

Article

Stable Oil-in-Water Nanoemulsions Stabilized by *Polygonatum sewerzowii* Extract: A Plant-Derived Alternative to Synthetic Surfactants

Dovletmyrat Porrykov ^{1,†}, Mahrijemal Annamyradova ^{1,*,†}, Dovran Shamamedov ² and Eszter Virág ^{3,4,*} 

¹ Department of Molecular Biology and Genetics, Oguz Han Engineering and Technology University, Ashgabat 744012, Turkmenistan; dovletmyrat.porrykov@etut.edu.tm

² Department of New Biology, Daegu Gyeongbuk Institute of Science and Technology (DGIST), Daegu 42988, Republic of Korea; dovran25@dgist.ac.kr

³ Research Institute for Medicinal Plants and Herbs Ltd., Lupaszigeti Str. 4, 2011 Budakalász, Hungary

⁴ Institute of Food Science, Faculty of Agricultural and Food Science and Environmental Management, University of Debrecen, Böszörményi Str. 138, 4032 Debrecen, Hungary

* Correspondence: annamyradovamahrijemal@gmail.com (M.A.); virag.e@gynki.hu (E.V.)

† These authors contributed equally to this work.

Featured Application

The natural emulsifying properties of *Polygonatum sewerzowii* extract highlight its potential as a plant-derived emulsifier for clean-label nanoemulsion systems. The present findings provide a basis for the further investigation of its application in food, cosmetic, and pharmaceutical formulations.

Abstract

Growing demand for clean-label ingredients has intensified the search for sustainable plant-derived emulsifiers capable of replacing synthetic surfactants in nanoformulations. In this study, the emulsifying potential of crude *Polygonatum sewerzowii* extract (PSE), obtained by optimized ultrasonic-assisted extraction, was evaluated for the preparation of oil-in-water nanoemulsions. PSE exhibited effective emulsifying properties and successfully produced stable nanoemulsions with droplet sizes of 191.7 ± 4.1 nm; $PDI = 0.034 \pm 0.023$. The formulations remained physically stable during 30 days of storage with minimal changes in droplet size and reduced the sunflower oil–water interfacial tension to 12.30 ± 0.44 mN/m at a concentration of 1% (w/w). Chemical characterization revealed that the extract contained $2.8 \pm 0.05\%$ saponins, $11.2 \pm 0.37\%$ phenolic compounds, $13.1 \pm 0.24\%$ proteins, $62.5 \pm 0.12\%$ carbohydrates, and $6.2 \pm 0.52\%$ ash, whose combined presence is likely responsible for the observed emulsifying behavior. Nanoemulsions prepared with 5% sunflower oil and 1% PSE exhibited satisfactory short-term physical stability during the investigated 30-day storage period, although changes in PDI, ζ -potential, and conductivity were observed at 25 °C. These findings identify *P. sewerzowii* extract as a promising plant-derived emulsifier for oil-in-water nanoemulsion systems. However, further studies addressing long-term stability, safety, biocompatibility, and application-specific performance are required before its suitability for food, cosmetic, or pharmaceutical applications can be established.

Keywords: *Polygonatum sewerzowii*; oil-in-water nanoemulsion; interfacial tension; droplet size; saponin; natural emulsifier



Academic Editor: Adem Gharsallaoui

Received: 1 July 2026

Revised: 10 September 2026

Accepted: 13 September 2026

Published: 17 September 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](https://creativecommons.org/licenses/by/4.0/)

[Attribution \(CC BY\)](https://creativecommons.org/licenses/by/4.0/) license.

1. Introduction

The increasing demand for sustainable and clean-label ingredients has accelerated the search for natural alternatives to synthetic emulsifiers used in nanoemulsion-based formulations for food, cosmetic, and pharmaceutical applications [1,2]. Nanoemulsions have attracted considerable attention due to their ability to improve the stability, bioavailability, and delivery efficiency of lipophilic compounds, making the selection of an appropriate emulsifier a critical factor in formulation design. Most commercially available emulsifiers are chemically synthesized and primarily serve technological functions without providing additional bioactive value [3–6]. Consequently, increasing research efforts are focused on identifying multifunctional plant-derived ingredients that combine effective surface activity with naturally occurring health-promoting compounds. Among these, saponin-rich botanical extracts have emerged as particularly promising candidates [7–9].

Saponins are naturally occurring glycosides widely distributed throughout the plant kingdom and are well-known for their amphiphilic structure and excellent surface-active properties [10,11]. Their molecular architecture, consisting of a hydrophilic sugar moiety linked to a hydrophobic steroidal or triterpenoid aglycone, enables adsorption at oil–water interfaces and facilitates the formation of stable emulsions [12,13]. The concentration and composition of saponins depend on plant species, environmental conditions, and extraction procedures, which can be optimized to maximize recovery and functionality [14,15]. Numerous studies have demonstrated that saponins not only reduce interfacial tension, but also contribute to the formation of physically stable nanoemulsions with improved resistance to coalescence and phase separation [16–18].

In recent years, considerable efforts have been devoted to identifying novel saponin-rich botanical sources suitable for use as natural emulsifiers [19–25]. Extracts obtained from plants such as Quillaja, tea, ginseng, *Tribulus terrestris*, sugar beet, *Pistacia lentiscus*, garlic, and licorice have successfully been applied to produce stable oil-in-water nanoemulsions and have demonstrated significant potential as sustainable alternatives to synthetic surfactants. Beyond applications in food, cosmetics, and pharmaceuticals, plant-derived saponins have also attracted interest in agricultural and biocontrol formulations owing to their multifunctional physicochemical and biological properties [26].

Polygonatum sewerzowii Regel is a perennial medicinal species belonging to the family *Asparagaceae* and is native to Central Asia, where its rhizomes have long been used in traditional medicine and as a functional food source [27]. Previous studies have demonstrated that *Polygonatum* species possess antioxidant, anti-inflammatory, antihyperglycemic, and anticancer activities, largely attributed to their diverse phytochemical composition [27–30]. Members of the genus are known to contain steroidal and triterpenoid saponins, flavonoids, alkaloids, and polysaccharides, suggesting that they may represent valuable sources of naturally derived emulsifying agents [31,32]. However, despite the increasing interest in plant-based emulsifiers, the emulsifying properties and nanoemulsion-forming capability of *P. sewerzowii* extracts have not yet been investigated.

The focus of the present study was to evaluate the feasibility of crude *P. sewerzowii* extract as a natural emulsifier for the preparation of oil-in-water nanoemulsions. The extract was chemically characterized with respect to its major surface-active constituents, and its emulsifying performance was assessed through measurements of interfacial tension, droplet size, particle size distribution, ζ -potential, and storage stability under different formulation conditions. This study provides the first comprehensive evaluation of *P. sewerzowii* extract as a plant-derived emulsifier for nanoemulsion systems, thereby expanding the portfolio of sustainable and clean-label ingredients for food, cosmetic, and pharmaceutical applications.

2. Materials and Methods

2.1. Plant Materials and Chemicals

Rhizomes were harvested from wild populations in the southwestern Kopetdag Mountains, Turkmenistan (38.4745701, 56.4250571) during the flowering season (Figure 1). After collection, rhizomes were rinsed with distilled water and dried at 40 °C in a convection oven until reaching a constant weight. The dried material was pulverized using an Ultra Centrifugal Mill ZM 200 (Retsch GmbH, Haan, Germany) operating at 6000 rpm to obtain fine powder (<0.5 mm). The resulting powder was stored in opaque, airtight containers at 4–5 °C to preserve its chemical integrity until further analysis.

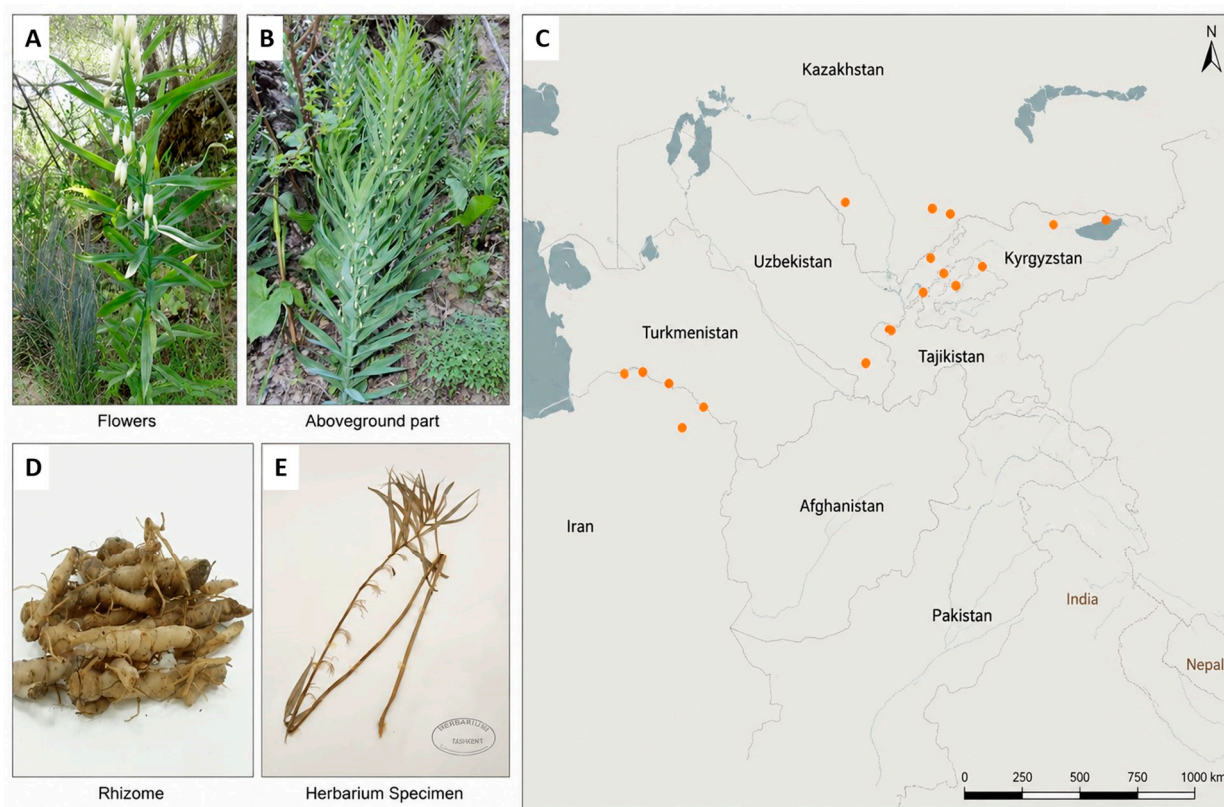


Figure 1. Morphological features and plant parts of *P. sewerzowii* Regel (Asparagaceae, Nolinoideae). Live flowering shoot with characteristic pendulous, greenish-white tubular flowers located in leaf axils (A). Fresh vegetative shoot exhibiting alternate leaf arrangement and lanceolate leaf shape with parallel venation (B). Harvested and cleaned rhizomes, which serve as storage organs for starch and secondary metabolites; used for subsequent phytochemical extraction and analysis (D). Herbarium sample, dried aerial parts showing unbranched, erect stems and withered flowers collected during the post-flowering stage, Herbarium Tashkent (E). Distribution map indicating the main growth area for this species concentrated within Central Asia. Orange dots indicate the reported geographical occurrence of *P. sewerzowii*. (C). Images were obtained by the authors.

All analytical-grade chemicals, including ethanol, diosgenin, chloroform, oleanolic acid, vanillin-acetic acid, perchloric acid, Folin–Ciocalteu reagent, gallic acid, sodium carbonate, D-glucose, phenol, sulfuric acid, DPPH reagent, and sunflower seed oil, were supplied by FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Ultrapure water used in all procedures was produced via the Alium® Comfort II purification system (Sartorius AG, Göttingen, Germany).

2.2. Sample and Extract Preparation

Crude extracts of *P. sewerzowii* rhizomes were prepared using ultrasonic-assisted extraction (UAE) as described by Gavrilă et al. and He et al. in 2022 [15,33], a widely recognized technique for efficiently isolating saponins and other bioactive phytochemicals from plant matrices. A systematic comparison of different solvent systems was beyond the scope of this study. Briefly, 10 g of powdered rhizome was extracted twice with 100 mL of 85% (v/v) aqueous ethanol (solid-to-solvent ratio 1:10 w/v) at 50 °C in a 40 kHz ultrasonic bath for 1 h. Each parameter was chosen based on optimized protocols reflecting the principle of polarity similarity and cavitation-enhanced mass transfer. The combined mixtures were centrifuged at $2280 \times g$ for 30 min (Kubota Corp., Tokyo, Japan), and the supernatant was clarified via vacuum filtration through Whatman No. 1 paper.

The choice of 85% aqueous ethanol was based on its proven efficacy in isolating saponins—amphipathic compounds with both hydrophilic and lipophilic moieties. This solvent composition offers an optimal balance between water and ethanol, effectively solubilizing both polar and non-polar constituents. Pure ethanol is less efficient at extracting hydrophilic compounds, while pure water lacks efficiency for non-polar constituents. Moreover, this ratio helps stabilize saponins and minimizes their degradation during extraction.

The filtrates were concentrated under vacuum (70 hPa, 40 °C) using a rotary evaporator (EYELA, Shanghai, China) and subsequently lyophilized at −80 °C and 5 Pa (EYELA) to obtain a dry powder. Freeze-drying was adopted to preserve thermolabile components, as evidenced by its effectiveness in retaining antioxidant and phenolic integrity in plant extracts [34]. The resultant extract was stored at −20 °C pending further analysis.

The extraction yield (EY), calculated on a dry-weight basis, was determined using Equation (1):

$$EY (\%) = \left(\frac{W_1}{W_0} \right) \times 100 \quad (1)$$

where W_1 is the weight of the freeze-dried extract, and W_0 is the initial dry weight of rhizome powder. All procedures were conducted in triplicate to ensure reproducibility.

2.3. Determination of Saponin Content

The total saponin content of the *P. sewerzowii* extract was quantified using two validated spectrophotometric techniques. In the first method where oleanolic acid was the standard, with a detection wavelength of 550 nm, the extract was refined following subsequent studies, and a standard calibration curve was constructed using oleanolic acid dissolved in chloroform (0–100 µg/mL; $R^2 = 0.9964$). Approximately 0.1 mL of extract was mixed with 0.1 mL of 5% vanillin–acetic acid reagent and 1.2 mL of 60% perchloric acid. The mixture was incubated at 60 °C for 30 min, then cooled in the dark for 10 min. An additional 5 mL of perchloric acid was added, and absorbance was immediately recorded at 550 nm against a reagent blank [35]. In the second method where diosgenin was the standard, with a detection wavelength of 240 nm, 50 mg of extract was dissolved in 3 mL chloroform. A calibration curve was prepared with diosgenin standards (1–5 mg/3 mL; $R^2 = 0.9789$). Absorbance measurements at 240 nm provided diosgenin-equivalent concentrations of steroidal saponins [36].

2.4. Determination of Total Phenolic Content

The total phenolic content (TPC) of the extract was determined using the Folin–Ciocalteu colorimetric method with gallic acid as the standard, as described by Sahu et al. (2013) [37]. A calibration curve ($R^2 = 0.9990$) was constructed using gallic acid solutions in ultrapure water at concentrations ranging from 0 to 100 µg/mL. In a test tube, 0.5 mL of the extract was mixed with 0.25 mL of Folin–Ciocalteu reagent, followed by the

addition of 2 mL of 7.5% sodium carbonate solution. The mixture was vortexed thoroughly and incubated in the dark at room temperature for 30 min. Absorbance was measured at 765 nm using a UV–Vis spectrophotometer (V-570, JASCO Co., Hachioji, Japan). A reagent blank was prepared under identical conditions without the sample extract.

2.5. Determination of Protein Content

Protein content was estimated based on total nitrogen content, determined using an organic elemental analyzer (Elementar UNICUBE, Langensfeld, Germany). Nitrogen analysis was conducted by the Chemical Analysis Division of the Research Facility Center for Science and Technology, University of Tsukuba. Protein content was calculated by multiplying the total nitrogen value by the nitrogen-to-protein conversion factor of 6.25 according to Mariotti et al. (2008) [38].

2.6. Determination of Total Carbohydrate Content

Total carbohydrate content was quantified using the phenol–sulfuric acid method with D (+)-glucose as the standard ($R^2 = 0.9964$) according to the Nielsen laboratory manual [39]. A calibration curve was constructed using glucose concentrations of 20–100 $\mu\text{g}/2\text{ mL}$ with corresponding absorbance values of 0.301, 0.519, 0.568, 0.725, and 0.834. In each assay, 2 mL of the sample extract was mixed with 0.05 mL of 80% phenol solution and 5 mL of concentrated sulfuric acid. The mixture was vortexed and incubated at 25 °C for 20 min, then cooled, and vortexed again. Absorbance was measured at 490 nm using a UV–Vis spectrophotometer. A blank containing only 2 mL ultrapure water (0 μg glucose) was used as a control.

2.7. Determination of Ash Content

Ash content was determined by incinerating the sample in a muffle furnace (Yamato FO100, Japan, Yamato Scientific Co., Ltd., Tokyo, Japan) at 600 °C for 24 h. Prior to ashing, 1 g of freeze-dried *P. sewerzowii* rhizome powder was oven-dried at 110 °C for 24 h to remove moisture. Moisture content was determined using a moisture analyzer (Shimadzu MOC63u, Japan, Shimadzu Corporation, Kyoto, Japan) at 120 °C. The ash content was calculated using Equation (2) according to Liu 2019 [40]:

$$\text{Ash content (\%, dry basis)} = \frac{W_1}{W_0} \times 100 \quad (2)$$

where W_1 is the weight of ash, and W_0 is the initial weight of the freeze-dried sample.

2.8. Determination of Antioxidant Activity

The antioxidant capacity of *Polygonatum sewerzowii* extract (PSE) was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay as described by Brand-Williams et al. (1995) [41]. Briefly, 1 mL of a 0.1 mM DPPH solution prepared in ethanol was mixed with 1 mL of PSE at concentrations of 10, 20, 30, and 40 $\mu\text{g}/\text{mL}$. The mixtures were incubated in the dark at room temperature for 30 min. The absorbance was then measured at 517 nm using a UV–Vis spectrophotometer. Ethanol served as the blank, and a DPPH solution without extract was used as the control. The radical scavenging activity was calculated as a percentage of inhibition using Equation (3):

$$\text{Inhibition(\%)} = \left(\frac{A_0 - A_1}{A_0} \right) \times 100 \quad (3)$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample. The half maximum inhibitory concentration (IC_{50} value) was determined by nonlinear regression analysis of the percentage inhibition ($I\%$) plotted against the various extract concentrations.

2.9. Interfacial Tension Measurement

Interfacial tension (IFT) of PSE solutions at different concentrations (0.1–3% w/w) was measured at 25 °C using the pendant drop method with an interfacial tensiometer (DMo-501, Kyowa Interface Science Co., Ltd., Saitama, Japan) at the sunflower oil/water interface. Measurements were taken at the sunflower oil–water interface. A 22-gauge syringe needle was used to introduce the extract solution into sunflower oil according to Rotenberg et al. (1983) [42]. The droplet image was captured at the point of maximum drop volume by a high-resolution camera, and the interfacial tension was calculated using FAMAS Format Version: 1.5 software based on the Young–Laplace equation.

2.10. Physical Characterization

The density of PSE solutions was measured using a portable densimeter (DA-130N, KEM Kyoto Electronics, Manufacturing Co., Ltd., Kyoto, Japan). The pH was determined using a digital pH meter (SevenCompact, Mettler Toledo, Greifensee, Switzerland). Viscosity was measured at 25 °C using a vibro viscometer (A&D Company, Ltd., Tokyo, Japan). Electrical conductivity was evaluated using a digital conductivity meter (SevenCompact, Mettler Toledo), and the refractive index was determined using a pocket refractometer (ATAGO, Tokyo, Japan) [43].

2.11. Preparation of Nanoemulsion

Nanoemulsions were prepared by mixing 1% (w/w) PSE in ultrapure water with sunflower oil at a 1:9 (v/v) ratio, as determined to be optimal in preliminary experiments [44,45]. To prevent microbial growth, 0.05% (w/w) sodium azide was added. Although a blank control was not included in the present study, previous studies have reported that sodium azide at this concentration has negligible influence on interfacial properties. The mixture was pre-emulsified using a high-speed homogenizer (Polytron® System PT 3100, Kinematica AG, Lucerne, Switzerland) at 10,000 rpm for 5 min. The resulting coarse emulsion was then processed using a high-pressure homogenizer (NanoVater NV200, Yoshida Kikai, Nagoya, Japan) at 100 MPa for four passes to form a nanoemulsion. The final nanoemulsion was stored at 5 °C until further analysis.

2.12. Characterization of Nanoemulsion

The volume mean droplet diameter ($d_{4,3}$) and droplet size distribution were measured using a static laser diffraction particle size analyzer (LS 13,320, Beckman Coulter, Brea, CA, USA). Zeta potential (ζ -potential) and particle size were further evaluated using a dynamic light scattering particle analyzer (Zetasizer Nano ZS, Malvern Instruments Ltd., Worcestershire, UK), after diluting the nanoemulsion 100-fold with ultrapure water. Both static light scattering (SLS) and dynamic light scattering (DLS) were employed because they provide complementary information about the nanoemulsion droplets. DLS was used to determine the hydrodynamic diameter, polydispersity index (PDI), and zeta potential of the droplets, whereas SLS provided complementary information on the particle size distribution. Using both techniques allowed us to verify the consistency of the particle size measurements and improve the reliability of the characterization.

2.13. Stability Test

Stability analysis was performed by monitoring the physicochemical properties of the nanoemulsion at Day 0 and after 30 days of storage at 5 and 25 °C. Changes in droplet size

and, where available, PDI and ζ -potential, were evaluated together with visual observations of aggregation, creaming, and phase separation.

2.14. Statistical Analysis

All experiments were performed in triplicate unless otherwise stated. Data are presented as the mean $\pm$ standard deviation (SD). Storage-stability data were evaluated descriptively, and no inferential statistical claims regarding differences between storage time points were made [46].

3. Results

3.1. Chemical Composition of PSE

Crude PSE was obtained by ultrasonic-assisted extraction using 85% (*v/v*) aqueous ethanol, resulting in an extraction yield of $21.6 \pm 0.07\%$ (*w/w*) (Table 1).

Table 1. Chemical composition of PSE.

Component	Content (% <i>w/w</i>)
Extraction yield	$21.6 \pm 0.07\%$
Saponins	2.8 ± 0.05
Phenolics	11.2 ± 0.37
Proteins	13.1 ± 0.24
Carbohydrates	62.5 ± 0.12
Ash	6.2 ± 0.52

The presence of saponins was confirmed by persistent foam formation and qualitative precipitation reactions, including the Salkowski test. Quantitative spectrophotometric analysis using oleanolic acid as the reference compound yielded a total saponin content of $2.8 \pm 0.05\%$ (*w/w*), corresponding to $28.8 \mu\text{g mg}^{-1}$ extract ($R^2 = 0.9964$). Analysis performed with diosgenin as an alternative reference standard produced comparable values ($R^2 = 0.9731$).

The total phenolic content determined by the Folin–Ciocalteu assay was $11.2 \pm 0.37\%$ (*w/w*), equivalent to $112.0 \text{ mg gallic acid equivalents (GAE) g}^{-1}$ dry extract. The calibration curve showed excellent linearity ($R^2 = 0.9990$).

Protein content, calculated from total nitrogen analysis using a conversion factor of 6.25, was $13.1 \pm 0.24\%$ (*w/w*). Non-protein nitrogen compounds may contribute to the calculated protein values. Complementary BCA or Bradford assays would provide a more accurate protein determination. Total carbohydrates, determined by the phenol–sulfuric acid assay, accounted for $62.5 \pm 0.12\%$ (*w/w*) of the extract ($R^2 = 0.9964$). The residual mineral fraction, expressed as total ash content, was $6.2 \pm 0.52\%$ (*w/w*).

The measured chemical composition of PSE is summarized in Table 1.

3.2. Antioxidant Activity

The antioxidant activity of PSE was evaluated using the DPPH radical scavenging assay. The extract exhibited concentration-dependent antioxidant activity, reaching a maximum radical scavenging activity (RSA) of $65.41 \pm 0.09\%$ at a concentration of $40 \mu\text{g mL}^{-1}$ (Figure 2).

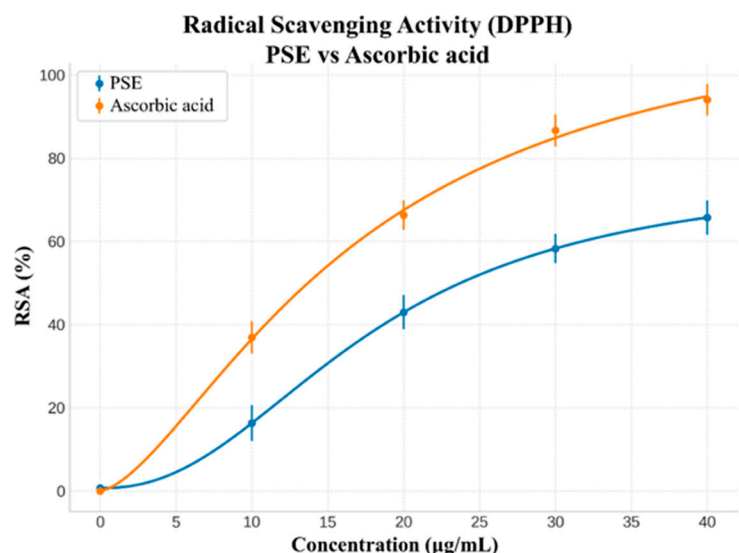


Figure 2. Dose–response curves for the RSA of PSE and the positive control (ascorbic acid) determined by the DPPH assay. Data are presented as the mean $\pm$ standard deviation (SD) of three independent measurements ($n = 3$). Curves were fitted using a four-parameter logistic (4PL) nonlinear regression model. Ascorbic acid exhibited higher RSA values across the tested concentration range, whereas both samples approached maximal scavenging activity at higher concentrations.

To evaluate the effect of nanoemulsion formulation on antioxidant properties, the DPPH radical scavenging activity of the corresponding nanoemulsion was also determined. The formulated nanoemulsion retained $57.56 \pm 0.37\%$ RSA at the equivalent concentration, corresponding to a reduction of only 7.85 percentage points compared with the free extract. This result demonstrates that the emulsification process preserved the majority of the antioxidant capacity of PSE despite the mechanical stresses associated with high-pressure homogenization.

The preservation of antioxidant activity after nanoemulsion preparation supports the further investigation of PSE as a multifunctional ingredient in nanoemulsion-based formulations.

3.3. Interfacial Tension

The interfacial activity of PSE was evaluated by measuring the sunflower oil–water IFT using the pendant drop method. As shown in Figure 3, PSE reduced the interfacial tension in a concentration-dependent manner, reaching a minimum value of $12.30 \pm 0.44 \text{ mN m}^{-1}$ at a concentration of 1% (w/w).

The observed IFT reduction demonstrates the pronounced surface activity of PSE and is consistent with the presence of amphiphilic constituents such as saponins, proteins, and phenolic compounds identified during chemical characterization. The interfacial tension obtained in the present study ($12.30 \pm 0.44 \text{ mN m}^{-1}$) is comparable to that reported for Quillaja saponins, *Verbascum nigrum* extract, ginseng saponins, and the *Limnophila aromatica* extract [10,11,16,20,24,25].

The concentration-dependent decrease in interfacial tension is also consistent with the measured saponin content of the extract ($2.8 \pm 0.05\%$, w/w), suggesting that these amphiphilic compounds play a major role in the adsorption of PSE molecules at the oil–water interface and contribute substantially to its emulsifying performance.

The measured interfacial tension of $12.30 \pm 0.44 \text{ mN m}^{-1}$ demonstrates that PSE possesses effective natural surface-active properties and supports its suitability for the stabilization of oil-in-water nanoemulsion systems.

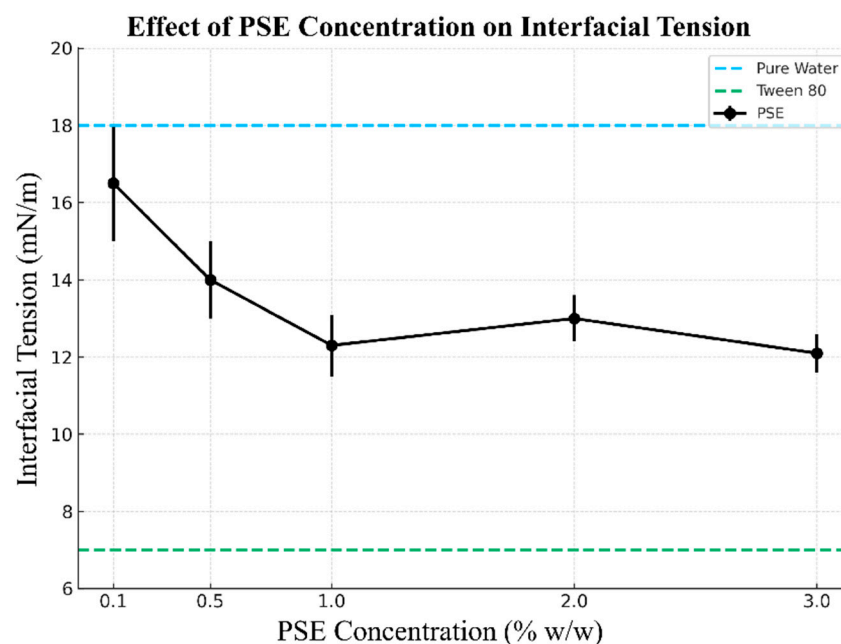


Figure 3. Interfacial tension at the sunflower oil–water interface as a function of *P. sewerzowii* extract concentration (0.1–3%, *w/w*).

3.4. Physicochemical Properties and Nanoemulsion Formation

The physicochemical properties of PSE at a concentration of 1% (*w/w*) are summarized in Table 2. The extract exhibited a density of 1.001 g cm^{−3}, viscosity of 1.46 mPa·s, total soluble solids (TSS) of 0.9 °Brix, pH of 4.65, electrical conductivity of 404.6 μS cm^{−1}, and a refractive index of 1.3330. The low viscosity and density close to that of water indicate that the extract can be readily dispersed in aqueous systems, while the moderately acidic pH and measurable electrical conductivity reflect the presence of dissolved ionic and bioactive constituents.

Table 2. Physicochemical properties of PSE at 1% (*w/w*) concentration.

Property	Value	Unit
Density	1.001	g/cm ³
Viscosity	1.46	mPa·s
TSS	0.9	°Brix
pH	4.65	—
Electrical Conductivity	404.6	μS/cm
Refractive Index	1.3330	—

The measured physicochemical parameters indicate that PSE forms a low-viscosity aqueous solution with properties favorable for high-pressure homogenization and nanoemulsion preparation. In particular, the combination of low viscosity, near-water density, and moderate conductivity is expected to facilitate efficient droplet disruption and stable dispersion formation during emulsification.

The visual appearance of the aqueous PSE solution is presented in Figure 4A,B. The extract formed a homogeneous, transparent brownish solution without visible sedimentation or phase separation, indicating good solubility under the selected preparation conditions. Such visual characteristics are advantageous for subsequent nanoemulsion preparation, as they promote uniform mixing with the oil phase prior to homogenization.

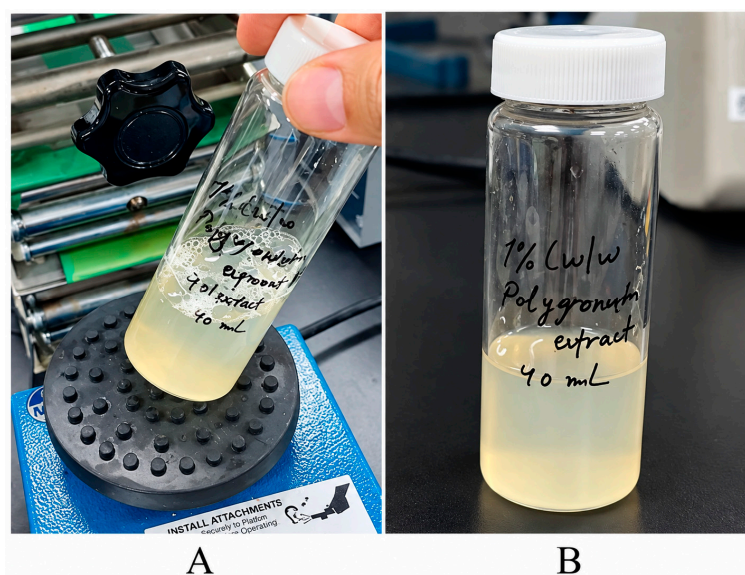


Figure 4. Visual appearance of aqueous *Polygonatum sewerzowii* extract (PSE) solution during (A) and after (B) thorough vortexing.

No macroscopic phase separation or droplet coalescence was observed, suggesting successful emulsification and the formation of a stable colloidal dispersion (Figure 5).

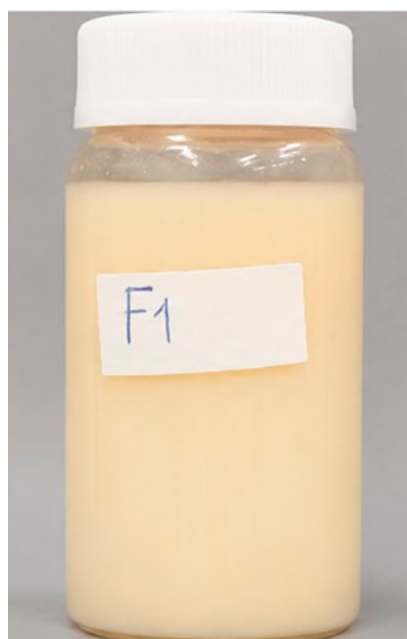


Figure 5. Macroscopic appearance of the PSE-stabilized nanoemulsion (F1).

3.5. Nanoemulsion Characteristics

The droplet size distribution of the PSE-based nanoemulsion was quantitatively characterized using laser diffraction and DLS. Laser diffraction revealed a narrow, unimodal distribution, with the highest volume fraction corresponding to droplet diameters of approximately 150–170 nm (Figure 6). No distinct secondary peak was detected within the measured size range, supporting the predominance of a nanoscale droplet population under the investigated conditions.

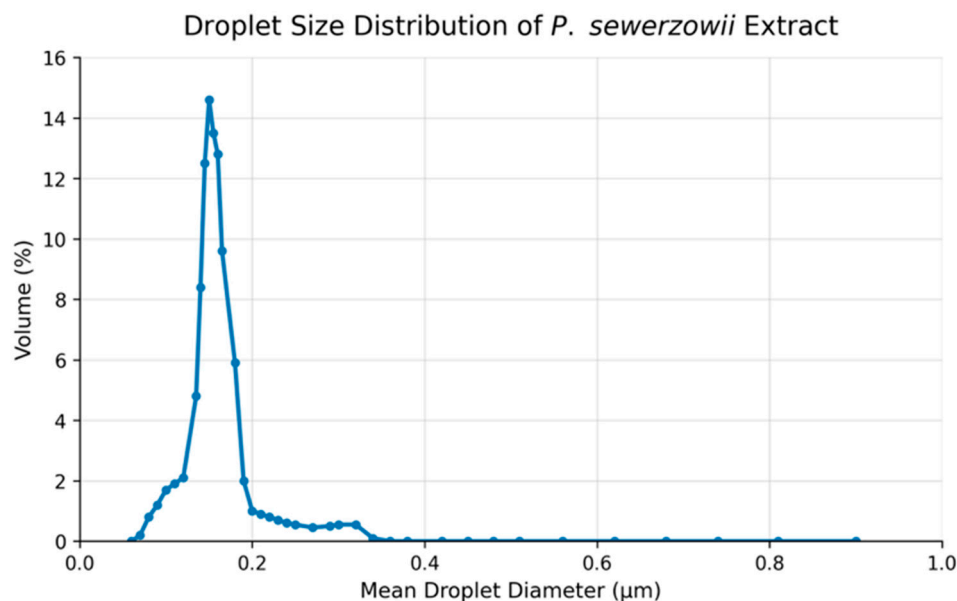


Figure 6. Droplet size distribution of the PSE-based nanoemulsion determined by laser diffraction.

Three independent batches were prepared and characterized by dynamic light scattering (DLS). The values reported in Figure 7 represent the mean $\pm$ SD of the three batches, and the individual measurements from the three independent batches have been included in Table S1 (Supplementary Materials). Analysis confirmed the uniformity of the formulation (Figure 7A). The nanoemulsion exhibited a Z-average particle diameter of 191.7 ± 4.1 nm with a low polydispersity index (PDI) of 0.034 ± 0.023 , indicating a narrowly distributed particle population. The corresponding intensity distribution consisted of a single peak accounting for 100% of the detected particles, with a standard deviation of 48.01 nm, demonstrating narrow size distribution.

The surface charge of the droplets was characterized by ζ -potential measurements (Figure 7B). The nanoemulsion displayed a mean ζ -potential of -26.7 ± 3.5 mV with a standard deviation of 4.64 mV, while the conductivity of the diluted system was 0.153 mS cm^{-1} . The negative surface charge indicates electrostatic repulsion between droplets, which contributes to colloidal stability and reduces the likelihood of droplet aggregation. The ζ -potential obtained in the present study (-26.7 ± 3.5 mV) is comparable to that reported for other plant extracts [10,11,16,20,24,25].

The low PDI together with the relatively high absolute ζ -potential value demonstrates that the PSE-based nanoemulsion possesses a homogeneous particle size distribution and favorable colloidal stability. Furthermore, the concentration-dependent reduction in interfacial tension observed in Section 3.3 and the measured saponin content of the extract ($2.8 \pm 0.05\%$, w/w) indicate that the naturally occurring amphiphilic constituents are responsible for the efficient stabilization of the oil–water interface.

The physicochemical characterization confirms that PSE enables the preparation of stable oil-in-water nanoemulsions with nanoscale droplet sizes, narrow size distribution, and appropriate electrostatic stabilization, highlighting its potential as a natural plant-derived emulsifier.

The physical storage stability of the PSE-based nanoemulsion was monitored for 30 days at 5 °C and 25 °C (Table 3). At 5 °C, minor changes in the measured physicochemical parameters were observed after 30 days. At 25 °C, the Z-average droplet size remained relatively stable, whereas the PDI increased and the absolute ζ -potential decreased, accompanied by a marked reduction in conductivity. Despite these changes, no visible phase separation, creaming, or extensive aggregation was observed during the inves-

tigated storage period. Overall, these findings support the short-term physical stability of the formulation, while indicating that storage at 25 °C resulted in greater physicochemical changes than storage at 5 °C.

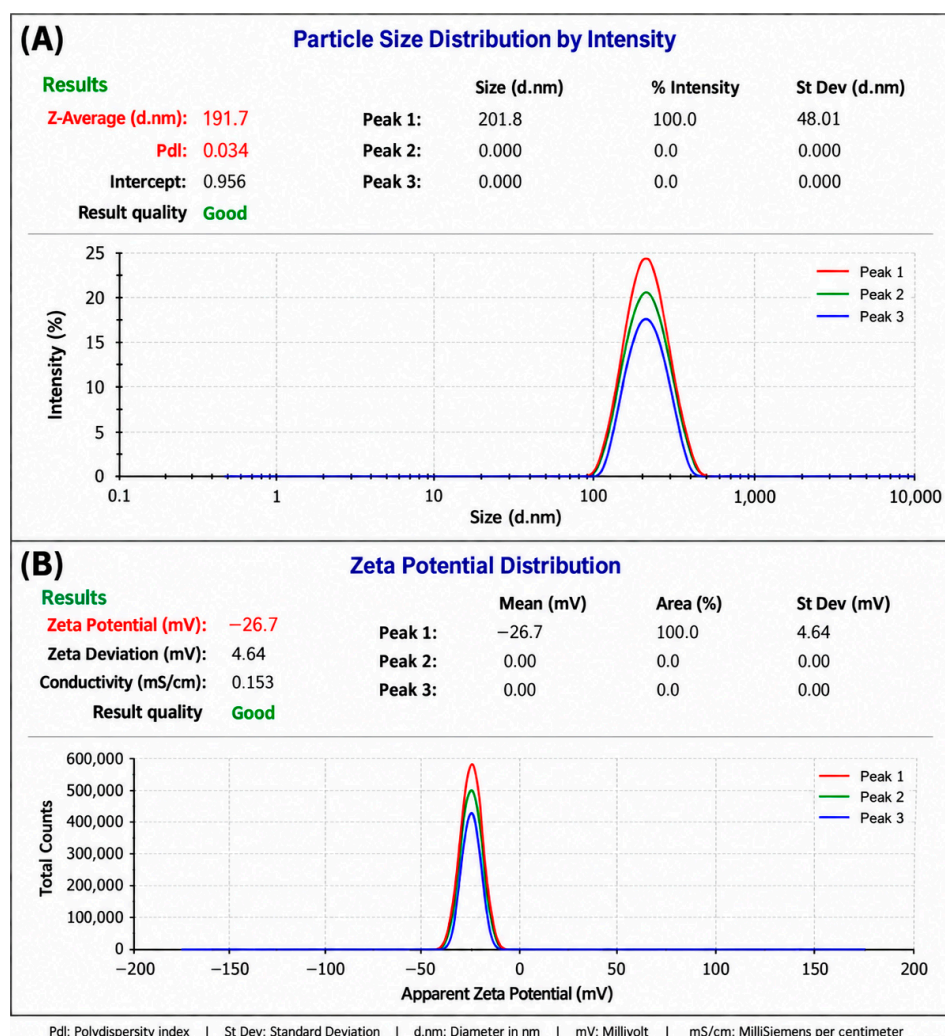


Figure 7. Representative DLS and ζ -potential distributions of the PSE nanoemulsion at day 0. The reported Z-average diameter, PDI, and ζ -potential values are expressed as mean $\pm$ standard deviation of three independently prepared batches ($n = 3$). Particle size distribution by intensity showing a Z-average diameter of 191.7 ± 4.1 nm and a polydispersity index (PDI) of 0.034 ± 0.023 (A). ζ -Potential distribution with a mean value of -26.7 ± 3.5 mV, indicating electrostatic stabilization of the nanoemulsion droplets (B).

Table 3. Physicochemical characteristics of PSE-stabilized nanoemulsions during 30 days of storage.

Temperature	Day	Z-Average (nm)	PDI	Intercept	Peak 1 Size (nm)	Peak 1 SD (nm)	Zeta Potential (mV)	Zeta Deviation (mV)	Conductivity (mS/cm)
5 °C	0	191.7	0.034	0.956	201.8	48.01	-26.7	4.64	0.1530
	SD	6.2	0.001	0.023	5.4	2.26	1.2	0.14	0.0057
5 °C	30	193.2	0.065	0.949	205.7	46.50	-24.8	4.30	0.1510
	SD	5.4	0.001	0.009	6.9	2.80	0.4	0.15	0.0067
25 °C	0	191.7	0.034	0.956	201.8	48.01	-26.7	4.64	0.1530
	SD	2.3	0.001	0.030	4.6	2.97	0.6	0.27	0.0066
25 °C	30	195.5	0.159	0.963	204.0	53.11	-20.7	7.46	0.0222
	SD	4.2	0.007	0.032	7.0	1.19	0.4	0.17	0.0008

3.6. Comparison with Literature-Reported Emulsifier Systems

To further evaluate the emulsifying performance of PSE, a comparative nanoemulsion was prepared using 1% (*w/w*) licorice (*Glycyrrhiza glabra*) root extract under identical preparation conditions. The licorice-stabilized nanoemulsion exhibited a mean droplet size of 224.3 ± 8.6 nm, a PDI of 0.169 ± 0.004 , and a zeta potential of -36.8 ± 1.4 mV ($n = 3$). These results indicate that both plant extracts are capable of producing stable nanoemulsions with narrow size distributions and high electrostatic stability. The comparative data further support the effectiveness of PSE extract as a promising natural emulsifier.

To place the performance of PSE into a broader formulation context, the physicochemical characteristics of the resulting nanoemulsion were compared with representative values reported for commonly used synthetic and plant-derived emulsifiers (Figure 8). The PSE-stabilized nanoemulsion exhibited a mean droplet diameter of 191.7 nm, a polydispersity index of 0.034 ± 0.023 , and a ζ -potential of -26.7 ± 3.5 mV. These values were comparable to those reported for nanoemulsions stabilized by Tween-20, Quillaja saponins, and tea saponins. As shown in Figure 8A, the droplet size obtained with PSE fell within the nanoscale range commonly reported for both synthetic and natural emulsifier systems. Although purified Quillaja saponins may produce somewhat smaller droplets under optimized conditions, the particle size achieved with PSE remained comparable to that of many commercial emulsifier formulations. More importantly, the PSE nanoemulsion exhibited a low PDI (Figure 8B), indicating a highly homogeneous droplet population. The measured PDI of 0.034 ± 0.023 was lower than many values reported for both Tween-20- and saponin-stabilized nanoemulsions, suggesting highly efficient droplet disruption and stabilization during high-pressure homogenization. The ζ -potential of the PSE nanoemulsion (-26.7 ± 3.5 mV) was more negative than values typically reported for Tween-20 formulations and approached the range observed for several plant-derived saponin systems (Figure 8C). This result indicates that electrostatic repulsion contributes substantially to colloidal stabilization and may complement steric stabilization mechanisms provided by surface-active constituents present in the extract. The positioning map (Figure 8D) further illustrates the relationship between droplet size and electrostatic stabilization among the evaluated emulsifier systems. PSE occupied a favorable region characterized by nanoscale droplet dimensions and sufficient surface charge to maintain colloidal stability. Despite containing only $2.8 \pm 0.05\%$ total saponins, the crude extract generated a nanoemulsion with physicochemical properties comparable to those of established synthetic and natural emulsifiers.

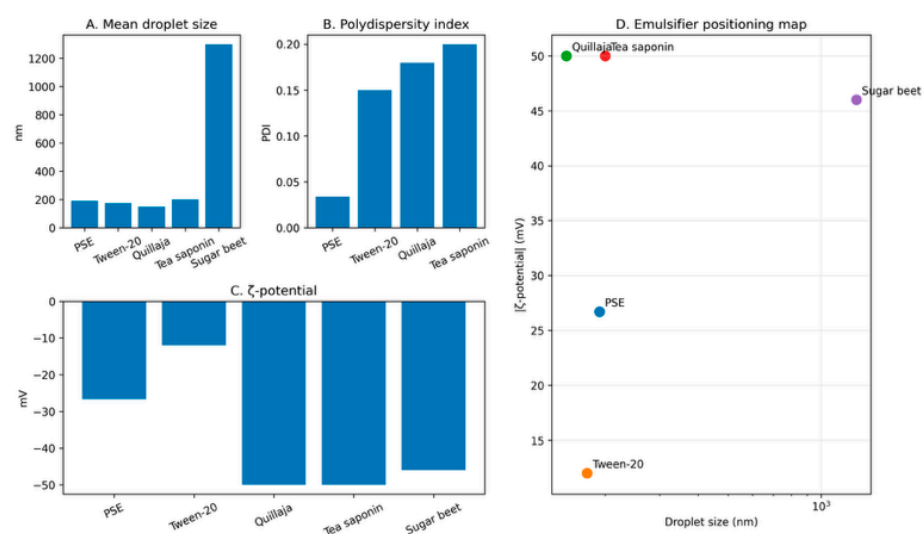


Figure 8. Comparative performance of PSE relative to representative synthetic and plant-derived emulsifiers. (A) Mean droplet size of nanoemulsions stabilized by PSE and selected literature-reported

emulsifiers. (B) Comparison of polydispersity index (PDI) values. (C) Comparison of ζ -potential values reflecting electrostatic stabilization. (D) Emulsifier positioning map showing the relationship between droplet size and absolute ζ -potential. The PSE-based nanoemulsion exhibited nanoscale droplet size (191.7 ± 4.1 nm), low polydispersity ($\text{PDI} = 0.034 \pm 0.023$), and favorable electrostatic stabilization (ζ -potential = -26.7 ± 3.5 mV), positioning it within the performance range of established synthetic and natural emulsifiers despite the use of a crude plant extract. Tween-20, Quillaja saponins, tea saponins, and sugar beet extract values were obtained from representative literature sources [6,19,21,43].

4. Discussion

4.1. Emulsifying Mechanisms of PSE

The development of sustainable and clean-label emulsifiers has become a major research focus in response to the increasing demand for naturally derived ingredients in food, cosmetic, and pharmaceutical formulations. Plant-derived saponins have attracted particular interest because their amphiphilic molecular structure enables efficient adsorption at oil–water interfaces while simultaneously providing additional biological functionalities [5,9–11,23]. In this context, the present study provides, to the best of our knowledge, the first comprehensive evaluation of crude PSE as a natural emulsifier for oil-in-water nanoemulsion systems.

A key finding of this study is that efficient nanoemulsion formation was achieved using a crude plant extract without purification of the surface-active constituents. This observation suggests that the emulsifying performance of PSE is not determined solely by its saponin content but rather by the combined action of multiple phytochemical classes. In addition to saponins, the extract contains proteins, phenolic compounds, and polysaccharides, all of which may contribute to interfacial adsorption and droplet stabilization through complementary mechanisms. Similar synergistic effects have been reported for other crude botanical extracts, including *Verbascum nigrum*, *Pistacia lentiscus*, *Limnophila aromatica*, and argan press-cake extracts, where different phytochemical groups collectively contributed to emulsion stability [16,19,20,25].

Unlike purified commercial emulsifiers, which rely predominantly on a single class of surface-active molecules, crude botanical extracts may provide multifunctional stabilization through the interaction of several naturally occurring constituents. This characteristic may represent an important advantage for the development of clean-label formulations, as it combines emulsifying functionality with the intrinsic biological activities of plant-derived compounds.

4.2. Interfacial Activity and Colloidal Stabilization

The ability of PSE to reduce the sunflower oil–water interfacial tension confirms the presence of surface-active phytochemicals capable of adsorbing at the oil–water interface. Saponins are considered the primary contributors to this behavior due to their amphiphilic structure, consisting of a hydrophobic triterpenoid or steroid aglycone linked to hydrophilic sugar residues. This molecular architecture facilitates adsorption at the interface, reduces interfacial free energy, and promotes droplet formation during homogenization [12,13]. The concentration-dependent decrease in interfacial tension observed in the present study is consistent with previous reports demonstrating enhanced interfacial adsorption and improved emulsification with increasing saponin concentration [18,47]. Although synthetic surfactants such as Tween-20 or Tween-80 generally achieve lower equilibrium interfacial tensions, the interfacial activity of PSE was comparable to that reported for several plant-derived emulsifiers, including Quillaja saponins, tea saponins, ginseng saponins, and other botanical extracts [6,11,48,49]. This finding further supports the potential of crude plant extracts as sustainable alternatives to synthetic surfactants in nanoemulsion formulations.

The favorable colloidal stability observed for the PSE-based nanoemulsion is likely the result of multiple complementary stabilization mechanisms. In addition to lowering the interfacial tension, naturally occurring proteins, polysaccharides, and phenolic compounds may contribute to the formation of a protective interfacial layer, while the negative surface charge provides electrostatic repulsion between droplets. Similar relationships between interfacial composition, surface charge, and nanoemulsion stability have been reported for systems stabilized with *Quillaja* saponins, tea saponins, ginseng saponins, and pea proteins [45,49–51].

The good physical stability observed during the 30-day storage period further supports the suitability of PSE for nanoemulsion stabilization. Nevertheless, additional stability studies under accelerated and stress conditions (e.g., centrifugation, pH, ionic strength, and shear stress) are required to further evaluate the robustness of the formulation for industrial applications.

4.3. Antioxidant Activity Retention

Another notable observation is the preservation of antioxidant activity following nanoemulsion preparation. PSE contains substantial amounts of antioxidant constituents, including phenolic compounds and polysaccharides, which are characteristic phytochemicals of the *Polygonatum* genus [52,53]. The nanoemulsion retained most of the radical scavenging activity of the free extract, indicating that the homogenization process did not substantially degrade the active phytochemicals. This finding agrees with previous reports showing that phenolic-rich plant extracts and *Polygonatum*-derived polysaccharides maintain their antioxidant capacity after encapsulation or processing [54–56]. Because phenolic compounds and polysaccharides constitute a substantial fraction of the present extract, their contribution to the measured antioxidant activity is likely significant. The preservation of antioxidant activity after homogenization further increases the practical value of PSE, as it combines emulsifying functionality with intrinsic antioxidant properties in a single natural ingredient.

4.4. Comparison with Synthetic and Natural Emulsifiers

The comparison with previously reported emulsifier systems highlights PSE as a promising candidate for further investigation as a plant-derived emulsifier. Although purified commercial saponins, particularly those derived from *Quillaja saponaria*, are widely regarded as benchmark natural emulsifiers because of their high saponin purity, the crude PSE extract produced nanoemulsions with physicochemical characteristics comparable to those reported for established plant-derived emulsifiers [9,10,48,50,51].

An important observation is that the emulsifying performance of PSE was achieved despite its relatively low total saponin content. This finding suggests that the stabilization mechanism of the crude extract is more complex than that of purified surfactants and likely involves synergistic interactions among saponins, proteins, polysaccharides, and phenolic compounds. Similar synergistic stabilization mechanisms have also been proposed for other botanical extracts, highlighting the importance of considering the entire phytochemical composition rather than individual compounds alone [16,19,20,25].

Direct experimental comparison with Tween-20 was not performed in the present study. Comparison with Tween-20 is therefore based exclusively on representative literature data and should be interpreted cautiously because formulation composition and processing conditions differ among studies. Within these limitations, the physicochemical characteristics of the PSE-stabilized nanoemulsion fell within ranges reported for nanoemulsions prepared using established synthetic and plant-derived emulsifiers.

While Tween-20 primarily stabilizes emulsions through steric hindrance, the negative surface charge observed for the PSE-based nanoemulsion suggests that electrostatic repulsion also contributes to droplet stabilization. The coexistence of steric and electrostatic stabilization mechanisms may contribute to the observed colloidal stability of the developed formulation.

In addition to its interfacial properties, the favorable physicochemical characteristics of the aqueous PSE solution, including its low viscosity and density close to that of water, likely facilitated efficient droplet disruption during homogenization [43,57]. Together with its multifunctional phytochemical composition, these properties highlight the potential of PSE as a sustainable emulsifier for the development of clean-label nanoemulsion systems in food, cosmetic, pharmaceutical, and other bio-based applications.

4.5. Future Research Directions and Application Prospects

Although the present study demonstrates the potential of PSE as a natural emulsifier, several limitations should be acknowledged. The emulsifying performance was evaluated using the crude extract, making it difficult to distinguish the contribution of individual phytochemical constituents. Furthermore, only one extraction solvent was investigated, and protein content was estimated by elemental nitrogen analysis without complementary protein-specific assays.

Future studies should optimize extraction conditions, investigate the interfacial behavior of individual extract fractions, evaluate long-term and accelerated storage stability, and assess the scalability and biological safety of the developed nanoemulsion, including biocompatibility, cytotoxicity, and hemolytic activity. Additional studies using different oil phases and formulation conditions would further support its industrial application.

Validation of the formulation at pilot and industrial scales will be necessary to confirm that the physicochemical properties and stability of the nanoemulsion can be maintained during large-scale production.

Overall, the combination of emulsifying capacity and intrinsic antioxidant activity highlights the potential of *P. sewerzowii* extract as a sustainable, plant-derived emulsifier and supports its further investigation for clean-label nanoemulsion formulations.

5. Limitation of the Study

Despite demonstrating the promising emulsifying performance of PSE, several limitations of the present study should be acknowledged. First, the experiments were conducted using a crude plant extract rather than purified or fractionated phytochemical components. Consequently, although the measured saponin content and interfacial activity strongly suggest an important role for these compounds, the relative contributions of saponins, proteins, phenolic compounds, and polysaccharides to emulsion stabilization could not be distinguished individually. Second, the physicochemical stability of the nanoemulsion was evaluated over a 30-day storage period under refrigerated and ambient conditions. While these results demonstrate good short-term stability, longer storage studies and accelerated stability tests under varying environmental conditions would provide additional information regarding shelf life and industrial applicability. Furthermore, the present work focused primarily on the physicochemical characterization of the nanoemulsion and did not investigate encapsulation efficiency, release kinetics, or the delivery of specific lipophilic bioactive compounds. Similarly, although antioxidant activity was retained after emulsification, no *in vitro* or *in vivo* studies were performed to evaluate potential improvements in bioavailability or biological efficacy.

The experiments were carried out using sunflower oil as the model lipid phase and a single optimized formulation. Future studies should investigate the applicability of PSE

with different oil matrices, formulation compositions, and processing conditions to further validate its versatility as a natural emulsifier across a broader range of food, cosmetic, and pharmaceutical systems.

Although only one extraction solvent was evaluated in this study, future work should systematically compare water, ethanol, and ethanol–water mixtures to optimize extraction efficiency and determine how solvent composition influences the emulsifying functionality of the resulting extract.

Biocompatibility, cytotoxicity, and hemolytic activity are important parameters for evaluating the potential biomedical and cosmetic applications of the developed nanoemulsions. However, the primary objective of the present study was to investigate the physicochemical properties and emulsifying performance of *Polygonatum sewerzowii* extract as a natural surfactant. Therefore, biological safety evaluations were beyond the scope of this work.

Despite these limitations, the present study provides the first comprehensive evidence that crude PSE possesses effective natural surface-active properties and can be successfully employed for the preparation of stable oil-in-water nanoemulsions, thereby establishing a foundation for future mechanistic and application-oriented investigations.

6. Conclusions

This study demonstrates, for the first time, that crude PSE can serve as an effective natural emulsifier for the preparation of stable oil-in-water nanoemulsions. The extract exhibited pronounced surface activity, reducing the sunflower oil–water interfacial tension to 12.30 mN m^{-1} and enabling the formation of homogeneous nanoemulsions with nanoscale droplet sizes, a low polydispersity index, and favorable ζ -potential, resulting in favorable colloidal stability.

The multifunctional composition of PSE, including saponins, phenolic compounds, proteins, and carbohydrates, is likely responsible for its emulsifying performance and preserved antioxidant activity after nanoemulsion preparation. These findings identify *P. sewerzowii* extract as a promising plant-derived emulsifier for oil-in-water nanoemulsion systems. However, further studies addressing long-term stability, formulation robustness, safety, biocompatibility, and application-specific performance are required before its suitability for food, cosmetic, or pharmaceutical applications can be established.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/app16189219/s1>, Table S1. Batch-to-batch reproducibility of PSE nanoemulsion.

Author Contributions: Conceptualization, M.A. and D.P.; methodology, M.A. and D.P.; validation, E.V., M.A. and D.P.; formal analysis, E.V.; investigation, M.A.; resources, E.V. and M.A.; data curation, E.V.; writing—original draft preparation, M.A. and D.P.; writing—review and editing, E.V.; visualization, D.S.; supervision, M.A.; project administration, D.P. and E.V.; funding acquisition, E.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the University of Debrecen Scientific Publication Research Support Program.

Data Availability Statement: The data supporting the findings of this study are available within the article. Additional data are available from the corresponding author upon reasonable request.

Conflicts of Interest: Dr. Eszter Virág was employed by Research Institute for Medicinal Plants and Herbs Ltd. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

PSE	<i>Polygonatum sewerzowii</i> extract
PDI	Polydispersity index
UAE	Ultrasonic-assisted extraction
TPC	Total phenolic content
IFT	Interfacial tension
GAE	Gallic acid equivalents
RSA	Radical scavenging activity
DLS	Dynamic light scattering

References

1. Ashaolu, T.J. Nanoemulsions for health, food, and cosmetics: A review. *Environ. Chem. Lett.* **2021**, *19*, 3381–3395. [[CrossRef](#)] [[PubMed](#)]
2. Aswathanarayan, J.B.; Vittal, R.R. Nanoemulsions and their potential applications in food industry. *Front. Sustain. Food Syst.* **2019**, *3*, 95. [[CrossRef](#)]
3. Dammak, I.; Sobral, P.J.d.A.; Aquino, A.; Neves, M.A.d.; Conte-Junior, C.A. Nanoemulsions: Using emulsifiers from natural sources replacing synthetic ones—A review. *Compr. Rev. Food Sci. Food Saf.* **2020**, *19*, 2721–2746. [[CrossRef](#)] [[PubMed](#)]
4. McClements, D.J.; Gumus, C.E. Natural emulsifiers—Biosurfactants, phospholipids, biopolymers, and colloidal particles: Molecular and physicochemical basis of functional performance. *Adv. Colloid Interface Sci.* **2016**, *234*, 3–26. [[CrossRef](#)] [[PubMed](#)]
5. McClements, D.J.; Bai, L.; Chung, C. Recent advances in the utilization of natural emulsifiers to form and stabilize emulsions. *Annu. Rev. Food Sci. Technol.* **2017**, *8*, 205–236. [[CrossRef](#)] [[PubMed](#)]
6. Zhu, Z.; Wen, Y.; Yi, J.; Cao, Y.; Liu, F.; McClements, D.J. Comparison of natural and synthetic surfactants at forming and stabilizing nanoemulsions: Tea saponin, Quillaja saponin, and Tween 80. *J. Colloid Interface Sci.* **2019**, *536*, 80–87. [[CrossRef](#)] [[PubMed](#)]
7. Adharsh Ashok, N.; Sontakke, M. Plant-based emulsifiers: Sources, extraction, properties and applications. *Pharma Innov.* **2023**, *12*, 8–16. [[CrossRef](#)]
8. Rai, S.; Acharya-Siwakoti, E.; Kafle, A.; Devkota, H.P.; Bhattarai, A. Plant-derived saponins: A review of their surfactant properties and applications. *Sci* **2021**, *3*, 44. [[CrossRef](#)]
9. Schreiner, T.B.; Dias, M.M.; Barreiro, M.F.; Pinho, S.P. Saponins as natural emulsifiers for nanoemulsions. *J. Agric. Food Chem.* **2022**, *70*, 6573–6590. [[CrossRef](#)] [[PubMed](#)]
10. Reichert, C.L.; Salminen, H.; Weiss, J. Quillaja saponin characteristics and functional properties. *Annu. Rev. Food Sci. Technol.* **2019**, *10*, 43–73. [[CrossRef](#)] [[PubMed](#)]
11. Schreiner, T.B.; Colucci, G.; Santamaria-Echart, A.; Fernandes, I.P.; Dias, M.M.; Pinho, S.P.; Barreiro, M.F. Evaluation of saponin-rich extracts as natural alternative emulsifiers: A comparative study with pure Quillaja Bark saponin. *Colloids Surf. A Physicochem. Eng. Asp.* **2021**, *623*, 126748. [[CrossRef](#)]
12. El Aziz, M.M.A.; Ashour, A.S.; Melad, A.S.G. A review on saponins from medicinal plants: Chemistry, isolation, and determination. *J. Nanomed. Res.* **2019**, *8*, 282–288. [[CrossRef](#)]
13. Timilsena, Y.P.; Phosanam, A.; Stockmann, R. Perspectives on saponins: Food functionality and applications. *Int. J. Mol. Sci.* **2023**, *24*, 13538. [[CrossRef](#)] [[PubMed](#)]
14. Fink, R.; Filip, S. Surface-active natural saponins. Properties, safety, and efficacy. *Int. J. Environ. Health Res.* **2023**, *33*, 639–648. [[CrossRef](#)] [[PubMed](#)]
15. He, S.; Wang, X.; Chen, J.; Li, X.; Gu, W.; Zhang, F.; Cao, G.; Yu, J. Optimization of the ultrasonic-assisted extraction Technology of Steroidal Saponins from *Polygonatum kingianum* Collett & Hemsl and evaluating its quality planted in different areas. *Molecules* **2022**, *27*, 1463. [[CrossRef](#)] [[PubMed](#)]
16. Taarji, N.; da Silva, C.A.R.; Khalid, N.; Gadhi, C.; Hafidi, A.; Kobayashi, I.; Neves, M.A.; Isoda, H.; Nakajima, M. Formulation and stabilization of oil-in-water nanoemulsions using a saponins-rich extract from argan oil press-cake. *Food Chem.* **2018**, *246*, 457–463. [[CrossRef](#)] [[PubMed](#)]
17. Jarzębski, M.; Siejak, P.; Smulek, W.; Fathordoobady, F.; Guo, Y.; Pawlicz, J.; Trzeciak, T.; Kowalczewski, P.L.; Kitts, D.D.; Singh, A. Plant extracts containing saponins affects the stability and biological activity of hempseed oil emulsion system. *Molecules* **2020**, *25*, 2696. [[CrossRef](#)] [[PubMed](#)]
18. Tsibranska, S.; Tcholakova, S.; Golemanov, K.; Denkov, N.; Pelan, E.; Stoyanov, S.D. Role of interfacial elasticity for the rheological properties of saponin-stabilized emulsions. *J. Colloid Interface Sci.* **2020**, *564*, 264–275. [[CrossRef](#)] [[PubMed](#)]

19. Feki, F.; Taarji, N.; Vodo, S.; Chamkha, M.; Bouallagui, Z.; Nakajima, M.; Isoda, H.; Neves, M.A.; Sayadi, S. Physicochemical Stability Enhancement of β -Carotene-Rich O/W Nanoemulsions Using a New Natural Emulsifier Developed from *Pistacia lentiscus* Fruit Residue. *Food Biophys.* **2023**, *18*, 249–261. [\[CrossRef\]](#)
20. Jarzębski, M.; Smułek, W.; Kosciński, M.; Białopiotrowicz, T.; Kaczorek, E. *Verbascum nigrum* L. (mullein) extract as a natural emulsifier. *Food Hydrocoll.* **2018**, *81*, 341–350. [\[CrossRef\]](#)
21. Ralla, T.; Salminen, H.; Edelman, M.; Dawid, C.; Hofmann, T.; Weiss, J. Stability of emulsions using a new natural emulsifier: Sugar beet extract (*Beta vulgaris* L.). *Food Biophys.* **2017**, *12*, 269–278. [\[CrossRef\]](#)
22. Salminen, H.; Kasapoğlu, K.N.; Özçelik, B.; Weiss, J. Stabilization of solid lipid nanoparticles with glycyrrhizin. *Eur. Food Res. Technol.* **2023**, *249*, 787–798. Correction in *Eur. Food Res. Technol.* **2023**, *249*, 1147. [\[CrossRef\]](#)
23. Schreiner, T.B.; Santamaria-Echart, A.; Peres, A.M.; Dias, M.M.; Pinho, S.P.; Barreiro, M.F. Study of binary mixtures of *Tribulus terrestris* extract and *Quillaja bark* saponin as oil-in-water nanoemulsion emulsifiers. *J. Surfactants Deterg.* **2024**, *27*, 123–133. [\[CrossRef\]](#)
24. Shu, G.; Khalid, N.; Chen, Z.; Neves, M.A.; Barrow, C.J.; Nakajima, M. Formulation and characterization of astaxanthin-enriched nanoemulsions stabilized using ginseng saponins as natural emulsifiers. *Food Chem.* **2018**, *255*, 67–74. [\[CrossRef\]](#) [\[PubMed\]](#)
25. Soeung, R.; Felipe, L.d.O.; Bouhoute, M.; Taarji, N.; Nakajima, M.; Neves, M.A. *Limnophila aromatica* crude extracts as natural emulsifiers for formation and stabilizing of oil-in-water (O/W) emulsions. *Colloids Interfaces* **2022**, *6*, 26. [\[CrossRef\]](#)
26. Syed-Ab-Rahman, S.F.; Carvalhais, L.C.; Omar, D. Development of plant-based emulsion formulations to control bacterial leaf blight and sheath brown rot of rice. *Heliyon* **2020**, *6*, e03151. [\[CrossRef\]](#) [\[PubMed\]](#)
27. Berdimuhamedov, G. *Medicinal Plants of Turkmenistan. I–XI Volumes*; TDNG: Ashgabat, Turkmenistan, 2009.
28. Li, X.-L.; Ma, R.-H.; Zhang, F.; Ni, Z.-J.; Thakur, K.; Wang, S.; Zhang, J.-G.; Wei, Z.-J. Evolutionary research trend of *Polygonatum* species: A comprehensive account of their transformation from traditional medicines to functional foods. *Crit. Rev. Food Sci. Nutr.* **2023**, *63*, 3803–3820. [\[CrossRef\]](#) [\[PubMed\]](#)
29. Luo, L.; Qiu, Y.; Gong, L.; Wang, W.; Wen, R. A review of *Polygonatum* Mill. genus: Its taxonomy, chemical constituents, and pharmacological effect due to processing changes. *Molecules* **2022**, *27*, 4821. [\[CrossRef\]](#) [\[PubMed\]](#)
30. Zhao, P.; Zhao, C.; Li, X.; Gao, Q.; Huang, L.; Xiao, P.; Gao, W. The genus *Polygonatum*: A review of ethnopharmacology, phytochemistry and pharmacology. *J. Ethnopharmacol.* **2018**, *214*, 274–291. [\[CrossRef\]](#) [\[PubMed\]](#)
31. Ning, H.; Yuan, M.; Wu, Q.; Ping, Y.; Zhou, Z.; Xu, Y.; Wu, Y.; Yin, H. Identification of chemical constituents from *Polygonatum cyrtoneura*. *Chin. J. Exp. Tradit. Med. Formulae* **2018**, *24*, 77–82.
32. Zhao, X.; Patil, S.; Qian, A.; Zhao, C. Bioactive compounds of *Polygonatum sibiricum*-therapeutic effect and biological activity. *Endocr. Metab. Immune Disord.-Drug Targets (Former. Curr. Drug Targets-Immune Endocr. Metab. Disord.)* **2022**, *22*, 26–37. [\[CrossRef\]](#) [\[PubMed\]](#)
33. Gavrilă, A.I.; Tatia, R.; Seciu-Grama, A.-M.; Tarcomnicu, I.; Negrea, C.; Calinescu, I.; Zalaru, C.; Moldovan, L.; Raiciu, A.D.; Popa, I. Ultrasound assisted extraction of saponins from *Hedera helix* L. and an in vitro biocompatibility evaluation of the extracts. *Pharmaceuticals* **2022**, *15*, 1197. [\[CrossRef\]](#) [\[PubMed\]](#)
34. da Silva Júnior, M.E.; Araújo, M.V.R.L.; Martins, A.C.S.; dos Santos Lima, M.; Da Silva, F.L.H.; Converti, A.; Maciel, M.I.S. Microencapsulation by spray-drying and freeze-drying of extract of phenolic compounds obtained from ciriguela peel. *Sci. Rep.* **2023**, *13*, 15222. [\[CrossRef\]](#) [\[PubMed\]](#)
35. Hiai, S.; Oura, H.; Nakajima, T. Color reaction of some saponins and saponins with vanillin and sulfuric acid. *Planta Medica* **1976**, *29*, 116–122. [\[CrossRef\]](#) [\[PubMed\]](#)
36. Zhaobao, X.; Chunhong, T.; Gang, C.; Zhisong, S. Studied on colorimetric determination of oleanolic acid in Chinese quince. *Nat. Prod. Res. Dev.* **2001**, *13*, 23–26.
37. Sahu, R.; Saxena, J. Screening of total phenolic and flavonoid content in conventional and non-conventional species of curcuma. *J. Pharmacogn. Phytochem.* **2013**, *2*, 176–179.
38. Mariotti, F.; Tomé, D.; Mirand, P.P. Converting nitrogen into protein—Beyond 6.25 and Jones' factors. *Crit. Rev. Food Sci. Nutr.* **2008**, *48*, 177–184. [\[CrossRef\]](#) [\[PubMed\]](#)
39. Nielsen, S.S. *Food Analysis*, 4th ed.; Springer: New York, NY, USA, 2010.
40. Liu, K. Effects of sample size, dry ashing temperature and duration on determination of ash content in algae and other biomass. *Algal Res.* **2019**, *40*, 101486. [\[CrossRef\]](#)
41. Brand-Williams, W.; Cuvelier, M.-E.; Berset, C. Use of a free radical method to evaluate antioxidant activity. *LWT-Food Sci. Technol.* **1995**, *28*, 25–30. [\[CrossRef\]](#)
42. Rotenberg, Y.; Boruvka, L.; Neumann, A. Determination of surface tension and contact angle from the shapes of axisymmetric fluid interfaces. *J. Colloid Interface Sci.* **1983**, *93*, 169–183. [\[CrossRef\]](#)
43. McClements, D.J. *Food Emulsions: Principles, Practices, and Techniques*; CRC Press: Boca Raton, FL, USA, 2004.
44. Mahdi Jafari, S.; He, Y.; Bhandari, B. Nano-emulsion production by sonication and microfluidization—A comparison. *Int. J. Food Prop.* **2006**, *9*, 475–485. [\[CrossRef\]](#)

45. Tadros, T.; Izquierdo, P.; Esquena, J.; Solans, C. Formation and stability of nano-emulsions. *Adv. Colloid Interface Sci.* **2004**, *108*, 303–318. [[CrossRef](#)] [[PubMed](#)]
46. Montgomery, D.C. *Design and Analysis of Experiments*; John Wiley & Sons: Hoboken, NJ, USA, 2017.
47. Mitra, S.; Dungan, S.R. Micellar properties of Quillaja saponin. 1. Effects of temperature, salt, and pH on solution properties. *J. Agric. Food Chem.* **1997**, *45*, 1587–1595. [[CrossRef](#)]
48. Ozturk, B.; McClements, D.J. Progress in natural emulsifiers for utilization in food emulsions. *Curr. Opin. Food Sci.* **2016**, *7*, 1–6. [[CrossRef](#)]
49. Ozturk, B.; Argin, S.; Ozilgen, M.; McClements, D.J. Formation and stabilization of nanoemulsion-based vitamin E delivery systems using natural surfactants: Quillaja saponin and lecithin. *J. Food Eng.* **2014**, *142*, 57–63. [[CrossRef](#)]
50. Shi, Z.-Y.; Zeng, J.-Z.; Wong, A.S.T. Chemical structures and pharmacological profiles of ginseng saponins. *Molecules* **2019**, *24*, 2443. [[CrossRef](#)] [[PubMed](#)]
51. Jarzębski, M.; Fathordoobady, F.; Guo, Y.; Xu, M.; Singh, A.; Kitts, D.D.; Kowalczewski, P.L.; Jeżowski, P.; Pratap Singh, A. Pea protein for hempseed oil nanoemulsion stabilization. *Molecules* **2019**, *24*, 4288. [[CrossRef](#)] [[PubMed](#)]
52. Liu, C.-x.; Cao, Y.; Xia, Q.-l. Study on the extraction processing and antioxidant activities of saponins from *Polygonatum cyrtoneura* Hua roots. *Acta Agric. Zhejiangensis* **2024**, *36*, 1144–1152.
53. Wang, Z.; Liu, H.; Fu, R.; Ou, J.; Wang, B. Structural characterization and anti-inflammatory activity of a novel polysaccharide PKP2-1 from *Polygonatum kingianum*. *Front. Nutr.* **2023**, *10*, 1156798. [[CrossRef](#)] [[PubMed](#)]
54. Sun, C.; Wang, G.; Sun, J.; Yin, J.; Huang, J.; Li, Z.; Mu, D.; He, M.; Liu, T.; Cheng, J. A new method of extracting *Polygonatum sibiricum* polysaccharide with antioxidant function: Ultrasound-assisted extraction-deep eutectic solvents method. *Foods* **2023**, *12*, 3438. [[CrossRef](#)] [[PubMed](#)]
55. Jiang, H.; Xu, Y.; Sun, C.; Adu-Frimpong, M.; Yu, J.; Deng, W.; Xu, X. Physicochemical properties and antidiabetic effects of a polysaccharide obtained from *Polygonatum odoratum*. *Int. J. Food Sci. Technol.* **2018**, *53*, 2810–2822. [[CrossRef](#)]
56. Li, J.; Wang, Z.; Fan, M.; Hu, G.; Guo, M. Potential Antioxidative and Anti-Hyperuricemic Components Targeting Superoxide Dismutase and Xanthine Oxidase Explored from *Polygonatum sibiricum* Red. *Antioxidants* **2022**, *11*, 1651. [[CrossRef](#)] [[PubMed](#)]
57. Mekala, S.; Silva, E.K.; Saldaña, M.D. Mechanism, kinetics, and physicochemical properties of ultrasound-produced emulsions stabilized by lentil protein: A non-dairy alternative in food systems. *Eur. Food Res. Technol.* **2022**, *248*, 185–196. [[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.