEs were diluted in methanol before the analysis. These results, thus, indicate that all formulations possess notable antioxidant potential; however, the markedly higher activity observed for ME-A suggests a more efficient solubilization, stabilization, or release of phenolic constituents in this specific ME system. Similar trends have been reported in previous studies [54,55], where variations in surfactant composition, oil phase, or droplet structure influenced the accessibility of encapsulated bioactive compounds to free radicals [55]. The close values for ME-E and ME-P (p = 0.3077) further imply that their internal organization provides a comparable environment for radical interaction, whereas ME-A may offer enhanced molecular mobility or more favorable partitioning of antioxidant compounds within the continuous phase.

Table 5. *In vitro* determined DPPH radical-scavenging activity and SPF values of the investigated ME samples.

Formulation Code	Radical-Scavenging Activity (%) (Mean ± SD, n = 3)	SPF Value (Mean ± SD, n = 3)
ME-A blank	2.06 ± 0.19	0.51 ± 0.03
ME-A	47.68 ± 0.18 [a]	14.34 ± 2.69 [c]
ME-E blank	1.61 ± 0.37	0.49 ± 0.04
ME-E	36.30 ± 0.90 [b]	11.55 ± 0.57
ME-P blank	1.94 ± 0.41	0.12 ± 0.01
ME-P	35.58 ± 0.21 [b]	11.12 ± 1.51
Ethanolic solution of pomegranate peel extract	23.91 ± 0.49 [a]	8.29 ± 1.31

[a] $p < 0.05$ compared to all other samples; [b] $p < 0.05$ compared to ME-A and ethanolic solution; [c] $p < 0.05$ compared to ethanolic solution.

Importantly, placebo MEs exhibited negligible antioxidant activity (Table 5), confirming that the observed radical-scavenging effect originates predominantly from the incorporated pomegranate peel extract rather than from the carrier system itself.

The improvement of antioxidant activity obtained with MEs was further supported by comparison with the ethanolic solution of pomegranate peel extract at the same concentration, which exhibited substantially lower antioxidant activity (23.91 ± 0.49%) (Table 5). This enhancement can be attributed to improved solubilization and dispersion of polyphenolic constituents within the ME systems, which likely increases their effective availability for radical scavenging. Moreover, the ME microstructure may protect sensitive polyphenols from degradation and promote synergistic interactions between extract components and formulation excipients, collectively contributing to the superior antioxidant performance compared to the simple ethanolic solution.

However, it should be emphasized that the DPPH assay provides information on chemical radical-scavenging capacity but does not directly reflect biological antioxidant effects in the skin, which depend on penetration, metabolism, and interaction with cellular systems.

3.5. Determination of Sun Protection Factor (SPF)

The sun protection factor (SPF) of the MEs was determined using the Mansur equation, based on their UV absorbance profiles. SPF represents the degree of protection a formulation provides against UV-induced erythema; in other words, it reflects the product's ability to reduce the skin-burning effects of solar radiation. It should be emphasized that the SPF values determined using the Mansur spectrophotometric method represent an *in vitro*

screening tool rather than a substitute for *in vivo* SPF testing. While this approach enables rapid assessment of UVB-absorbing capacity, it does not account for formulation spreading, film thickness, photostability, or biological skin responses. Therefore, the calculated SPF values should be interpreted as indicators of photoprotective potential, rather than as definitive evidence of sunscreen efficacy. In this context, the developed MEs are not proposed as standalone sunscreens but as multifunctional topical systems that may enhance skin protection by combining antioxidant activity with intrinsic UV-absorbing properties.

The results of this part of the study are presented in Table 5. Hence, based on the calculated SPF values, all MEs demonstrated notable photoprotective potential, without significant differences between them. Numerical differences in SPF values were observed among the formulations, with ME-A exhibiting the highest mean value (14.34 $\pm$ 2.69), although without statistical significance. According to the European Commission's classification of sun protection levels [33], all investigated formulations corresponded to the category of low protection (SPF 10.0–14.9). ME-E and ME-P exhibited comparable SPF values (11.55 $\pm$ 0.57 and 11.12 $\pm$ 1.51, respectively). Using the same experimental setting, blank samples exhibited negligible SPF values (0.12–0.51), confirming that the UV protection mainly originated from the incorporated active extract rather than the formulation itself.

Also, a conventional ethanolic solution of pomegranate peel extract, prepared in parallel and analyzed under identical conditions, exhibited a lower SPF value (8.29 $\pm$ 1.31). This observed difference highlights the critical role of the ME system in enhancing photoprotective performance. The improved SPF of ME formulations can be attributed to more effective UV absorption resulting from improved solubilization and stability of polyphenolic constituents, as well as possible synergistic action between the extract and the ME excipients.

However, before the ME formulations were assessed in terms of their SPFs, the SPF of ethanolic solutions of pomegranate peel extract at increasing concentrations was preliminarily assessed without dilution steps of the solutions. As the concentration of the extract increased from 10 μg/mL to 200 μg/mL, the SPF values also increased from 1.3 to 20.2. Moreover, a strong linear correlation was observed between the calculated SPF values and the concentration of the pomegranate peel extract, with an excellent coefficient of determination (r^2 = 0.9994) (Figure 4). This nearly ideal linearity indicates that the UV-protective effect of the extract increases proportionally with its concentration, confirming the polyvalent contribution of phenolic constituents to UV absorption. Such behavior is expected for polyphenol-rich formulations, as the aromatic ellagitannin structures, including punicalagin, exhibit pronounced absorption in the UV region due to their conjugated ring systems. These findings are consistent with previously published observations showing that the SPF values of pomegranate peel-containing formulations increased proportionally with extract concentration [3,54,56]. Besides that, hydrogels enriched with black pomegranate peel extract [54] demonstrated notable antioxidant activity but considerably lower SPF values than lotions containing red pomegranate peel extract [56], highlighting the influence of both extract type and formulation composition on photoprotection.

According to this part of the study, pomegranate peel extract may contribute meaningfully to multifunctional topical formulations, not only as an antioxidant but also as an intrinsic photoprotective agent with a desired level of UV protection. These findings, hence, support the potential of pomegranate peel, an abundant agro-industrial by-product, as a valuable source of bioactive compounds that can be efficiently valorized in dermal delivery systems.

Although the study provided valuable insights into the dermal delivery potential of the investigated formulations, the applied *in vitro* assays provide only preliminary indications of biological activity and cannot fully predict *in vivo* performance. In particular, antioxidant activity was evaluated solely using the DPPH assay, while photoprotective

potential was assessed exclusively by spectrophotometric SPF determination under *in vitro* conditions. Therefore, the obtained results should be interpreted cautiously and regarded as indicative rather than definitive evidence of biological efficacy. Future research should include a more comprehensive characterization of the phenolic profile of the extract, investigation of possible synergistic effects among individual constituents, and evaluation of the formulations in relevant *ex vivo* and *in vivo* models to better predict their overall efficacy, skin behavior and safety under physiological conditions.

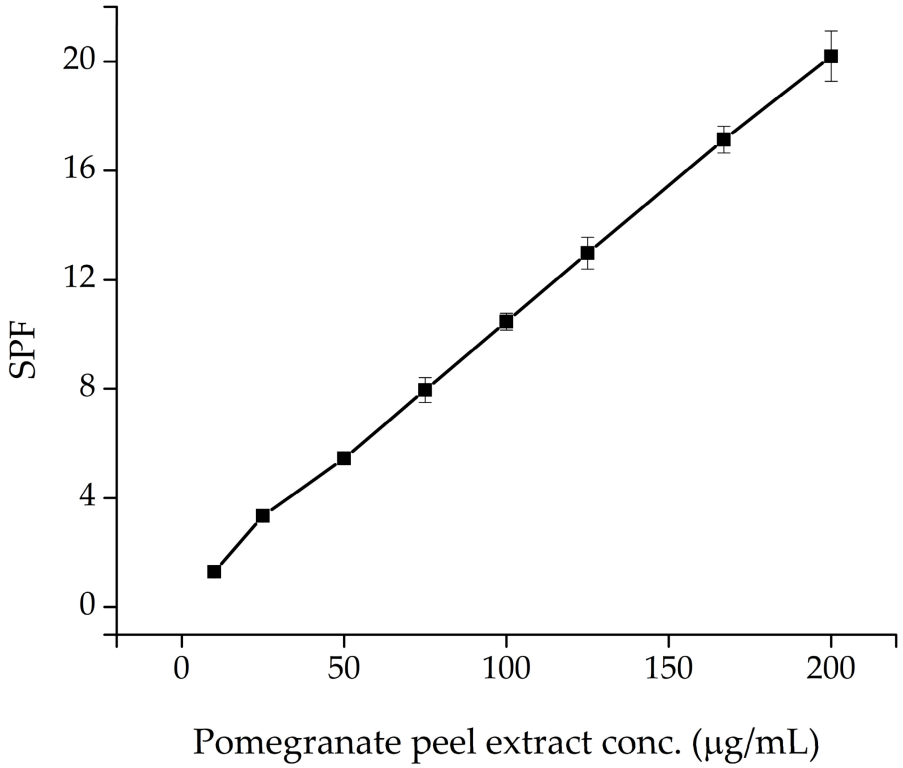


Figure 4. *In vitro* calculated SPF values of ethanolic solutions with varying concentrations of pomegranate peel extract (mean ± SD, n = 3).

4. Conclusions

The developed MEs effectively accommodated polar, high-molecular-weight phenolics from pomegranate peel and enabled controlled release of punicalagin. Statistically significant differences among formulations were observed for release behavior and antioxidant activity, while differences in skin delivery and SPF values were generally not statistically significant. Although ME-A exhibited the highest numerical values for total intra- and transdermal delivery, as well as SPF, these findings indicate only a tendency rather than a definitive superiority of this formulation.

Importantly, the formulations preserved the antioxidant activity of the extract, as assessed by the DPPH assay, and demonstrated measurable *in vitro* photoprotective potential corresponding to low UV protection (SPF ≈ 11–14).

Although punicalagin was used as a marker compound, other phenolic constituents may exhibit different release and penetration behavior, which should be addressed in future studies. Overall, the obtained results support the potential of pomegranate peel extract-loaded MEs as dermal delivery systems capable of improving solubilization and modulating skin delivery of phenolic compounds. In a broader context, the combination of bioactive compounds derived from agro-industrial waste with biocompatible surfactant systems represents a promising approach toward the development of sustainable dermal formulations.

Author Contributions: Conceptualization, N.B.P. and R.Š.; methodology, N.B.P., K.Š., J.Ž. and V.A.; software, N.B.P.; validation, D.K.R.; formal analysis, N.B.P., T.T., D.K.R. and V.A.; investigation, N.B.P., T.T., D.K.R. and V.A.; resources, K.Š. and R.Š.; data curation, D.K.R. and J.Ž.; writing—original draft preparation, N.B.P. and T.T.; writing—review and editing, N.B.P., D.K.R., K.Š. and R.Š.; visualization, N.B.P.; supervision, R.Š. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: This study did not require ethical approval according to national regulations. The Ethics Committee for the Protection and Welfare of Experimental Animals in Biomedical Research, Faculty of Medicine, University of Banja Luka, has provided a formal exemption, confirming that the procedures involved in this research do not fall under activities requiring ethical approval. All experimental procedures were conducted in accordance with ethical standards and with the aim of minimizing any potential harm.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available in this article.

Acknowledgments: The authors are grateful to Gattefosse SAS, BASF and Kao Chemicals for their kind donations of samples of Capryol™ 90 and Transcutol® P, Plantacare® 810 UP and Emanon® EV-E, respectively, and the Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-136/2025-03/200003).

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviation

The following abbreviation is used in this manuscript:

ME Microemulsion

References

1. Abdellatif, A.A.; Alawadh, S.H.; Bouazzaoui, A.; Alhowail, A.H.; Mohammed, H.A. Anthocyanins rich pomegranate cream as a topical formulation with anti-aging activity. *J. Dermatol. Treat.* **2021**, *32*, 983–990. [CrossRef]
2. Dimitrijevic, J.; Tomovic, M.; Bradic, J.; Petrovic, A.; Jakovljevic, V.; Andjic, M.; Živković, J.; Milošević, S.Đ.; Simanic, I.; Dragicevic, N. *Punica granatum* L. (Pomegranate) extracts and their effects on healthy and diseased skin. *Pharmaceutics* **2024**, *16*, 458. [CrossRef]
3. Tumbarski, Y.; Ivanov, I.; Vrancheva, R.; Mazova, N.; Nikolova, K. Pomegranate Peels: A Promising Source of Biologically Active Compounds with Potential Application in Cosmetic Products. *Cosmetics* **2025**, *12*, 169. [CrossRef]
4. Šavikin, K.; Aradski, A.A.; Jovanović, A.; Pirković, A.; Radan, M.; Nikolić, N.Ć.; Živković, J. From food waste to anti-aging solution: Harnessing the bioactive potential of pomegranate peel microparticles in skincare. *Bioorg. Chem.* **2025**, *164*, 108818. [CrossRef]
5. Bonifácio, B.V.; da Silva, P.B.; Ramos, M.A.d.S.; Negri, K.M.S.; Bauab, T.M.; Chorilli, M. Nanotechnology-based drug delivery systems and herbal medicines: A review. *Int. J. Nanomed.* **2014**, *9*, 1–15.
6. Radan, M.; Ćujić Nikolić, N.; Kuzmanović Nedeljković, S.; Mutavski, Z.; Krgović, N.; Stević, T.; Marković, S.; Jovanović, A.; Živković, J.; Šavikin, K. Multifunctional pomegranate peel microparticles with health-promoting effects for the sustainable development of novel nutraceuticals and pharmaceuticals. *Plants* **2024**, *13*, 281. [CrossRef]
7. Parisi, O.I.; Puoci, F.; Restuccia, D.; Farina, G.; Iemma, F.; Picci, N. Polyphenols and their formulations: Different strategies to overcome the drawbacks associated with their poor stability and bioavailability. In *Polyphenols in Human Health and Disease*; Watson, R.R., Preedy, V.R., Zibadi, S., Eds.; Elsevier/Academic Press: Amsterdam, The Netherlands, 2014; pp. 29–45.
8. Baccarin, T.; Lemos-Senna, E. Potential application of nanoemulsions for skin delivery of pomegranate peel polyphenols. *AAPS PharmSciTech* **2017**, *18*, 3307–3314. [CrossRef]
9. Tuncay Tanriverdi, S.; Gokce, E.H.; Filiz, S.B.; Kose, F.A.; Ozer, O. Protection of hair color with pomegranate peel extract-loaded liposomal formulation: Preparation, characterization, and ex-vivo activity studies. *J. Cosmet. Dermatol.* **2022**, *21*, 6292–6301. [CrossRef]
10. Teaima, M.H.; Badawi, N.M.; Attia, D.A.; El-Nabarawi, M.A.; Elmazar, M.M.; Mousa, S.A. Efficacy of pomegranate extract loaded solid lipid nanoparticles transdermal emulgel against Ehrlich ascites carcinoma. *Nanomed. Nanotechnol. Biol. Med.* **2022**, *39*, 102466. [CrossRef]

11. Badawi, N.M.; Teaima, M.H.; El-Say, K.M.; Attia, D.A.; El-Nabarawi, M.A.; Elmazar, M.M. Pomegranate extract-loaded solid lipid nanoparticles: Design, optimization, and in vitro cytotoxicity study. *Int. J. Nanomed.* **2018**, *13*, 1313–1326. [CrossRef]
12. Baccarin, T.; Lemos-Senna, E. Pomegranate seed oil nanoemulsions encapsulating pomegranate peel polyphenol-rich ethyl acetate fraction: Development and antioxidant assessment. *J. Nanopharm. Drug Deliv.* **2014**, *2*, 333–343. [CrossRef]
13. Fleck, A.; Cabral, P.F.; Vieira, F.F.; Pinheiro, D.A.; Pereira, C.R.; Santos, W.C.; Machado, T.B. *Punica granatum* L. hydrogel for wound care treatment: From case study to phytomedicine standardization. *Molecules* **2016**, *21*, 1059. [CrossRef]
14. Leanpolchareanchai, J.; Teeranachaideekul, V. Topical microemulsions: Skin irritation potential and anti-inflammatory effects of herbal substances. *Pharmaceuticals* **2023**, *16*, 999. [CrossRef]
15. Souto, E.B.; Cano, A.; Martins-Gomes, C.; Coutinho, T.E.; Zielińska, A.; Silva, A.M. Microemulsions and nanoemulsions in skin drug delivery. *Bioengineering* **2022**, *9*, 158. [CrossRef]
16. Lopes, L.B. Overcoming the cutaneous barrier with microemulsions. *Pharmaceutics* **2014**, *6*, 52–77. [CrossRef]
17. Barjaktarević, A.; Coneac, G.; Cupara, S.; Kostić, O.; Kostić, M.; Olariu, I.; Vlaia, V.; Cotan, A.-M.; Neamu, Ş.; Vlaia, L. Novel Alkyl-Polyglucoside-Based Topical Creams Containing Basil Essential Oil (*Ocimum basilicum* L. Lamiaceae): Assessment of Physical, Mechanical, and Sensory Characteristics. *Pharmaceutics* **2025**, *17*, 934. [CrossRef]
18. Parveen, R.; Akhtar, N.; Mahmood, T. Topical microemulsion containing *Punica granatum* extract: Its control over skin erythema and melanin in healthy Asian subjects. *Postep. Dermatol. Alergol.* **2014**, *31*, 351–355. [CrossRef]
19. Wahyuni, S.; Aisyah, R.; Bestari, R.S.; Ryanuranti, R.G.; Anggreheni, P.D. Potential of Natural Larvicide Microemulsion Based on Pomegranate Peel Extract (*Punica granatum* L) against Dengue Haemorrhagic Fever Vectors. *Biomedika* **2025**, *17*, 22–30. [CrossRef]
20. Bubic Pajic, N.Z.; Todosijevic, M.N.; Vuleta, G.M.; Cekic, N.D.; Dobricic, V.D.; Vucen, S.R.; Calija, B.R.; Lukic, M.Z.; Ilic, T.M.; Savic, S.D. Alkyl polyglucoside vs. ethoxylated surfactant-based microemulsions as vehicles for two poorly water-soluble drugs: Physicochemical characterization and in vivo skin performance. *Acta Pharm.* **2017**, *67*, 415–439. [CrossRef]
21. Grabež, M.; Škrbić, R.; Stojiljković, M.P.; Rudić-Grujić, V.; Paunović, M.; Arsić, A.; Petrović, S.; Vučić, V.; Mirjanić-Azarić, B.; Šavikin, K. Beneficial effects of pomegranate peel extract on plasma lipid profile, fatty acids levels and blood pressure in patients with diabetes mellitus type-2: A randomized, double-blind, placebo-controlled study. *J. Funct. Foods* **2020**, *64*, 103692. [CrossRef]
22. European Directorate for the Quality of Medicines & HealthCare. *European Pharmacopoeia*, 9th ed.; European Directorate for the Quality of Medicines & HealthCare: Strasbourg, France, 2017.
23. WHO. Pericarpium Granati. In *WHO Monographs on Selected Medicinal Plants*; WHO: Geneva, Switzerland, 2009; Volume 4, pp. 117–126.
24. Sahle, F.F.; Metz, H.; Wohlrab, J.; Neubert, R.H. Polyglycerol fatty acid ester surfactant–based microemulsions for targeted delivery of ceramide AP into the stratum corneum: Formulation, characterisation, in vitro release and penetration investigation. *Eur. J. Pharm. Biopharm.* **2012**, *82*, 139–150. [CrossRef]
25. USP37-NF32 (United States Pharmacopoeia 37—National Formulary 32). *Chapter 1724: Semisolid Drug Products—Performance Tests*; United States Pharmacopeial Convention: Rockville, MD, USA, 2014; pp. 1273–1284.
26. Mo, J.; Kaewnopparat, N.; Songkro, S.; Panichayupakaranant, P.; Reanmongkol, W. Physicochemical properties, in vitro release and skin permeation studies of a topical formulation of standardized pomegranate rind extract. *Pak. J. Pharm. Sci.* **2015**, *28*, 29–36.
27. Mota Ferreira, L.; Gehrcke, M.; Ferrari Cervi, V.; Eliete Rodrigues Bitencourt, P.; Ferreira da Silveira, E.; Hofstatter Azambuja, J.; Prates Ramos, A.; Nascimento, K.; Beatriz Moretto, M.; Braganhol, E. Pomegranate seed oil nanoemulsions with selective antiglioma activity: Optimization and evaluation of cytotoxicity, genotoxicity and oxidative effects on mononuclear cells. *Pharm. Biol.* **2016**, *54*, 2968–2977. [CrossRef]
28. Mansur, J.d.S.; Breder, M.; Mansur, M.; Azulay, R.D. Determination of sun protection factor by spectrophotometry. *An. Bras. Dermatol.* **1986**, *61*, 121–124.
29. Sayre, R.M.; Agin, P.P.; LeVee, G.J.; Marlowe, E. A comparison of in vivo and in vitro testing of sunscreening formulas. *Photochem. Photobiol.* **1979**, *29*, 559–566. [CrossRef]
30. Feng, L.; Yin, Y.; Fang, Y.; Yang, X. Quantitative determination of punicalagin and related substances in different parts of pomegranate. *Food Anal. Methods* **2017**, *10*, 3600–3606. [CrossRef]
31. Omer, H.A.; Abdel-Magid, S.S.; Awadalla, I.M. Nutritional and chemical evaluation of dried pomegranate (*Punica granatum* L.) peels and studying the impact of level of inclusion in ration formulation on productive performance of growing Ossimi lambs. *Bull. Natl. Res. Cent.* **2019**, *43*, 182. [CrossRef]
32. Tartaro, G.; Mateos, H.; Schirone, D.; Angelico, R.; Palazzo, G. Microemulsion microstructure (s): A tutorial review. *Nanomaterials* **2020**, *10*, 1657. [CrossRef]
33. Azarmi, S.; Roa, W.; Löbenberg, R. Current perspectives in dissolution testing of conventional and novel dosage forms. *Int. J. Pharm.* **2007**, *328*, 12–21. [CrossRef]
34. de Campos Araújo, L.M.P.; Thomazine, J.A.; Lopez, R.F.V. Development of microemulsions to topically deliver 5-aminolevulinic acid in photodynamic therapy. *Eur. J. Pharm. Biopharm.* **2010**, *75*, 48–55. [CrossRef]

35. Naoui, W.; Bolzinger, M.A.; Fenet, B.; Pelletier, J.; Valour, J.P.; Kalfat, R.; Chevalier, Y. Microemulsion microstructure influences the skin delivery of an hydrophilic drug. *Pharm. Res.* **2011**, *28*, 1683–1695. [CrossRef]
36. Delgado-Charro, M.B.; Iglesias-Vilas, G.; Blanco-Méndez, J.; López-Quintela, M.A.; Marty, J.-P.; Guy, R.H. Delivery of a hydrophilic solute through the skin from novel microemulsion systems. *Eur. J. Pharm. Biopharm.* **1997**, *43*, 37–42. [CrossRef]
37. Kraszni, M.; Marosi, A.; Larive, C.K. NMR assignments and the acid–base characterization of the pomegranate ellagitannin punicalagin in the acidic pH-range. *Anal. Bioanal. Chem.* **2013**, *405*, 5807–5816. [CrossRef] [PubMed]
38. Bubic Pajic, N.; Nikolic, I.; Mitsou, E.; Papadimitriou, V.; Xenakis, A.; Randjelovic, D.; Dobricic, V.; Smitran, A.; Cekic, N.; Calija, B.; et al. Biocompatible microemulsions for improved dermal delivery of sertaconazole nitrate: Phase behavior study and microstructure influence on drug biopharamaceutical properties. *J. Mol. Liq.* **2018**, *272*, 746–758. [CrossRef]
39. Li, J.; Mooney, D.J. Designing hydrogels for controlled drug delivery. *Nat. Rev. Mater.* **2016**, *1*, 16071. [CrossRef]
40. Korsmeyer, R.W.; Gurny, R.; Doelker, E.; Buri, P.; Peppas, N.A. Mechanisms of solute release from porous hydrophilic polymers. *Int. J. Pharm.* **1983**, *15*, 25–35. [CrossRef]
41. Dash, S. Kinetic modeling on drug release from controlled drug delivery systems. *Acta Pol. Pharm.* **2010**, *67*, 217–223.
42. Heuschkel, S.; Goebel, A.; Neubert, R.H. Microemulsions—Modern colloidal carrier for dermal and transdermal drug delivery. *J. Pharm. Sci.* **2008**, *97*, 603–631. [CrossRef]
43. Mahrhauser, D.S.; Kahlig, H.; Partyka-Jankowska, E.; Peterlik, H.; Binder, L.; Kwizda, K.; Valenta, C. Investigation of microemulsion microstructure and its impact on skin delivery of flufenamic acid. *Int. J. Pharm.* **2015**, *490*, 292–297. [CrossRef]
44. Shah, V.P.; Yacobi, A.; Radulescu, F.S.; Miron, D.S.; Lane, M.E. A science based approach to topical drug classification system (TCS). *Int. J. Pharm.* **2015**, *491*, 21–25. [CrossRef]
45. Klang, V.; Schwarz, J.C.; Lenobel, B.; Nadj, M.; Aubock, J.; Wolzt, M.; Valenta, C. In vitro vs. in vivo tape stripping: Validation of the porcine ear model and penetration assessment of novel sucrose stearate emulsions. *Eur. J. Pharm. Biopharm.* **2012**, *80*, 604–614. [CrossRef]
46. Jacobi, U.; Kaiser, M.; Toll, R.; Mangelsdorf, S.; Audring, H.; Otberg, N.; Sterry, W.; Lademann, J. Porcine ear skin: An in vitro model for human skin. *Ski. Res. Technol.* **2007**, *13*, 19–24. [CrossRef]
47. Escribano, E.; Calpena, A.C.; Queralt, J.; Obach, R.; Doménech, J. Assessment of diclofenac permeation with different formulations: Anti-inflammatory study of a selected formula. *Eur. J. Pharm. Sci.* **2003**, *19*, 203–210. [CrossRef] [PubMed]
48. Virani, A.; Dholaria, N.; Mohd, H.; Albayati, N.; Michniak-Kohn, B. Effect of chemical penetration enhancers on the transdermal delivery of olanzapine in human skin in vitro. *AAPS Open* **2024**, *10*, 4. [CrossRef]
49. Kreilgaard, M. Influence of microemulsions on cutaneous drug delivery. *Adv. Drug Deliv. Rev.* **2002**, *54*, S77–S98. [CrossRef]
50. Houston, D.M.; Robins, B.; Bugert, J.J.; Denyer, S.P.; Heard, C.M. In vitro permeation and biological activity of punicalagin and zinc (II) across skin and mucous membranes prone to Herpes simplex virus infection. *Eur. J. Pharm. Sci.* **2017**, *96*, 99–106. [CrossRef] [PubMed]
51. Pantelic, I.; Milic, J.; Vuleta, G.; Dragicevic, N.; Savic, S. Natural emulsifiers of the alkyl polyglucoside type and their influence on the permeation of drugs. In *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*; Dragicevic, N., Maibach, H.I., Eds.; Springer: Berlin/Heidelberg, Germany, 2015; pp. 231–250.
52. Carrer, V.; Alonso, C.; Pont, M.; Zanuy, M.; Córdoba, M.; Espinosa, S.; Barba, C.; Oliver, M.A.; Martí, M.; Coderch, L. Effect of propylene glycol on the skin penetration of drugs. *Arch. Dermatol. Res.* **2020**, *312*, 337–352. [CrossRef] [PubMed]
53. Rudecká, K. The Antiinflammatory Effects of Creams Based on a Pomegranate Extract and Their Penetration to the Skin. Diploma Thesis, Faculty of Pharmacy, Charles University, Hradec Kralove, Czech Republic, 2014.
54. Chasanah, U.; Sugiyanto, A.C.; Anggraeni, N.; Yuniar, V.; Ermawati, D. Characteristics, Antioxidant Activity, and Sun Protection Factor of Black Pomegranate Peel Ethanolic Extract Hydrogel. *KnE Med.* **2022**, *2*, 231–243. [CrossRef]
55. Arunprasert, K.; Pinyocheep, B.; Pratoomsuwan, P.; Rung-ananchai, R.; Satiraphan, M.; Nuntharatanapong, N.; Panomsuk, S. Optimization and evaluation of microemulsions containing grape seed oil. *Thai Bull. Pharm. Sci.* **2023**, *18*, 87–95.
56. Faizatun, F.; Tanoto, V.; Maharani, S.C. Formulation, characterization, antioxidant and sun protection factor activity of red pomegranate (*Punica granatum* L.) peel extract in sunscreen lotion. *J. Nat. Prod. Degener. Dis.* **2025**, *2*, 91–98. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.