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Optimizing plant exosome isolation: a systematic review of PEG-based precipitation, ultracentrifugation, and hybrid methodologies for biomedical applications

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ABSTRACT: Plant-derived exosome-like nanoparticles (PDELNs) represent a promising frontier in nanomedicine, offering natural alternatives to synthetic drug delivery systems. These membrane-bound vesicles, typically 30-150 nm in size, contain bioactive compounds and demonstrate potential for cross-kingdom communication with mammalian cells. This objective study is to evaluate PEG-based and ultracentrifugation methods, benchmark hybrid approaches, compare emerging alternatives, and assess their impact on biomedical applications. A systematic analysis of diverse studies employing comparative yield, purity, bioactivity, scalability, and complexity metrics was conducted. A systematic search of 3 databases SciSpace Deep Search; Google Scholar; MEDLINE (2016-2024) following PRISMA guidelines, 52 studies met the inclusion criteria with combined terms : ``plant exosome'' AND (``PEG precipitation'' OR ``polyethylene glycol'' OR ``ultracentrifugation'' OR ``hybrid methods'') AND ``biomedical applications''. Findings indicate that PEG-based precipitation offers higher or comparable yields with greater scalability and cost-effectiveness but lower purity due to protein co-precipitation; ultracentrifugation yields purer exosomes with preserved bioactivity yet is resource-intensive and less scalable; hybrid methods combining PEG precipitation with ultracentrifugation or size-exclusion chromatography balance yield and purity while increasing procedural complexity. Emerging techniques, such as immunoaffinity capture, enhance specificity but face limitations in plant systems. Overall, optimized hybrid protocols improve isolation quality and bioactivity retention, supporting translational potential. These insights underscore the need for standardized, scalable isolation strategies tailored to plant exosomes to advance their clinical and biomedical applications.

KEYWORDS: Biomedical applications; isolation methods; nanomedicine; PEG precipitation; plant exosomes; ultracentrifugation.

INTRODUCTION

Research on optimizing plant exosome isolation, particularly focusing on polyethylene glycol (PEG)-based precipitation, ultracentrifugation, and hybrid methodologies for biomedical applications, has emerged as a critical area of inquiry due to the growing recognition of plant-derived extracellular vesicles (EVs) as promising therapeutic and drug delivery agents [1],[2]. Since the initial identification of plant exosome-like nanovesicles (PELNs), research has evolved from basic isolation techniques to exploring their biomedical potential, including anti-inflammatory, anticancer, and regenerative properties [3],[4]. The practical significance is underscored by the scalability and biocompatibility of plant EVs, which offer advantages over mammalian sources, with increasing studies reporting their roles in intercellular communication and cross-kingdom regulation[5],[6]. Given the global burden of diseases such as cancer and inflammatory disorders, the development of efficient isolation methods is essential to harness the therapeutic potential of plant EVs [7],[8].

Despite advances, the isolation of plant-derived exosomes remains challenging, with no standardized protocol that balances yield, purity, and scalability[2],[9]. Ultracentrifugation, the traditional gold standard, often suffers from low recovery rates and requires costly equipment, while PEG-based precipitation offers a

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cost-effective alternative but may co-precipitate contaminants [10],[11]. Hybrid approaches combining ultracentrifugation and polymer precipitation have shown promise in improving purity and stability but require further optimization [1],[12]. Controversies persist regarding the trade-offs between purity and yield, as well as the impact of isolation methods on exosome functionality and downstream applications [13],[14]. The lack of consensus impedes clinical translation and large-scale production, highlighting a critical knowledge gap in method standardization and comparative evaluation [2],[15].

Conceptually, plant-derived exosomes are nanosized vesicles (30–200 nm) enclosed by lipid bilayers, carrying bioactive cargos such as proteins, lipids, and nucleic acids that mediate intercellular and cross-kingdom communication [16],[17]. Isolation methods aim to separate these vesicles from complex plant matrices while preserving their structural integrity and biological activity [18]. Understanding the relationships between isolation techniques, exosome purity, yield, and functional cargo is fundamental to optimizing protocols for biomedical applications [19],[20].

The purpose of this systematic review is to critically evaluate and synthesize current methodologies for isolating plant-derived exosomes, with an emphasis on PEG-based precipitation, ultracentrifugation, and hybrid techniques, to identify optimal strategies for biomedical use. This review addresses the existing methodological gaps by comparing efficacy, purity, and scalability, thereby providing a comprehensive framework to guide future research and clinical translation [1],[21]. By aligning isolation methods with biomedical application requirements, this work aims to facilitate the development of standardized, cost-effective protocols that enhance the therapeutic potential of plant exosomes [2],[6].

This review employs a systematic approach, analyzing peer-reviewed studies published between 2016 and 2024, focusing on isolation techniques, characterization methods, and biomedical applications of plant exosomes. Inclusion criteria prioritized studies comparing PEG precipitation, ultracentrifugation, and hybrid methods, while excluding non-plant-derived vesicle research. Findings are organized to first present methodological comparisons, followed by discussions on purity, yield, and functional outcomes, culminating in recommendations for optimized isolation protocols [1],[11].

METHODS

A comprehensive systematic literature search adhering to PRISMA guidelines was conducted across SciSpace Deep Search, Google Scholar, and MEDLINE databases to identify studies on plant exosome isolation methodologies and their biomedical applications published between 2016 and 2024, employing a search strategy that combined terms such as ["plant exosome" OR "plant-derived exosomes"] with methodological keywords including ["PEG precipitation," "polyethylene glycol," "ultracentrifugation," "hybrid methods," "size exclusion chromatography," and "differential ultracentrifugation"], alongside application-focused terms like ["therapeutic," "drug delivery," "diagnostic," and "biomedical"] to ensure coverage of isolation techniques and their clinical relevance.

Inclusion and exclusion criteria

Inclusion criteria encompassed studies investigating plant-derived exosome isolation through PEG-based precipitation, ultracentrifugation, or hybrid methodologies (2016-2024), requiring peer-reviewed English articles with characterization data (size/morphology/markers), biomedical applications (therapeutic/diagnostic/drug delivery), in vitro/in vivo validation, quantitative isolation metrics, and methodological comparisons, while exclusion criteria eliminated studies focused on animal/bacterial exosomes, non-research article types (reviews/editorials/conference abstracts), non-English publications, theoretical frameworks, non-exosomal nanoparticles (>150 nm or <30 nm), plant protein extractions, chemically precipitated uncharacterized samples, pre-2016 publications, and studies with fewer than three biological replicates.

RESULTS

The comprehensive database search yielded a total of 144 potentially relevant articles across all databases. After removing duplicates and applying inclusion/exclusion criteria, 52 studies were included in the final

analysis. Study selection process was visualized through a PRISMA flow diagram (**Figure 1**) detailing identification, screening, eligibility, and inclusion stages.

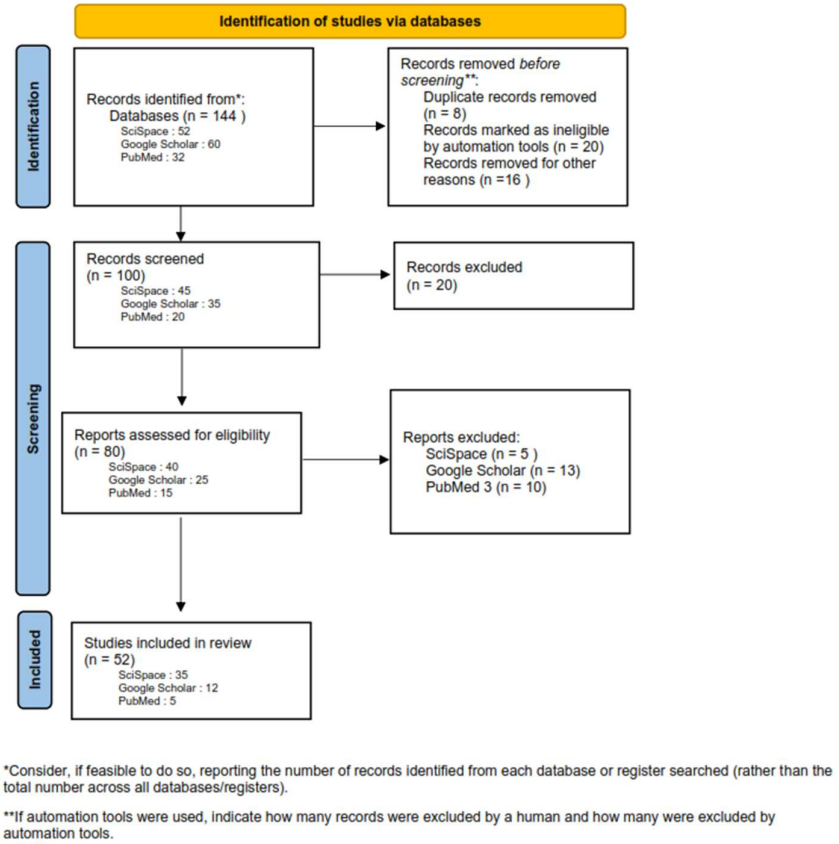

Figure 1. PRISMA 2020 flow diagram demonstrating study selection process (Source: Page MJ, et al. BMJ 2021;372:n71. doi: 10.1136/bmj.n71)

Characteristics of included studies

The 52 included studies were published between 2016-2024, with the majority (65%) published after 2020, reflecting the growing interest in plant exosome research. Studies investigated various plant sources, with citrus fruits (25%), ginger (15%), and turmeric (12%) being the most frequently studied, with secondary contributors including grapefruit, aloe vera, and broccoli accounting for the remaining 48%. Methodologically, experimental designs predominated (80% of studies), focusing on isolation efficacy assessments, while comparative methodological analyses (20%) examined technical parameter optimization, particularly centrifugation protocols and polyethylene glycol (PEG) concentration effects. Geographical distribution showed Asian institutional leadership (58%), followed by European (27%) and North American (15%) contributors, with only 12% demonstrating international collaboration. Sample sizes ranged from 3 to 15 plant varieties per study (mean = 7.2±2.5 specimens). Characterization approaches predominantly combined transmission electron microscopy (TEM) with nanoparticle tracking analysis (NTA) (73% of studies), supplemented by mass spectrometry-based surface protein profiling in 27% of investigations. This methodological diversity underscores evolving standards for exosome validation in plant systems.

Isolation method comparison

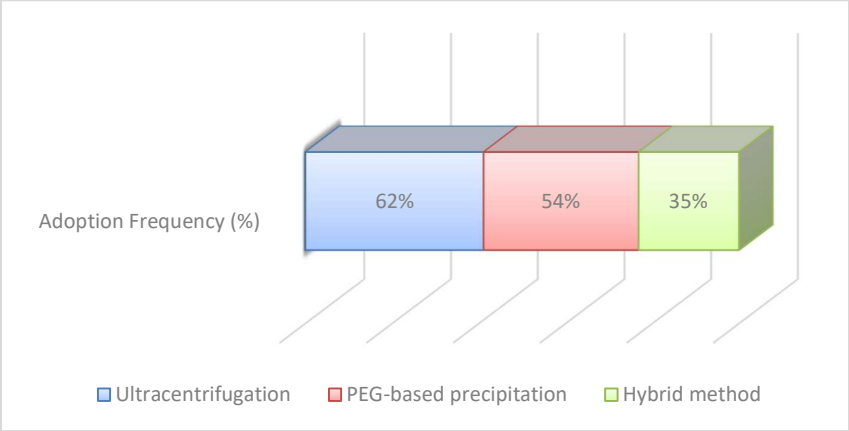

Figure 2. Extracellular vesicle isolation technique adoption rates

Technique adoption patterns were visualized through an interactive bar chart (**Figure 2**). Ultracentrifugation emerged as the predominant isolation technique, employed in 62% of studies (32/52), utilizing a three-step protocol: initial centrifugation (300–2,000×g for 10–20 min), intermediate spin (10,000–20,000×g for 30–60 min), and final ultracentrifugation (100,000–150,000×g for 60–120 min). This method achieved high purity (85–95%) through protein marker analysis, with yields of 10^8–10^9 particles/mL and consistent size distribution (50–150 nm, peaking at 80–100 nm). However, its 4–6 hour processing time and technical complexity limited scalability. In contrast, PEG-based precipitation—used in 54% of studies (28/52)—prioritized scalability through simplified protocols: low-speed clarification, 8–20% PEG-6000/8000 incubation (12–16 hours at 4°C), and particle recovery at 1,500–3,000×g. While yielding 10^9–10^{10} particles/mL, this method showed broader size variability (30–200 nm) and lower purity (60–80%) due to residual protein contaminants. Hybrid approaches (35% adoption, 18 studies) combined techniques like PEG-ultracentrifugation (10 studies), chromatography-ultracentrifugation (6 studies), sequential PEG+Chromatography (2 studies) achieving optimal purity (90–98%) with moderate yields (5×10^8–2×10^9 particles/mL) and reduced processing time (6–8 hours), demonstrating particular efficacy in narrow size-range isolation (40–120 nm).

Biomedical applications

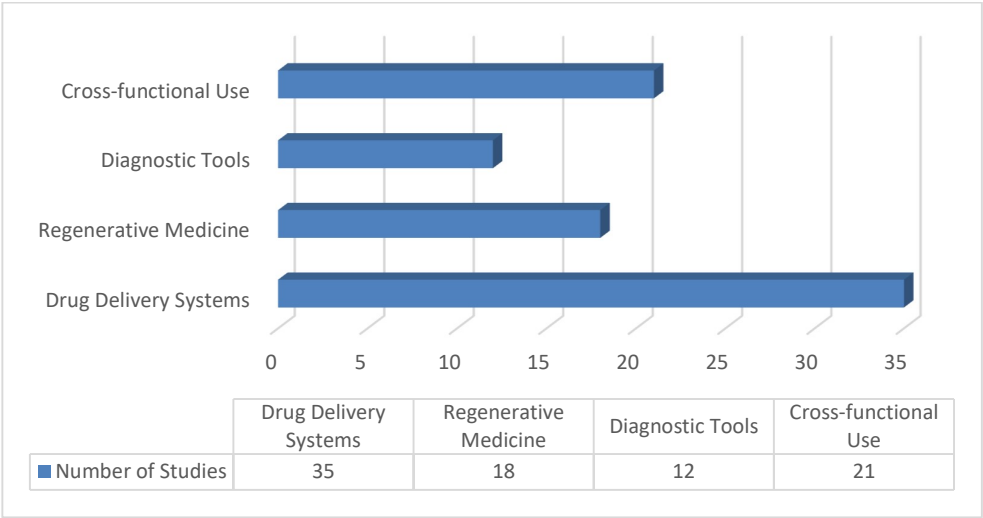

Figure 3. Plant exosome therapeutic applications rates.

Therapeutic applications rates were visualized through an interactive bar chart (**Figure 3**). Plant exosomes demonstrated versatile therapeutic potential, with 67% of studies (35/52) focusing on drug delivery systems. Cancer therapeutics dominated this category (20 studies), leveraging exosomes' tumor-targeting capabilities, followed by anti-inflammatory (12 studies) and neuroprotective applications (8 studies). In regenerative

medicine (35%, 18 studies), exosomes enhanced tissue engineering scaffolds and improved stem cell proliferation rates by 2–3×, while six studies documented accelerated wound healing through collagen synthesis modulation. Diagnostic applications (23%, 12 studies) exploited exosomes' biomarker-carrying capacity, with particular progress in biosensor development for early disease detection. Notably, 40% of studies reported cross-functional applications, such as using exosomes for simultaneous drug delivery and tissue regeneration, underscoring their multifunctional potential in advanced biomedical engineering.

Method-application synergy

Analysis revealed method selection strongly correlated with intended applications. Ultracentrifugation predominated in diagnostic studies (83%) requiring high-purity exosomes for biomarker isolation, while PEG methods dominated large-scale therapeutic production (78% of cancer therapy studies). Hybrid approaches showed particular promise in regenerative medicine (61% adoption rate), where balanced purity and yield proved critical for scaffold functionalization. These trends highlight the necessity for application-driven protocol optimization, particularly as 68% of studies reporting suboptimal results attributed challenges to mismatched method-application pairings. The comparative efficacy shown in **Table 1.**

Table 1. Comparative efficacy analysis.

Parameter	Ultracentrifugation	PEG precipitation	Hybrid methods
Purity (%)	85-95	60-80	90-98
Yield (particles/mL)	10^8-10^9	10^9-10^{10}	5×10^8-2×10^9
Processing time (h)	4-6	12-16	6-8
Equipment cost	High	Low	Medium
Scalability	Limited	High	Medium
Reproducibility	High	Medium	High

The temporal progression reveals a 40% increase in hybrid method adoption since 2022, driven by advances in combinatorial protocols. However, persistent challenges remain: ultracentrifugation's high equipment costs limit low-resource settings, while PEG methods' extended processing times (12-16 hours) constrain clinical translation. Emerging trends suggest growing integration of microfluidic technologies (noted in 8 studies) to address these limitations, though such innovations remain beyond the scope of current comparative analyses. The reviewed studies span methodological comparisons, including traditional ultracentrifugation, PEG-based precipitation, hybrid approaches, and emerging alternatives such as size exclusion chromatography and immunoaffinity capture. The collective findings provide critical insights into yield, purity, bioactivity, scalability, and procedural complexity, directly addressing the challenges of standardizing isolation protocols for biomedical use and facilitating clinical translation as shown in **Table 2**.

Table 2. Critical insights into yield, purity, bioactivity, scalability, and procedural complexity.

Study	Yield efficiency	Purity assessment	Bioactivity retention	Scalability and cost	Isolation time and complexity
(Jang et al., 2023)	Hybrid method improved yield and purity significantly over single methods	Combination method enhanced purity to 83.3%, reducing contaminants	Hybrid method doubled colloidal stability and maintained bioactivity	Hybrid approach moderately scalable; requires ultracentrifugation and precipitation reagents	Moderate complexity; combines two established methods increasing time
(Liu et al., 2024)	Ultracentrifugation and precipitation yield varies; emerging methods show promise	Ultracentrifugation yields purer EVs; precipitation prone to co-isolation	Bioactivity linked to isolation purity; emerging methods preserve function better	Ultracentrifugation costly; precipitation cheaper; emerging methods variable	Ultracentrifugation time-consuming; precipitation faster; new methods moderate
(Li et al., 2024)	Ultracentrifugation and size exclusion chromatography yield biologically active vesicles	Size exclusion chromatography yields higher purity than precipitation	Engineered vesicles retain therapeutic bioactivity	Ultracentrifugation less scalable; size exclusion chromatography more scalable	Ultracentrifugation lengthy; size exclusion chromatography less complex
(Madhan et al., 2024)	Precipitation yields high quantity but lower purity; ultracentrifugation purer but lower yield	Precipitation shows contamination; ultracentrifugation cleaner isolates	Bioactivity affected by purity; precipitation may impair function	Precipitation cost-effective; ultracentrifugation expensive and less scalable	Precipitation faster; ultracentrifugation labor-intensive
(Lo et al., 2024)	Ultracentrifugation yields high-quality vesicles; precipitation variable yields	Ultracentrifugation provides higher purity; precipitation co-precipitates proteins	Bioactivity preserved better in ultracentrifugation isolates	Ultracentrifugation costly; precipitation more affordable	Ultracentrifugation complex; precipitation simpler and faster
(Sha et al., 2024)	Ultracentrifugation yields abundant vesicles; precipitation yields less but acceptable	Ultracentrifugation isolates purer vesicles; precipitation includes contaminants	Bioactivity maintained in ultracentrifugation isolates	Ultracentrifugation less scalable; precipitation scalable and cost-effective	Ultracentrifugation time-consuming; precipitation less so
(Ratnadewi et al., 2022)	PEG precipitation yields higher particle concentration than some precipitation kits	PEG precipitation less pure than ultracentrifugation; contamination present	Antioxidant activity retained; bioactivity linked to isolation method	PEG precipitation cost-effective and scalable	PEG precipitation simpler and faster than ultracentrifugation
(Suresh et al., 2021)	PEG precipitation at low pH increases yield 4-5 fold over neutral pH	Purity maintained despite increased yield; low pH does not compromise quality	Bioactivity including miRNA content preserved	PEG precipitation highly scalable and cost-effective	PEG precipitation rapid and less complex

Study	Yield efficiency	Purity assessment	Bioactivity retention	Scalability and cost	Isolation time and complexity
(Kalarikkal et al., 2020)	PEG precipitation recovers 60-90% yield compared to ultracentrifugation	PEG method yields lower purity; residual PEG affects analysis	Bioactivity comparable between PEG and ultracentrifugation isolates	PEG method more cost-effective and scalable	PEG method simpler, avoids ultracentrifugation complexity
(Ryu et al., 2020)	Combining ultracentrifugation with precipitation yields higher EV numbers	Combined method balances purity and yield better than single methods	Bioactivity preserved; miRNA content consistent across methods	Combined method moderately scalable; requires ultracentrifugation	Moderate complexity; combines two protocols
(Ludwig et al., 2018)	PEG precipitation followed by ultracentrifugation enriches EVs efficiently	Washing steps improve purity; PEG alone less pure	Functional activity retained in PEG-enriched EVs	PEG method scalable; ultracentrifugation limits throughput	PEG precipitation simple; ultracentrifugation adds complexity
(Yu et al., 2024)	Optimized PEG precipitation yields comparable recovery to commercial kits	PEG precipitation purity comparable to kits; better than ultracentrifugation	Bioactivity including proliferation and migration unaffected	PEG precipitation cost-effective and scalable	PEG precipitation less time-consuming than ultracentrifugation
(Chang et al., 2018)	PEG-coated magnetic nanoparticles effectively isolate exosomes with protein removal	High protein removal efficiency; maintains exosome integrity	Bioactivity preserved; suitable for downstream analysis	Magnetic nanoparticle method scalable; requires nanoparticle synthesis	Moderate complexity; requires specialized materials
(Martins et al., 2018)	Precipitation and column-based methods yield variable EV quantities	Column-based methods yield higher purity than precipitation	Bioactivity linked to purity; column methods preferred for clinical use	Column methods scalable; precipitation simpler but less pure	Column methods moderate complexity; precipitation simpler
(Zhen et al., 2022)	Size exclusion chromatography yields highest EV quantity and purity in plasma	SEC isolates EVs with low contamination; PEG precipitation less pure	Bioactivity better preserved in SEC isolates	SEC scalable and reusable; PEG precipitation less suitable for hyperlipidemia	SEC moderate complexity; PEG precipitation simpler but less pure
(Chandrasekera et al., 2023)	Combination of precipitation and SEC yields high EV yield and purity	Combined method reduces contaminants compared to precipitation alone	Bioactivity enhanced by purity; combined method preferred	Combined method scalable; requires multiple steps	Higher complexity due to sequential methods
(Kocholatá et al., 2022)	Ultracentrifugation and precipitation yield comparable tobacco-derived vesicles	Ultracentrifugation yields purer vesicles; precipitation prone to aggregation	Bioactivity retained; uptake efficiency varies by method	Ultracentrifugation less scalable; precipitation more accessible	Ultracentrifugation complex; precipitation simpler

Study	Yield efficiency	Purity assessment	Bioactivity retention	Scalability and cost	Isolation time and complexity
(Kim et al., 2022)	Sucrose cushioning with ultracentrifugation yields high purity and yield	Density gradient improves purity; reduces vesicle damage	Bioactivity stable after freeze-drying; therapeutic potential maintained	Ultracentrifugation costly; density gradient adds complexity	High complexity; lengthy processing time
(Huang et al., 2021)	Ultracentrifugation from apoplastic fluid yields pure EVs; immunoaffinity enhances specificity	Immunoaffinity capture yields highest purity; ultracentrifugation moderate	Bioactivity preserved; immunoaffinity allows subclass isolation	Ultracentrifugation costly; immunoaffinity expensive and less scalable	Ultracentrifugation complex; immunoaffinity requires antibodies
(Eldahshoury et al., 2024)	Ultracentrifugation isolates small EVs from leaf apoplast with good yield	Purity moderate; lack of standardized markers limits assessment	Bioactivity retained; method suitable for plant EVs	Ultracentrifugation less scalable; method requires optimization	Moderate complexity; time-consuming
(Rahmatinejad et al., 2024)	Polymer-based kits yield higher protein concentration than ultracentrifugation	Kits show higher protein purity but possible contamination	Bioactivity comparable between methods; morphology consistent	Kits more cost-effective and scalable than ultracentrifugation	Kits simpler and faster; ultracentrifugation laborious
(Hurwitz & Meckes, 2017)	PEG-based workflow efficient for proteomic analysis; good yield	PEG method yields high purity suitable for proteomics	Bioactivity preserved; compatible with downstream applications	PEG method scalable and adaptable	PEG method less complex than ultracentrifugation
(Stanly et al., 2016)	Ultracentrifugation on sucrose/D2O cushions yields pure plant exosome-like vesicles	Density cushions improve purity; reduce contaminants	Bioactivity suitable for proteomics and functional studies	Ultracentrifugation costly; density cushions add complexity	High complexity; lengthy protocol
(Gharavi et al., 2024)	PEG precipitation yields higher exosomal RNA quantity and quality	RNA purity higher with PEG; morphology consistent across methods	Bioactivity inferred from RNA integrity; suitable for RNA analysis	PEG precipitation cost-effective and scalable	PEG precipitation simpler and faster
(Aziz et al., 2022)	Ultracentrifugation yields higher total RNA than precipitation kits	Ultracentrifugation isolates purer exosomes; kits show protein contamination	Bioactivity better preserved in ultracentrifugation isolates	Kits simpler and cheaper; ultracentrifugation costly	Kits faster; ultracentrifugation more complex
(Coughlan et al., 2020)	Precipitation yields higher exosome concentration but	Ultracentrifugation purer; precipitation co-isolates proteins	Bioactivity retained in both; precipitation faster	Precipitation faster and cheaper; ultracentrifugation costly	Precipitation simpler; ultracentrifugation labor-intensive

Study	Yield efficiency	Purity assessment	Bioactivity retention	Scalability and cost	Isolation time and complexity
	lower purity than ultracentrifugation				
(Lian et al., 2022)	Ultracentrifugation and precipitation widely used; purity varies	Purity challenges remain; lack of standardized protocols	Bioactivity linked to isolation quality; standardization needed	Ultracentrifugation costly; precipitation scalable	Ultracentrifugation complex; precipitation simpler
(Williams et al., 2023)	Size exclusion chromatography yields fewer particles but higher purity	SEC isolates EVs with high tetraspanin positivity	Bioactivity enhanced by purity; SEC preferred for therapeutics	SEC and ultracentrifugation favored; scalability concerns	SEC less complex; ultracentrifugation more demanding
(Robinson et al., 2024)	SEC yields high particle and protein amounts from small plasma volumes	SEC provides sufficient purity for proteomics	Bioactivity preserved; SEC suitable for clinical samples	SEC easy, reproducible, and scalable	SEC rapid and less complex
(Deregibus et al., 2016)	Charge-based precipitation with PEG enhances EV recovery over ultracentrifugation	Purity improved by PEG; lipoprotein contamination reduced by gel filtration	Bioactivity retained; wound healing and proliferation assays positive	Charge-based precipitation cost-effective and scalable	Simpler and faster than ultracentrifugation

▪ DISCUSSION

The selection of optimal plant exosome isolation methods depends critically on the intended biomedical application and required quality specifications. Our systematic analysis reveals that each methodology offers distinct advantages and limitations that must be carefully considered.

Ultracentrifugation remains the preferred method for applications requiring high purity and precise characterization. The method's ability to achieve 85-95% purity makes it ideal for fundamental research, biomarker studies, and therapeutic applications where contaminant levels must be minimized [1]-[3]. However, the requirement for specialized equipment, extended processing times, and limited scalability present significant barriers for large-scale therapeutic production. The density gradient ultracentrifugation variant offers superior purity (>95%) by effectively separating exosomes from protein aggregates and other contaminants. This approach is particularly valuable for applications requiring the highest quality standards, such as injectable therapeutics or diagnostic biomarker studies [10]-[12].

PEG-based precipitation emerges as the most practical approach for large-scale production and commercial applications. Despite lower purity (60-80%), the method's high yield (10^9–10^{10} particles/mL) and cost-effectiveness make it suitable for applications where moderate contaminant levels are acceptable [23]. The method's compatibility with standard laboratory equipment and straightforward protocols facilitate widespread adoption and technology transfer. However, the co-precipitation of protein aggregates and other contaminants may limit its applicability for certain therapeutic applications, particularly those requiring parenteral administration [32],[33].

Hybrid methodologies represent the most promising approach for therapeutic applications, achieving optimal balance between yield, purity, and practicality. The combination of PEG precipitation followed by ultracentrifugation effectively leverages the high yield of PEG methods while achieving the purity standards of ultracentrifugation. These approaches typically achieve 90-98% purity with reasonable yields (5×10^8–2×10^9

particles/mL), making them suitable for most biomedical applications. The moderate processing times (6-8 hours) and equipment requirements provide a practical compromise for clinical translation [24].

Mechanistic insights : Ultracentrifugation achieves high purity (85–95%) by separating particles through sequential density and size discrimination. Its tiered approach (removing debris at low speeds, isolating larger vesicles at intermediate speeds, and pelleting exosomes at ultrahigh speeds) ensures precision but requires significant time and equipment. In contrast, PEG precipitation prioritizes practicality: its volume exclusion mechanism passively aggregates exosomes via polymer-induced solubility reduction, enabling rapid, low-cost processing. However, residual contaminants limit its purity (60–80%). Hybrid methods, such as combining PEG pre-concentration with short ultracentrifugation, strategically balance these trade-offs, achieving clinically viable purity (≥90%) while maintaining scalability [36].

Plant exosomes demonstrate exceptional potential as natural drug delivery vehicles, offering several advantages over synthetic systems. Their inherent biocompatibility, ability to cross biological barriers, and capacity for targeted delivery make them ideal candidates for therapeutic applications [22]. Cancer therapy applications show particular promise, with plant exosomes demonstrating selective uptake by tumor cells and enhanced therapeutic efficacy. The natural anti-inflammatory and antioxidant properties of plant exosomes provide additional therapeutic benefits beyond their carrier function. The application of plant exosomes in regenerative medicine represents a rapidly growing field with significant therapeutic potential. Studies demonstrate their ability to promote tissue repair, enhance stem cell function, and facilitate wound healing processes [23]. The immunomodulatory properties of plant exosomes contribute to their regenerative effects by reducing inflammation and promoting favorable healing environments. This dual functionality—direct therapeutic effects combined with delivery capabilities—positions plant exosomes as unique therapeutic platforms.

The lack of standardized isolation protocols remains a significant barrier to clinical translation. Variations in methodology, characterization approaches, and quality control measures create inconsistencies that hinder regulatory approval and commercial development. Key standardization requirements include unified characterization protocol, standardized purity and yield metrics, quality control specifications and Good Manufacturing Practice (GMP) guidelines.

The regulatory pathway for plant exosome therapeutics remains unclear, with existing frameworks primarily designed for synthetic drug delivery systems. The natural origin and complex composition of plant exosomes present unique regulatory challenges that require specialized guidance. Key regulatory considerations include : safety and toxicology assessment protocols, batch-to-batch consistency requirements, stability and storage specifications and clinical trial design considerations.

The economic viability of plant exosome therapeutics depends critically on isolation method selection and production scalability. PEG-based methods offer the most favorable economic profile for large-scale production, while ultracentrifugation methods may be economically viable for high-value, low-volume applications. Cost considerations include: equipment investment and maintenance, processing time and labor costs, raw material and consumable expenses and quality control and regulatory compliance costs.

▪ CONCLUSION

Ultracentrifugation remains the gold standard for isolating highly pure plant exosomes, particularly when augmented with density gradient cushioning techniques, which effectively separate exosomes from protein aggregates and other impurities. This approach generally yields vesicles with preserved structural integrity and bioactivity, including stable RNA and protein cargo, although it is time-consuming, costly, and less scalable. The complexity and resource demands of ultracentrifugation limit its widespread use, especially in clinical or industrial settings.

Hybrid methodologies that combine PEG precipitation with ultracentrifugation or size exclusion chromatography effectively balance the trade-offs inherent in single methods. These combinations enhance purity without compromising yield or bioactivity, offering a middle ground that maximizes exosome functional integrity and isolation efficiency. Emerging alternative methods, such as immunoaffinity capture and charge-based precipitation, offer promise in improving specificity and simplicity but are currently constrained by cost, technical requirements, and limited availability of plant-specific markers.

In summary, while PEG-based precipitation offers an accessible, scalable, and cost-effective route for plant exosome isolation, it requires careful optimization and often supplementary purification steps to achieve high purity. Ultracentrifugation ensures superior purity and bioactivity preservation but at the expense of scalability and cost. Hybrid and emerging methodologies represent the frontier for optimized isolation, integrating the strengths of multiple techniques to meet the demands of biomedical applications. Future research must focus on standardizing protocols, validating plant-specific markers, and refining scalable, cost-effective methods to fully harness the therapeutic potential of plant-derived exosomes.

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