University of Jiroft

Biospecies Research

Journal homepage: bsr.ujiroft.ac.ir

Iranian Biology Society

Comparative characterization of extracellular vesicles isolated from Aloe vera leaf peel and gel using ultracentrifugation and PEG precipitation

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https://doi.org/10.22034/bsr.2026.568098.1011

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ARTICLE INFO

Article Type
Original Article

Article History
Received: 22 December 2025
Accepted: 07 February 2026
Published: 15 February 2026

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ABSTRACT

This study aimed to evaluate different isolation methods, including ultracentrifugation (UC) and polyethylene glycol (PEG)-based precipitation of extracellular vesicles (EVs) from Aloe vera and investigate their yield, purity, and physicochemical properties. Plant-derived extracellular vesicles (PDEVs) have attracted increasing attention as natural nanocarriers for biomedical, nutraceutical, and food-related applications. In this study, EVs were isolated from Aloe vera leaf peel and gel using UC and PEG methods. The EVs were characterized by nanoparticle tracking analysis (NTA), NanoDrop protein quantification, and zeta potential measurements. Aloe vera gel-derived EVs using the UC method showed higher particle concentrations, more consistent size distributions, and lower protein contamination compared with peel and gel PEG-derived samples. The PEG isolation markedly increased apparent protein concentration, especially in the gel, indicating co-precipitation of non-vesicular plant components and polymer-associated artifacts. Zeta potential analysis further revealed significant surface charge variation on PEG-derived vesicles, both in peel and gel, particularly after high-speed centrifugation. In conclusion, these results demonstrate that UC provides superior purity and physicochemical stability of Aloe vera EVs, while PEG precipitation inflates protein yield and alters vesicle surface properties. The present study highlights the importance of the isolation method, which affects its potential applications.

Keywords: Aloe vera, Extracellular vesicle, Polyethylene glycol, Zeta potential

How to cite this paper

Ramzani Ghara, A., Ezzati Ghadi, F., Hosseini, S.H., 2026. Comparative characterization of extracellular vesicles isolated from Aloe vera leaf peel and gel using ultracentrifugation and PEG precipitation. *Biospecies Research*, 1, pp. 70-79.

Introduction

Extracellular vesicles (EVs) are nanoscale lipid bilayer-enclosed particles secreted by virtually all living cells (Abdal Dayem et al., 2025). EVs are included: apoptotic bodies, microvesicles, and exosomes. EVs play essential roles in intracellular communication by transferring proteins, lipids, nucleic acids, and other bioactive

molecules (Battistelli & Falcieri, 2021). Although EVs have been extensively studied in mammalian systems, recent research has revealed that plants also produce extracellular vesicles, often referred to as plant-derived nanovesicles (PDNVs) (Mu et al., 2023). PDNVs are cost-effective, sustainable, non-toxic, non-immunogenic, and suitable for therapeutic application. In contrast, mammalian EVs, such as Human cell-derived EVs, revealed batch-to-batch variability, high costs, and safety concerns (Fawzy et al., 2025, Ruf et al. 2022) suggested that the biogenesis mechanisms of EVs from plants and mammals are structurally similar, and also their intercellular communication is functionally analogous (Ruf et al., 2022). Plant-derived EVs exhibit unique stability compared to animal and bacterial-derived EVs due to their lipid composition and bioactive compounds (Langellotto et al., 2024). PDNVs are increasingly recognized for their potential in therapeutics, agriculture, and nutrition. Plant-derived nanovesicles are enriched with biologically active compounds like microRNAs, enzymes, lipids, and secondary metabolites, which can modulate physiological and biochemical processes in recipient organisms. PDNVs isolated from fruits, vegetables, and medicinal plants have shown anti-inflammatory, antioxidant, and immunomodulatory properties (Lo et al., 2024). Plant-derived nanovesicles protect sensitive molecules from enzymatic degradation, allowing more efficient delivery to target cells. Plant exosomes are considered safer for human consumption than their animal-derived counterparts, making them attractive candidates for drug delivery and nutraceutical applications (Rawat et al., 2025).

Aloe vera, a succulent plant widely used in traditional medicine and skincare, is a particularly auspicious source of plant nanovesicles. The leaves of Aloe vera contain a rich array of bioactive molecules, including polysaccharides (Matei et al., 2025), proteins (Mitra et al., 2023), phenolic compounds, and antioxidants (Solaberrieta et al., 2022). Studies have reported that Aloe vera showed therapeutic effects in wound healing, anti-inflammatory activity, and immunomodulation (Naini et al., 2021). A study indicated that nanovesicles isolated from Aloe vera can retain bioactive compounds, potentially enhancing their stability, bioavailability, and cellular uptake compared to crude plant extracts (Kim et al., 2021). Nanovesicles derived from Aloe vera have demonstrated potential to modulate cellular pathways associated with inflammation and oxidative stress, supporting their application in nutraceutical and biomedical properties (Ramírez et al., 2024; Choi et al., 2023).

Despite their growing importance, the field of plant exosomes is still in its infancy. A significant challenge in plant EV research is the lack of standardized isolation techniques. EVs isolation from plant sources, including affinity-based methods, differential centrifugation, density gradient centrifugation, filtration method, polymer-based precipitation, and size-exclusion chromatography. Differential centrifugation and polymer-based precipitation using polyethylene glycol are the most commonly used methods for EVs isolation from plant sources. Variations in isolation procedures can lead to differences in yield, purity, and the functional integrity of the nanovesicles, complicating their characterization and downstream applications. Understanding how different isolation methods affect the biophysical properties of plant-derived nanovesicles is crucial for both basic research and applied purposes. Comparative studies are essential for determining optimal protocols that maximize vesicle recovery while preserving their native structure and bioactivity. The present study aims to investigate different isolation methods, including UC and PEG-based precipitation of EVs across different tissue sources of Aloe vera to evaluate the impact of isolation techniques on EV yield, purity, and physicochemical properties.

Materials and Methods

Chemicals and reagents

All chemicals used in the present study were purchased from Merck & Co., Inc.

Isolation of Aloe vera gel and peel

Fresh Aloe vera plants were obtained from a local market and thoroughly washed three times with sterile distilled water to remove surface contaminants. Leaves were sterilized by 70% ethanol and allowed to dry for a few seconds. Leaves were separated into inner gel and outer peel (rind) using a sterile knife. Gel (44g) and peel (62g) tissues were processed independently in all subsequent steps under a sterile laminar flow hood (Heraguard™). All equipment and reagents used in this study were sterilized. Each tissue was mixed with 1X standard fresh-chilled phosphate-buffered saline (pH: 7.4) at a weight-to-volume ratio of 1:3 and incubated at room temperature for one hour to facilitate the release of soluble components. The mixtures were then homogenized using a blender (Ninja 1200W) until a uniform suspension was obtained.

Gel homogenates were centrifuged (Eppendorf 5415R) first at 300 × g for 3 min, then 500 × g for 10 min, and finally 2000 × g for 20 min at 4 ºC. The supernatant after each centrifugation step was collected and used for the next centrifugation step. For peel samples, centrifugation was carried out three times. At first, centrifugation was carried out at 1000 × g for 10 min, and the supernatant was collected. The second step of centrifugation was at 3000 × g for 30 min. Finally, 10000 × g for 60 min, the supernatant was collected after each step. The final supernatants in peel-derived samples were passed through a 0.45 μm filter (Whatman uniflo) to remove large particles.

Ultracentrifugation-based isolation of Aloe vera EVs from gel and peel

Clarified supernatants obtained from gel or peel homogenates were subjected to high-speed centrifugation according to established EV isolation protocols (Shen et al., 2025). The supernatants were centrifuged (Beckman Coulter Optima™ L-90k) at 150000 × g twice, and the resulting pellets were collected and resuspended in PBS, stored at -4 ºC for short-term use before characterization.

PEG-based precipitation of Aloe vera EVs from gel and peel

For polyethylene glycol (PEG)-based isolation, a twofold concentrated PEG stock solution (8% w/v PEG 6000 in 1 M NaCl) was prepared and mixed with clarified supernatants at a 1:1 volume ratio (Kim & Park, 2022). Mixtures were incubated at 4 ºC for 16 hours to allow EV precipitation. Samples were centrifuged at 1500 × g for 30 min, then at 10000 × g for 60 min to collect PEG-precipitated EVs. The pellets were gently washed with PBS to remove residual PEG, then resuspended in PBS. The preparations were designated as PEG-isolated Aloe vera EVs and stored at -4 ºC for short-term use before characterization.

Protein quantification by NanoDrop

Total protein concentration of EVs preparations was measured using a NanoDrop spectrophotometer (Thermoscientific NanoDrop one C microvolume UV-Vis spectrophotometer w/cuvette). Protein content was estimated by measuring absorbance at 280 nm. A260/280 ratios were recorded to assess sample purity and potential nucleic acid contamination.

Nanoparticle tracking analysis

Particle size and distribution, and concentration of Aloe vera EVs were determined by nanoparticle tracking analysis (NTA) using a Particle Metrix system (Nanoparticle tracking NS300). The EV preparations were appropriately diluted in PBS before measurement. All samples were recorded at a camera level (CL) of 15, except for selected gel-derived PEG samples evaluated at a camera level (CL) of 12 due to signal saturation.

Zeta potential and pH measurement

Zeta potential measurements were performed (using Zeta potential Malven Zetasizer zs) to assess the surface charge and colloidal stability of Aloe vera EVs. Measurements were conducted on multiple preparations for each isolation method and tissue source. Also, the pH

of the EV suspensions was recorded concurrently to evaluate the chemical environment of the samples.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical analyses among groups were performed using One-Way Analysis of Variance (ANOVA). P-values less than 0.05 were considered statistically significant.

Result

NanoDrop Protein Quantification

Protein concentration and absorbance ratios measured by NanoDrop are summarized in Table 1. Apparent differences were observed between isolation methods and tissue sources. EVs isolated by UC exhibited substantially lower protein concentrations compared with PEG-isolated samples for both peel and gel. In peel samples from Aloe vera, UC yielded a protein concentration of 0.713 ± 0.001 mg/ml, PEG precipitation significantly increased ($p < 0.05$) apparent protein levels to 2.543 ± 0.00 mg/ml ($1500 \times g$) and further to 3.313 ± 0.00 mg/ml ($10000 \times g$). Gel-derived EVs showed a similar trend, with UC samples containing 2.359 ± 0.00 mg/ml protein compared to 4.321 ± 0.00 mg/ml and 5.427 ± 0.00 mg/ml, which significantly increased ($p < 0.05$) for PEG isolates collected at $1500 \times g$ and $10000 \times g$, respectively.

Measurements of A260/280 ratios showed UC-isolated EVs displayed significantly lower ratios ($p < 0.05$), particularly in peel samples (0.90 ± 0.01) compared to gel samples (1.38 ± 0.01). PEG-derived samples, in comparison, showed significantly elevated A260/280 ratios ($p < 0.05$), especially in gel EVs isolated at $10000 \times g$ (1.79 ± 0.01).

Table 1. NanoDrop protein concentration and purity ratios

Sample	Isolation	Centrifugation (g)	Protein concentration (mg/ml)	A280	A260/280
Peel	UC	150000	0.713 ±0.001*	0.71±0.001	0.90± 0.01*
Peel	PEG	1500	2.543 ±0.000 *	2.54±0.000*	1.26± 0.01*
Peel	PEG	10000	3.313±0.000*	3.31±0.000*	1.40± 0.01*
Gel	UC	150000	2.359±0.000	2.36±0.000	1.38± 0.01
Gel	PEG	1500	4.321±0.000*	4.32±0.000*	1.50± 0.01*
Gel	PEG	10000	5.427±0.000*	5.42±0.000*	1.79± 0.01*

All data are presented as mean ± standard deviation (SD).

Statistical comparisons among groups were performed using One-way ANOVA. * $p < 0.05$ was considered statistically significant.

Nanoparticle tracking analysis (NTA)

Particle size distribution and concentration data obtained by NTA are presented in Table 2. Gel-derived UC EVs exhibited the highest ($p < 0.05$) particle concentration (4.97×10^{11} ± 0.01 particles/ml) with a mean size of 125.8 nm. In contrast, peel-derived UC EVs showed a significantly smaller mean size (85 nm) and a concentration of 1.63×10^{11} particles/ml.

PEG precipitation for peel-derived EVs resulted in 4.71×10^{9} ± 0.01 particles/ml at $1500 \times g$ and decreased to 2.80×10^{10} ± 0.01 particles/ml at $10000 \times g$. Gel-derived PEG EVs showed 1.53×10^{10} ± 0.01 particles/ml at $1500 \times g$ and decreased to 1.01×10^{10} ± 0.01 particles/ml at $10000 \times g$. Notably, the gel EV sample measured at camera level (CL) 12 showed a size distribution (125.7 nm) comparable to UC isolates.

Table 2. NTA Size and Concentration of EVs

Sample	Isolation	Centrifugation (g)	Size (nm)	Concentration (particles/ml)
Peel	UC	150000	85 ± 1.00	$1.63 \times 10^{11} \pm 0.05$*
Peel	PEG	1500	83.7 ± 1.00 *	$4.71 \times 10^{9} \pm 0.01$*
Peel	PEG	10000	86.6 ± 1.00	$2.80 \times 10^{10} \pm 0.01$*
Gel	UC	150000	125.8 ± 1.00 *	$4.970 \times 10^{11} \pm 0.01$*
Gel	PEG	1500	106.2 ± 1.00 *	$1.53 \times 10^{10} \pm 0.01$
Gel	PEG	10000	48.1 ± 1.00 *	$1.01 \times 10^{10} \pm 0.01$*
Gel	PEG	10000 (CL12)	125.7 ± 1.00	$3.33 \times 10^{10} \pm 0.01$*

All data are presented as mean ± standard deviation (SD).

Statistical comparisons among groups were performed using One-way ANOVA. * $p < 0.05$ was considered statistically significant.

Zeta potential and pH

Zeta potential and pH values are summarized in Table 3. UC-derived gel EVs exhibited strongly positive zeta potentials (38.85 ± 0.95 mv), indicating good colloidal stability. In contrast, the zeta potential of UC-derived peel EVs showed a significant decrease of 10.78 ±1.02 ($p < 0.05$) compared to UC-derived gel EVs. PEG precipitation significantly altered surface charge characteristics. Gel-derived PEG EVs collected at 1500 × g displayed 24.95 ± 1.17 mv, whereas samples collected at 10000 ×g exhibited a significant increase in zeta potential (40.40 ±2.11 mv). A similar pattern was observed for peel-derived EVs, where low-speed PEG pellets showed a zeta potential value of 11.26 ±1.60 mv, and high-speed PEG pellets demonstrated significantly increased surface charge ($p < 0.05$) of 39.90 ± 2.87 mv when compared to low-speed PEG peel-derived EVs. Results from Table 3 showed that the pH of all EV suspensions remained acidic and relatively stable (pH 4.5 to 4.7), indicating that differences in zeta potential were primarily attributable to isolation method and centrifugal force rather than pH variation. Collectively, these results demonstrate that both tissue source and isolation strategy significantly influence surface charge and colloidal stability of EVs.

Table 3. Zeta potential and pH of Aloe vera EVs

Sample	**Isolation**	**Centrifugation (g)**	**Zeta Potential (mV)**	**pH**
Gel	UC	150000	38.850 ± 0.95*	4.6
Peel	UC	150000	10.78 ± 1.02	4.7
Gel	PEG	1500	24.950 ± 1.17*	4.5
Gel	PEG	10000	40.40 ± 2.11*	4.6
Peel	PEG	1500	11.26 ± 1.60	4.6
Peel	PEG	10000	39.90 ± 2.87*	4.6

All data are presented as mean ± standard deviation (SD).

Statistical comparisons among groups were performed using One-way ANOVA. * $p < 0.05$ was considered statistically significant.

Discussion

Plant-derived extracellular vesicles exhibit intrinsic bioactive properties, including anti-inflammatory and antioxidant activities, which can contribute directly to their biological efficacy. The isolation strategy of PDEVs is a key factor influencing vesicle purity, yield, and biological functionality, due to the complex nature of

plant tissues and coexisting biomolecules. Therefore, selecting and optimizing an appropriate isolation method is essential to ensure reproducibility and maximize the translational potential of PDEVs across biomedical and industrial applications.

The present study aimed to investigate how different isolation techniques influence the physicochemical characteristics and functional potential of Aloe vera-derived nanovesicles. Previous studies have shown that Aloe vera is rich in phenolic compounds, including cinnamic acids, chromones, anthracene derivatives, and flavonoids, many of which contribute to its well-documented antioxidant and anti-inflammatory (Mensah et al., 2025; Abid et al., 2025). In this study, EVs were isolated from Aloe vera peels and gel using both UC and PEG precipitation methods.

As summarized in Table 1, NanoDrop analysis revealed marked differences in protein concentration and absorbance ratios between UC and PEG precipitation methods for both Aloe vera gel and peel-derived EVs. UC-isolated peel EVs exhibited a relatively low protein concentration (0.713 mg/ml). In contrast, gel-derived EVs showed higher protein levels (2.359 mg/ml), indicating intrinsic differences in vesicle abundance and soluble protein content between the two plant tissues. In contrast, PEG precipitation resulted in substantially higher apparent protein concentrations across all samples (Table 1). A study has shown that the PEG precipitation method yields a higher protein concentration (Rahmatinejad et al., 2024). PEG precipitation relies on chemical aggregation to isolate vesicles at lower centrifugal forces, offering a faster and more scalable approach, with slightly lower purity due to co-isolated proteins and plant polysaccharides (Ludwig et al., 2018; Zhu et al., 2024).

Peel-derived EVs isolated by PEG showed protein concentrations ranging from 2.54 mg/ml at 1500 × g to 3.31 mg/ml at 10000 × g, while gel-derived PEG isolates reached even higher values (4.32 to 5.43 mg/ml). These findings are consistent with previous reports indicating that PEG-based isolation enhances total recoverable material by soluble proteins, polysaccharides, and other macromolecules (Huang et al., 2025).

The A260/280 ratios presented in Table 1 further support this interpretation. UC-isolated peel EVs displayed a low A260/280 ratio, reflecting relatively high protein purity and minimal nucleic acid or carbohydrate contamination. In contrast, PEG-isolated samples showed elevated A260/280 ratios, particularly in gel-derived EVs, suggesting co-isolation of nucleic acid, phenolic compounds, and polysaccharides, which are abundant in Aloe vera gel. Previous studies have shown that PEG precipitation can lead to lower purity of the isolated EV population (Martínez-Greene et al., 2021). It has been demonstrated in previous studies that ultracentrifugation is the method of choice for applications demanding high purity and precise characterization of extracellular vesicles (Mu et al., 2023).

The NTA results presented in Table 2 highlight apparent differences in particle size distribution and vesicle concentration between isolation methods and plant tissues. UC-isolated peel-derived EVs exhibited a mean particle size of approximately 85 nm with a concentration of 1.63 × 1011 particles/ml, and UC-isolated gel EVs showed a larger size (125.8 nm) and substantially higher concentration (4.97 × 1011 particles/ml). These values fall within the expected size range of small EVs and indicate efficient enrichment of intact vesicles using UC. PEG-isolated samples, as shown in Table 2, exhibited more variable size distributions and lower particle concentrations compared to UC isolates, which may reflect selective pelleting of smaller vesicles or interference from PEG residues during NTA measurement. Notably, despite higher protein concentrations observed in PEG isolates (Table 1), particle concentrations measured by NTA were consistently lower than those obtained by UC (Table 2). In contrast to the results obtained in the present study, another researcher indicates that the concentration of EV particles is higher when using PEG precipitation (Gharavi et al., 2024). This discrepancy emphasizes that protein content alone does not accurately reflect EV yield and reinforces the necessity of particle-based quantification when evaluating isolation efficiency. The gel EV sample measured at CL12 highlighting the sensitivity of PEG-derived samples to

measurement conditions. Overall, UC produced more concentrated and size-consistent EV populations, whereas PEG precipitation resulted in lower particle recovery and greater variability in size measurements, particularly at higher centrifugal forces.

Zeta potential is a key indicator of the surface charge and colloidal stability of EVs in a dispersed system. The net negative charge of nonfunctionalized EVs influences particle-particle and particle-medium interactions, affecting their tendency to aggregate. A higher absolute zeta potential promotes electrostatic repulsion and improves dispersion stability. Surface charge also plays a vital role in biological processes such as cellular uptake and cytotoxicity. Therefore, zeta potential provides valuable insight into the stability and in vivo behaviour of EVs, supporting their application in nanomedicine (Midekessa et al., 2020; Rogers et al., 2023).

This study, zeta potential and pH values reported in Table 3 provide insight into the colloidal stability and surface charge characteristics of Aloe vera-derived EVs. UC-isolated gel EVs demonstrated consistently high positive zeta potentials, indicative of strong electrostatic repulsion and excellent colloidal stability. Such values suggest that vesicle membranes and preserved surface chemistry following UC isolation. In contrast, UC-isolated peel EVs exhibited significantly lower zeta potentials, suggesting lower electrostatic stability, a higher tendency toward aggregation, and possible differences in membrane composition between gel- and peel-derived vesicles. This difference likely reflects tissue-specific variations in lipid composition, surface proteins, and associated phytochemicals among PDEVs from different tissues (Huang et al., 2025). PEG-isolated EVs showed zeta potential values that varied with centrifugal force, as summarized in Table 3. Low-speed PEG isolates exhibited moderate zeta potentials for gel-derived EVs and lower values for peel-derived EVs. Notably, high-speed PEG pelleting resulted in zeta potential values comparable to or exceeding those of UC-isolated gel EVs, suggesting selective enrichment of more stable vesicle populations or removal of loosely bound contaminants.

PDEVs have been reported to have higher stability against enzymatic degradation compared to mammalian drive EVs; thus, they are a potential candidate for oral drug administration due to their stability in both normal and acidic pH (Feng et al., 2023). Yang et al. (2020) reported that PDEVs from lemon using PEG precipitation increase their circulation time due to the attachment of PEG to the surface of EVs. Also, PEG improved EVs' stability in acidic pH (Yang et al., 2020).

Variations in PEG concentration can strongly affect precipitation efficiency and the extent of co-isolated proteins and polysaccharides, thereby impacting EV purity. A study has shown that concentration range of PEG (8%, 10%, 12%, and 15%) could increase the exosomes size (Kalarikkal et al., 2020). Finally, differences between Aloe vera gel and peel tissues (matrix complexity and biochemical composition) may contribute to variability in EV yield and physicochemical properties.

In the present study, the pH of all samples remained stable between 4.5 and 4.7 (Table 3), consistent with the native acidity of Aloe vera tissues. The absence of significant pH variation across isolation methods indicates that neither UC nor PEG precipitation altered the chemical environment of the EV preparations. Thus, results demonstrated that both the isolation method and tissue source profoundly influence the physicochemical properties of Aloe vera EVs. UC consistently produced preparations with higher particle concentrations, lower protein contamination, and more predictable zeta potential profiles, supporting its suitability for high-purity EV research. PEG precipitation, while yielding higher apparent protein concentrations, resulted in lower particle counts and greater variability in surface charge, reflecting co-precipitation of non-vesicular components. Nevertheless, PEG offers advantages in scalability and operational simplicity, making it attractive for applications where yield is prioritized over purity.

Gel-derived EVs consistently outperformed peel-derived EVs across all parameters, including particle concentration, protein yield, and colloidal stability, underscoring Aloe vera gel as a superior source of plant-derived nanovesicles for downstream applications.

Conclusion

This study provides a comprehensive comparative evaluation of ultracentrifugation and polyethylene glycol precipitation for the isolation of extracellular vesicles (EVs) from Aloe vera gel and peel tissues. Integrating protein quantification, nanoparticle tracking analysis, and zeta potential measurements demonstrated that both the isolation method and plant tissue source significantly influence the yield, purity, and physicochemical stability of plant-derived nanovesicles. UC consistently produced EV preparations with higher particle concentrations, narrower size distributions within the expected range of small EVs, and lower levels of co-isolated proteins, indicating superior purity and structural integrity. These characteristics make UC-isolated Aloe vera EVs particularly suitable for mechanistic studies, molecular profiling, and applications requiring well-defined vesicle populations. In contrast, PEG precipitation resulted in substantially higher apparent protein concentrations but lower particle counts, reflecting co-precipitation of soluble proteins, polysaccharides, and other plant macromolecules. While this reduces purity, PEG-based isolation offers practical advantages in terms of simplicity, reduced equipment requirement, and scalability, which are critical for translational and industrial applications. The isolation of Aloe vera tissues highlights the gel as a superior and more reliable source of Aloe vera nanovesicles, likely due to its rich biochemical composition and lower structural complexity compared to peel tissue. Methodological limitations include the lack of standardized EV isolation protocols, which reduces reproducibility and cross-study comparability. In addition, ultracentrifugation has limited industrial scalability due to high cost and low throughput, while PEG precipitation is more scalable but suffers from lower purity and requires further steps. Future studies should focus on integrating molecular cargo analysis, functional bioactivity assays, industrial scalability, standardization of methods, and additional purification steps to optimize Aloe vera EVs.

Acknowledgments

The authors express their gratitude to the University of Jiroft for their support.

Conflict of Interest

The authors declare that there is no conflict of interest.

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