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"Cosmetic Potential of Stellaria alsine Extract: Anti-Aging, Anti-Inflammatory, and Brightening Properties of an Under-rated Botanical"

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1. Introduction

The modern cosmetics industry is evolving rapidly beyond mere skin care, reflecting consumer demand for health, sustainability, and high-functionality. In particular, interest in natural plant-derived ingredients has grown substantially alongside a broader preference for clean beauty, leading to the exploration and evaluation of underutilized plant resources as potential bioactive ingredients for cosmetics. Plants belonging to the Stellaria genus are distributed globally and have been traditionally used in folk medicine for treating skin inflammation, diuresis, and wound healing. While Stellaria media has been relatively well-studied, Stellaria alsine var. undulata, a subspecies native to East Asia including Korea, remains poorly explored despite its similar or potentially superior bioactivities. [2](Lim WT et al). Scientific studies have demonstrated that the antioxidant and anti-inflammatory properties of plant extracts are primarily attributed to the presence of phenolic and flavonoid compounds. [4] (Mishra AP et al). These act by scavenging reactive oxygen species (ROS), regulating melanin synthesis, and modulating inflammatory mediators, thereby promoting skin health. However, these effects are highly dependent on extraction conditions, which makes optimization of extraction methods a critical step in developing effective natural ingredients. Among various extraction technologies, ultrasonic-assisted extraction (UAE) and supercritical fluid extraction (SFE) are gaining attention as eco-friendly methods. [3] (Xu Y et al). UAE offers high efficiency at low temperatures in short time periods, while SFE with ethanol as a co-solvent can recover both polar and non-polar compounds with minimal solvent residue. These methods are ideal for preserving thermolabile bioactives.To overcome limitations such as poor skin permeability and low stability of natural extracts, nanoencapsulation technology has been increasingly applied in recent years. Nano-sized delivery systems enhance the stability of bioactives, improve dermal penetration, and

significantly increase bioavailability. [7] (Cho Y et al). Prior studies have shown that nanoformulation of botanical extracts can augment their whitening, anti-aging, and anti-inflammatory efficacy. In this study, we aimed to systematically evaluate the cosmeceutical potential of Stellaria alsine var. undulata, a scientifically underexplored plant. Optimized ultrasonic ethanol extraction (UAE 80%) and supercritical ethanol extraction (SFE-EtOH) were applied to obtain functional extracts. The extracts were analyzed for active compound content (notably Roseoside), and their antioxidant, anti-inflammatory, anti-aging, and skin-brightening effects were evaluated using in vitro assays. In addition, nanoencapsulation was carried out to enhance functional efficacy and potential applicability in cosmetic formulations.This research highlights the value of an underrated botanical and introduces a novel candidate for future high-performance natural cosmetics. It contributes to the development of next-generation skincare solutions that meet both sustainability and innovation goals.

2. Materials and Methods

2.1. Plant Material and Extraction

Stellaria alsine var. undulata was collected in April 2024 from Jangseong-gun, Jeollanam-do, Republic of Korea. The plant was taxonomically identified and a voucher specimen was deposited in the laboratory. After ultrasonic washing (5–10 minutes), the samples were dried at 45°C for 24 hours and ground into fine powder. Two types of extracts were prepared: (1) ultrasonic-assisted extraction using 80% ethanol (UAE), and (2) supercritical fluid extraction with ethanol as co-solvent (SFE-EtOH). UAE was performed by treating 1 g of dried powder with 10 mL of 80% ethanol under ultrasonication at 37°C for 90 minutes (two cycles), followed by filtration and rotary vacuum concentration. SFE-EtOH was conducted at 400 bar and 50°C for 120 minutes using CO_2 with 5 mL ethanol as co-solvent. All extracts were filtered through a 0.2 μm PTFE membrane and stored at 1°C.

2.2. Bioactive Compound Analysis

The identification and quantification of bioactive compounds in the extracts were conducted using ultra-high-performance liquid chromatography coupled with Orbitrap mass spectrometry (UHPLC-Orbitrap-MS). Roseoside, a sesquiterpenoid compound with strong antioxidant potential and lipid oxidation inhibition activity, was selected as a marker compound. [1] (Kim CK et al). Analyses were performed at Sunchon National University using a Vanquish UHPLC system and an Orbitrap Exploris 120 mass spectrometer.

2.3. Quantification of Roseoside

Quantitative analysis of Roseoside was carried out on the two extracts of Stellaria alsine var. undulata: (1) UAE (80% ethanol ultrasonic extract), and (2) SFE-EtOH (supercritical fluid extract using ethanol as co-solvent).

2.4. Functional Assays

Functional evaluations included: (1) total phenolic content (TPC) analysis using the Folin–Ciocalteu method; (2) antioxidant activity by DPPH radical scavenging assay; (3) SOD-like

activity assay. Whitening efficacy was assessed via tyrosinase inhibition assay, and anti-aging activity was determined by elastase inhibition assay. Anti-inflammatory effects were evaluated by measuring nitric oxide (NO) production in LPS-induced RAW264.7 macrophage cells. All assays were conducted in triplicate with appropriate positive controls.

2.5. Nanoencapsulation

To enhance the bioavailability and stability of bioactive compounds, nanoencapsulation was performed using agar-based nanoemulsion technology. The average particle size was adjusted to 100–200 nm. Morphological evaluation was conducted by transmission electron microscopy (TEM), and zeta potential was measured to assess colloidal stability. These analyses were carried out at Chonnam National University.

3. Results

3.1. Roseoside Content in Extracts

UHPLC-Orbitrap-MS analysis revealed that the content of Roseoside, a sesquiterpenoid compound with known antioxidant potential, was significantly higher in the SFE-EtOH extract (0.41 ± 0.02 mg/g) than in the UAE extract (0.07 ± 0.03 mg/g) ($p < 0.05$). This indicates that the use of supercritical ethanol as a co-solvent effectively enhanced the extraction efficiency of lipophilic bioactives, including Roseoside. Table 1 compares the Roseoside content between both extracts, and Figure 1a displays the Extracted Ion Chromatogram (XIC) confirming the presence and relative abundance of Roseoside. In addition, Figure 1b provides a visual comparison of the quantified Roseoside levels, clearly demonstrating the enhanced yield in SFE-EtOH.

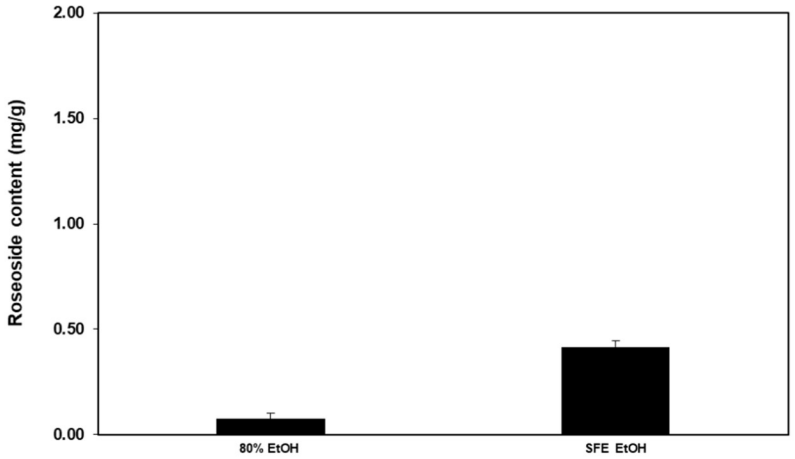

Table 1. Roseoside Content Analysis in UAE and SFE-EtOH Extracts.

Marker compounds	Amount (ppm)	Content (mg/g)
80% EtOH	7.70	0.07
SFE-EtOH	43.61	0.41

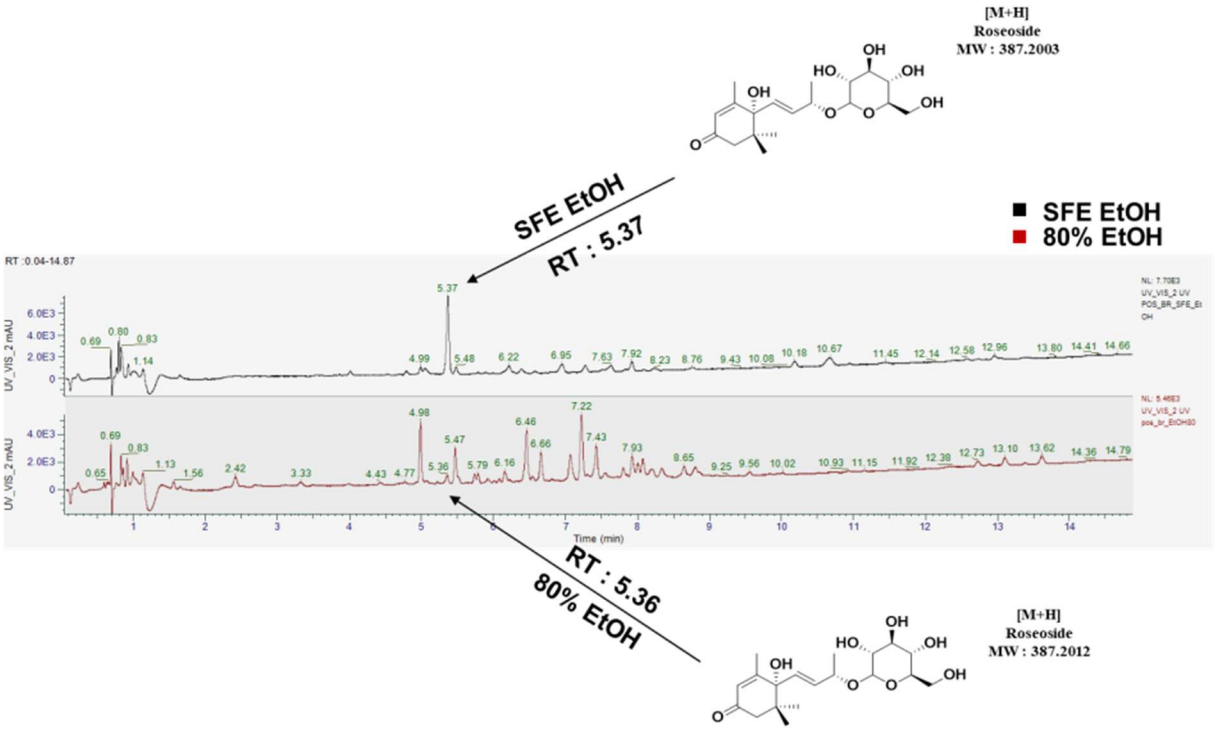

Figure 1a. UHPLC-UV (254 nm) Chromatograms of Stellaria alsine Extracts (UAE and SFE-EtOH)

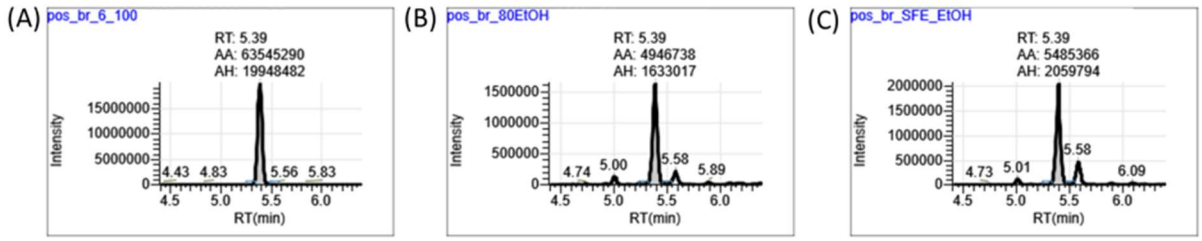

Figure 1b. Extracted Ion Chromatograms (XIC) of Roseoside : (A) Roseoside standard solution, (B) Roseoside in UAE extract; (C) Roseoside in SFE-EtOH extract.

3.2. Antioxidant Activities

The antioxidant potential of *Stellaria alsine var. undulata* extracts was evaluated using three assays: total phenolic content (TPC), DPPH radical scavenging activity, and superoxide dismutase (SOD)-like activity. Among the two extract types, the ultrasonic-assisted ethanol extract (UAE) consistently exhibited superior antioxidant effects compared to the supercritical ethanol extract (SFE-EtOH). [4] (Mishra AP et). al In the TPC assay (Figure 2), the UAE extract showed the highest value of **25.16 mg TAE/g extract**, while the SFE-EtOH extract yielded **17.49 mg TAE/g extract**, indicating a higher concentration of phenolic compounds in the UAE extract. Phenolic compounds are known to act as primary antioxidants that can donate hydrogen to neutralize free radicals. The DPPH radical scavenging activity (Figure 3) of the UAE extract reached **75.12% at 20 mg/mL**, which was significantly higher than that of the

SFE-EtOH extract (**61.84% at the same concentration**). This assay reflects the electron-donating ability of the extracts and their capacity to neutralize reactive oxygen species (ROS), highlighting the superior antioxidant profile of the UAE extract. Similarly, the SOD-like activity (Figure 4) of the UAE extract was measured at **48.89%**, while the SFE-EtOH extract exhibited only **17.21%** activity. As this assay mimics the function of the endogenous antioxidant enzyme SOD, the results suggest that the UAE extract has a higher potential to mitigate oxidative stress through enzyme-like mechanisms. Taken together, these findings demonstrate that ultrasonic-assisted extraction yields an extract with significantly stronger antioxidant capacity than the supercritical ethanol extraction method.

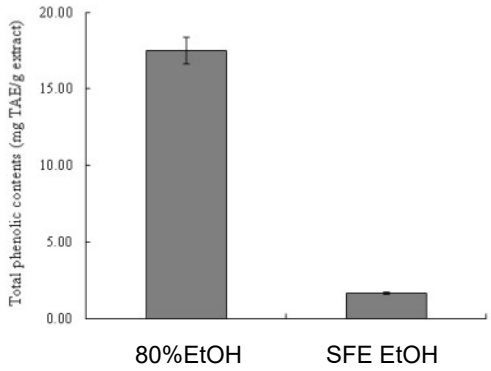

Figure 2. Total Phenolic Content of UAE and SFE-EtOH Extracts

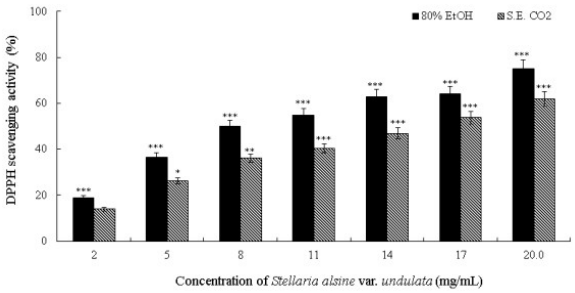

Figure 3. DPPH Radical Scavenging Activity

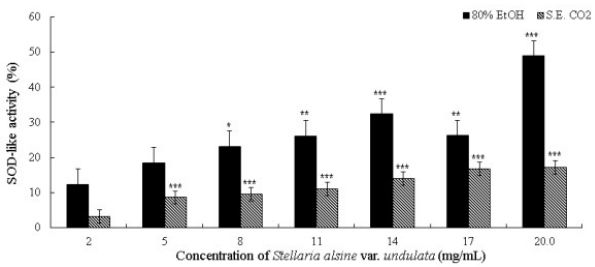

Figure 4. **SOD-like Activity of Extracts**

3.3. Whitening and Anti-aging Effects

Whitening and anti-aging effects of the extracts were assessed by tyrosinase and elastase inhibition assays. [5] (Park J et al). The UAE 80% ethanol extract exhibited a **tyrosinase inhibition rate of 17.16% at 17 mg/mL**, indicating moderate skin-brightening activity. Elastase inhibition, which is associated with the prevention of wrinkle formation and collagen degradation, was markedly higher in the UAE extract (**111.12% at 17 mg/mL**) compared to the SFE-EtOH extract (**85.26% at 20 mg/mL**). These results suggest that the UAE extract possesses strong anti-aging potential, likely due to its rich antioxidant constituents. Overall, the UAE extract demonstrated superior performance in both whitening and anti-aging assays compared to the SFE-EtOH extract, supporting its application as a multifunctional cosmetic ingredient.

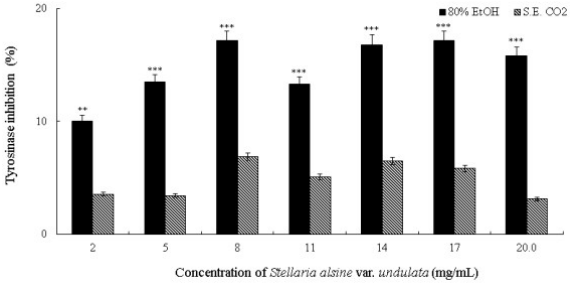

Figure 5. Tyrosinase Inhibition

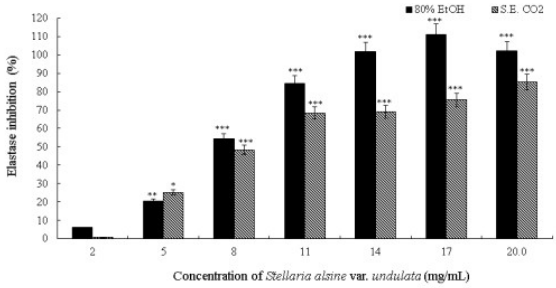

Figure 4. Elastase Inhibition

3.4. Anti-inflammatory Effects

To assess the anti-inflammatory activity of the extracts, cell viability and nitric oxide (NO) production were measured in LPS-stimulated RAW264.7 macrophages. [9] (Kwon YJ et al) MTT assays confirmed that both UAE and SFE-EtOH extracts maintained cell viability above 80% at concentrations below 31.3 μg/mL, indicating no cytotoxicity under the experimental conditions. The UAE extract demonstrated a significant inhibitory effect on NO production, reducing levels by **26.82%** at 31.3 μg/mL. In contrast, the SFE-EtOH extract did not produce a statistically significant reduction in NO levels. These findings suggest that the UAE extract has notable anti-inflammatory activity likely related to its phenolic constituents.

Figure 7 (a)

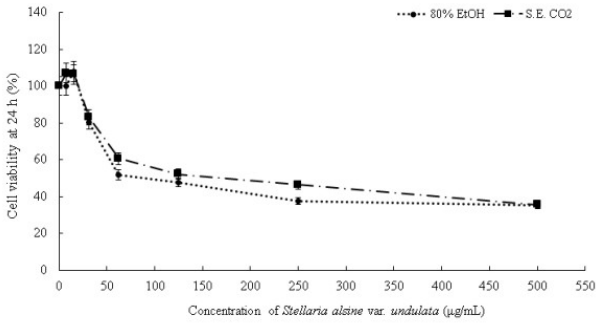

Figure 7 (b)

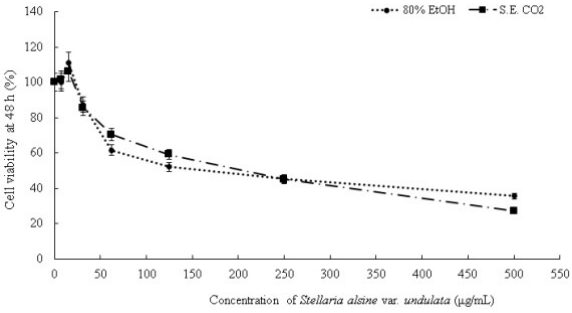

Figure 7. Cell viability of RAW264.7 macrophages treated with Stellaria alsine extracts. (a) Viability after 24 hours (based on MTT assay). (b) Viability after 48 hours (based on MTT assay).

Figure 8 (a)

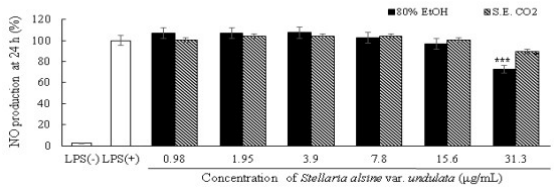

Figure 8 (b)

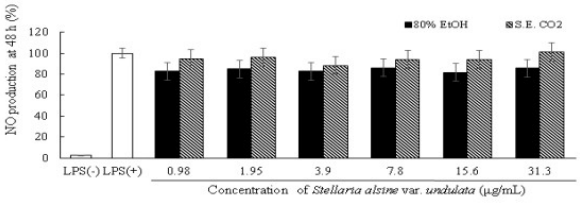

Figure 8. Nitric oxide (NO) production in RAW264.7 cells treated with Stellaria alsine extracts. (a) NO production after 24 hours, (b) NO production after 48 hours.

3.5. Nanoencapsulation Efficiency

Nanoencapsulation of *Stellaria alsine* extract was performed using agar and gelatin-based nanoemulsion formulations. Transmission electron microscopy (TEM) analysis confirmed the formation of spherical nanocapsules with diameters ranging from **100 to 200 nm**. [8] (Zhang Q et al). Zeta potential measurements ranged from **–35.8 to –40.2 mV**, indicating a stable colloidal dispersion with potential for enhanced dermal delivery. [3] (Xu Y et al). or [7] (Cho Y et al).

Sample condition	Zeta Potential (mV)	Stability Assessmen
Post-initial emulsification	-22.5 mV	Moderate stability
Following emulsifier concentration adjustment	-35.8 mV	High stability
Post-treatment with preservatives and stabilizing agents	-40.2 mV	Highly stable colloidal dispersion

Table 2. Zeta Potential Measurements of Nanocapsules

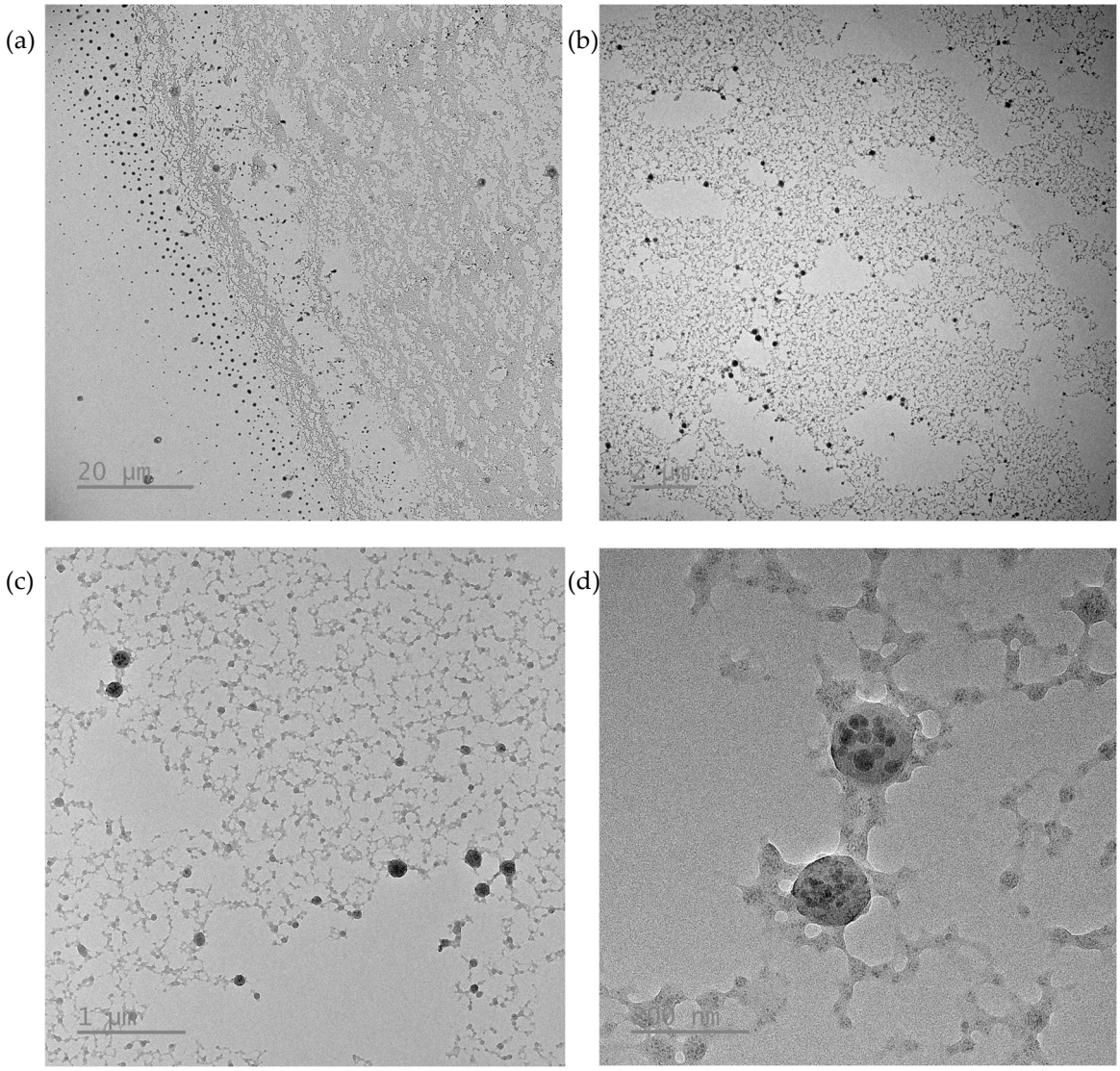

Figure 9. Transmission electron microscopy (TEM) images of nanocapsules at different magnifications. (a) ×500: Uniform distribution of nanocapsules observed. (b) ×3,000: Well-dispersed nanocapsules identified. (c) ×10,000: Distinct nanocapsule dispersion confirmed. (d) ×50,000: Particle size estimated to range from 150 to 180 nm

4. Discussion

This study demonstrates the cosmeceutical potential of *Stellaria alsine var. undulata* through a comprehensive evaluation of its antioxidant, whitening, anti-aging, and anti-inflammatory properties. Two extraction methods, ultrasonic-assisted ethanol extraction (UAE) and supercritical ethanol extraction (SFE-EtOH), were compared to determine their efficiency in isolating bioactive compounds. The SFE-EtOH extract yielded significantly higher levels of Roseoside, a marker compound with strong antioxidant and anti-inflammatory effects, suggesting its suitability for targeting specific lipophilic actives. However, the UAE extract exhibited higher total phenolic content, stronger DPPH radical scavenging activity, and superior SOD-like activity, indicating broader antioxidant potential. In the whitening and anti-aging assays, UAE showed stronger tyrosinase and elastase inhibition than SFE-EtOH, suggesting its effectiveness in mitigating melanin synthesis and collagen degradation—key processes in skin pigmentation and wrinkle formation, respectively. Anti-inflammatory assays further confirmed that UAE extract suppressed NO production more effectively than SFE-EtOH in LPS-stimulated macrophages. Furthermore, the application of agar-based nanoemulsion technology successfully produced nanocapsules with sizes ranging from 100–200 nm and zeta potentials between –35.8 to –40.2 mV. These physicochemical properties indicate excellent colloidal stability and suggest that the nanocapsules may enhance the delivery and bioavailability of active compounds in topical formulations. Taken together, UAE extraction combined with nanoencapsulation appears to be the most suitable approach for maximizing the cosmetic functionality of Stellaria alsine var. undulata. These findings not only validate the traditional medicinal value of this plant but also provide a promising strategy for developing innovative, multifunctional cosmetic formulations based on underutilized botanical resources.

5. Conclusion

This study highlights the cosmeceutical potential of *Stellaria alsine var. undulata*, an underutilized botanical species with demonstrated antioxidant, whitening, anti-aging, and anti-inflammatory activities. Among the extraction methods evaluated, the ultrasonic-assisted ethanol extraction (UAE) was found to be superior in preserving and delivering bioactivity, particularly when combined with agar-based nanoencapsulation.

These findings support the development of multifunctional, natural cosmetic formulations using UAE-derived *Stellaria* extracts, and provide a scientific foundation for their application in skin health enhancement. Moreover, the approach presented in this study may serve as a model for the valorization of other traditional and under-researched botanical ingredients.

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