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Review article

Revolutionizing medicine: Harnessing plant-derived vesicles for therapy and drug transport

Li Lv [a], Zhenkun Li [a], Xin Liu [b], Wenhui Zhang [a], Yi Zhang [c], Ying Liang [c], Zhixian Zhang [a], Yueqiao Li [d], Mingxia Ding [a,**], Rongqing Li [e,***], Jie Lin [a,*]

[a] *Department of Medical Oncology, The Second Affiliated Hospital of Kunming Medical University, 374 Dianmian Avenue, Wuhua District, Kunming, 650101, Yunnan, China*
[b] *Department of Thoracic Surgery, The Second Affiliated Hospital of Kunming Medical University, 374 Dianmian Avenue, Wuhua District, Kunming, 650101, Yunnan, China*
[c] *Department of Thyroid - Breast Surgery, The Second Affiliated Hospital of Kunming Medical University, 374 Dianmian Avenue, Wuhua District, Kunming, 650101, Yunnan, China*
[d] *Department of Medical Oncology, Yanjin Country People's Hospital, No. 87, Pingjie Street, Yanjin County, Zhaotong, 657500, Yunnan, China*
[e] *Department of Radiation Oncology, The First Affiliated Hospital of Kunming Medical University, 295 Xichang Road, Wuhua District, Kunming, 650032, Yunnan, China*

A R T I C L E I N F O

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A B S T R A C T

The emergence of extracellular vesicles (EVs), which are natural lipid bilayer membrane structures facilitating intercellular substance and information exchange, has sparked innovative approaches in drug development and carrier enhancement. Plant-derived EVs notably offer advantages including low preparation cost, low immunogenicity, flexible drug delivery, high stability, good tissue permeability, and high inherent medicinal value compared to their animal-derived counterparts. Despite these promising attributes, the research on plant-derived EVs remains fragmented and lacks comprehensive synthesis. This review aims to address this gap by summarizing the isolation methods, biological characteristics, and storage techniques of plant-derived EVs. Additionally, we explore the potential of plant-derived EVs as therapeutic agents and drug carriers for treating various diseases. Finally, we delineate the current impediments to plant-derived EV development and highlight future research directions. By providing a detailed overview, we hope to facilitate further research and application in this emerging field.

1. Introduction

In recent years, the investigation of plant-derived extracellular vesicles (EVs) has attracted considerable interest due to their potential in therapeutic applications and drug delivery. The initial observation of EVs in barley leaf cells affected by fungi was documented in 1967 using transmission electron microscopy [1]. However, comprehensive studies on EVs commenced with mammalian cells in the 1980s, focusing primarily on their role in cell communication and disease mechanisms [2]. Over the past few decades, research on mammalian-derived EVs has advanced significantly, leading to the recognition of their potential as drug carriers in the

* Corresponding author.
** Corresponding author.
*** Corresponding author.
E-mail addresses: dmx7166@126.com (M. Ding), lrqmxl@126.com (R. Li), linjie@kmmu.edu.cn (J. Lin).

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field of nanomedicine [3,4]. In the 2010s, interest in plant-derived EVs began to grow as researchers discovered their structural and compositional similarities to mammalian-derived EVs [5]. Moreover, plant-derived EVs have demonstrated specific advantages, including high biocompatibility, cost-effectiveness, and ease of production, which may complement or expand the applications of mammalian-derived EVs in drug delivery and therapeutic contexts [6,7].

Key milestones in the development of plant-derived EVs include the identification of their non-immunogenic nature, the discovery of their inherent medicinal properties, and the development of methods for their large-scale isolation and purification. Recently, plant-derived EVs have emerged as effective drug carriers because of their structural and compositional resemblances to mammalian-derived EVs [8]. Additionally, plant-derived EVs are regarded as nonimmunogenic and safe, as plants do not harbor zoonotic or human pathogens. Furthermore, plant-derived EVs retain various biological activities from their source plants [9]. For instance, mulberry (Morus alba L.) root bark is recognized for its anti-inflammatory effects. Edible EVs derived from mulberry bark are being investigated as novel agents for the prevention and treatment of intestinal-associated inflammatory diseases by activating COPS8 in intestinal epithelial cells [10]. Likewise, strawberries and their derived EVs, celebrated for their antioxidant properties, have demonstrated efficacy in reducing oxidative stress in human mesenchymal cells [11]. Importantly, less than 300 g of plant material is sufficient to yield 1 g of high-purity plant-derived EVs [12]. These high-yield, cost-effective natural drug carriers undoubtedly hold significant promise for clinical translation.

Although plant-derived EVs present numerous advantages, research in this field is still in its infancy and remains fragmented across various independent studies, lacking a comprehensive synthesis. To address this fragmentation, we conducted a systematic review of the literature, utilizing the PubMed, Embase, and Web of Science databases. The keywords employed for the search included 'extracellular vesicles' and 'plant'. The inclusion criteria consisted of: 1) studies focused on plant-derived vesicles; 2) original research published in English; and conference abstracts were excluded. This review seeks to bridge this gap by offering a thorough overview of the latest techniques for isolating plant-derived EVs, their biological properties, relevant characterization methods, and storage strategies. Additionally, we emphasize the utility of plant-derived EVs in diseases treatment and drug delivery. By consolidating existing knowledge and identifying key challenges, we aspire to pave the way for future research and clinical applications of plant-derived EVs.

2. Isolation of plant-derived EVs

Effective isolation of plant-derived EVs is a prerequisite for their research and application. Current methods for isolating EVs from mammalian cells are relatively mature [13]. Given the structural similarities between plant-derived EVs and their mammalian counterparts, these methods serve as valuable references for isolating plant-derived EVs. Before extracting plant-derived EVs, the raw material should be pre-processed, *i.e.*, the plant surface should be washed off with PBS or ultrapure water to eliminate contaminants

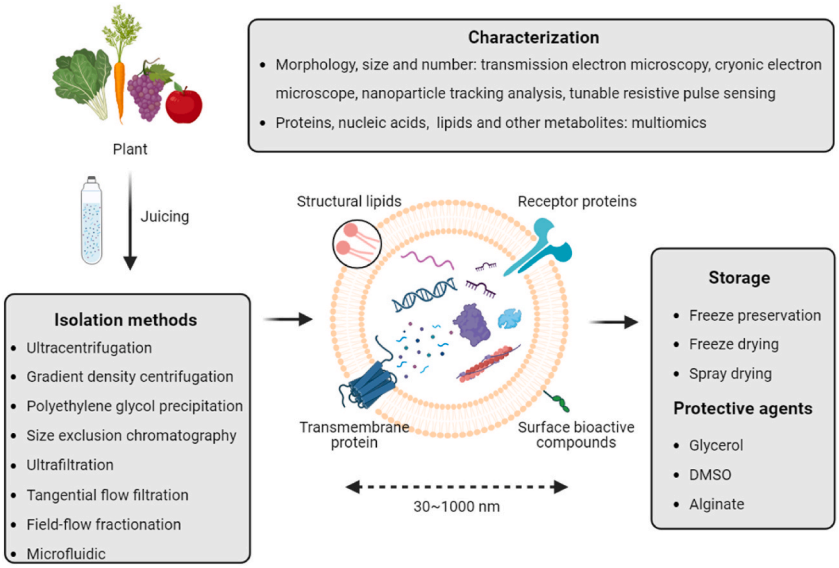

Fig. 1. Isolation of plant-derived EVs and their characterization and storage methods. This figure illustrates the process of extracellular vesicles from plants. Initially, plant materials such as leafy greens, carrots, grapes, and tomatoes are juiced. The exosomes are then isolated using various methods including ultracentrifugation, gradient density centrifugation, polyethylene glycol precipitation, size exclusion chromatography, ultrafiltration, tangential flow filtration, field-flow fractionation, and microfluidics. The structure of exosomes, ranging from 30 to 1000 nm, is depicted with components such as structural lipids, receptor proteins, transmembrane proteins, and surface bioactive compounds. Characterization of exosomes is performed using transmission electron microscopy, cryo-electron microscopy, nanoparticle tracking analysis, tunable resistive pulse sensing, and multiomics for proteins, nucleic acids, lipids, and other metabolites. For storage, exosomes can be preserved through freeze preservation, freeze drying, and spray drying, using protective agents like glycerol, DMSO, and alginate.

such as soil. Plant juice is obtained using a juicer and then appropriately diluted, followed by differential centrifugation to remove fibrous debris and particles, yielding a supernatant containing plant-derived EVs and processed in the next step [14]. In this section, we will introduce several commonly used methods, including ultracentrifugation, gradient density centrifugation, polyethylene glycol precipitation, size exclusion chromatography, and tangential flow filtration (Fig. 1).

2.1. Ultracentrifugation

Ultracentrifugation remains the predominant technique for extracting plant-derived EVs. This process begins with the low-speed centrifugation of the previously obtained supernatant at 1000–10,000×*g* for 20–60 min, effectively removing dead cells, cell debris, and large particulates. Subsequently, high-speed centrifugation at 100,000–150,000×*g* for 60–120 min is performed to pellet the EVs, which are then resuspended in phosphate-buffered saline (PBS) for further analysis [15]. Recent advancements in rotor design and centrifugation protocols have significantly enhanced EV yield and separation efficiency by minimizing protein contamination and improving purity [16]. Despite its widespread application, ultracentrifugation presents limitations, including the requirement for costly ultracentrifuges and potential for damage to EVs due to high shear forces, ultimately leading to a reduced yield of viable EVs [17].

2.2. Gradient density centrifugation

Gradient density centrifugation is employed to purify plant-derived EVs by leveraging the principle that particles will settle in distinct regions based on varying density gradients [18]. Initially, a sucrose solution is prepared with a concentration gradient of 8 %, 15 %, 30 %, 45 %, and 60 %. The supernatant obtained through differential centrifugation is then added to the sucrose gradient solution, followed by ultracentrifugation. Plant-derived EVs are primarily enriched within the sucrose layer ranging from 30 % to 45 % concentration [19,20]. Although effective, the high viscosity of sucrose solutions can pose challenges. Recent studies have proposed the use of iodixanol or potassium bromide gradients as alternatives, which may enhance separation efficiency and mitigate viscosity-related issues [21,22].

2.3. Polyethylene glycol (PEG) precipitation

PEG precipitation is conducted by modifying the solubility and dispersion of plant-derived EVs, resulting in their precipitation from solution [23,24]. Typically, a suitable amount of PEG6000 is added to the supernatant obtained through differential centrifugation, followed by incubation at 4 °C with shaking overnight. The following day, the treated solution is resuspended in PBS after low-speed centrifugation for 30 min to recover plant-derived EVs. While PEG precipitation is straightforward and facilitates large-scale EVs isolation, it necessitates further optimization due to its relatively low purification efficiency [25].

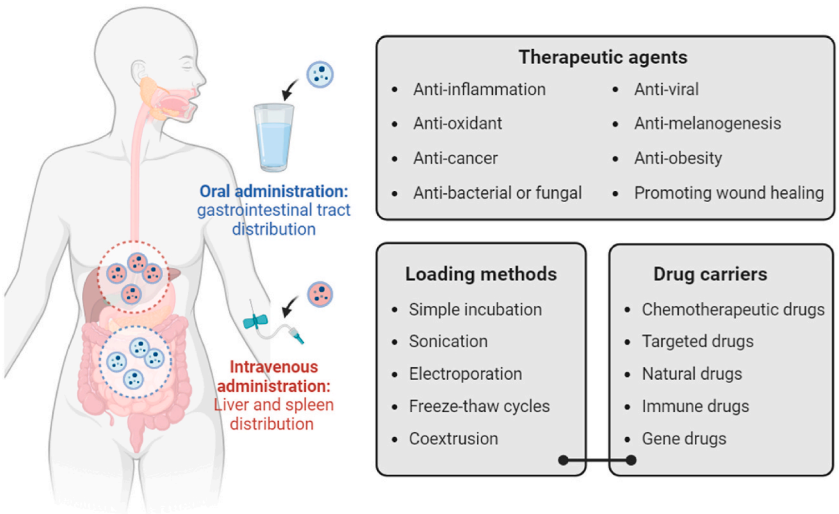

Fig. 2. Plant-derived EVs as therapeutic agents and drug carriers. This figure illustrates the application of plant-derived EVs in therapeutic delivery through two main administration routes: oral and intravenous. Oral administration targets the gastrointestinal tract, while intravenous administration primarily distributes plant-derived EVs to the liver and spleen. Plant-derived EVs can carry various therapeutic agents, including anti-inflammatory, antioxidant, anti-cancer, anti-bacterial or antifungal, anti-viral, anti-melanogenesis, anti-obesity agents, and those promoting wound healing. Loading methods for plant-derived EVs include simple incubation, sonication, electroporation, freeze-thaw cycles, and coextrusion. They can be used as carriers for chemotherapeutic drugs, targeted drugs, natural drugs, immune drugs, and gene drugs.

2.4. Size-exclusion chromatography (SEC)

The isolation of plant-derived EVs using SEC depends on the selectivity of the pore sizes in the resin beads of the column for particles of different radii [26]. As with the previous method, the supernatant obtained through differential centrifugation is pass through the column. Plant-derived EVs will preferentially elute from of the column, while smaller impurities remain within the column for an extended duration. SEC is straightforward to operate and relatively cost-effective, allowing the isolated EVs to maintain their structural integrity and biological activity. However, SEC may be time-consuming and less effective in removing large impurity particles [27,28].

2.5. Tangential flow filtration

Tangential flow filtration (TFF) represents an advanced method for the isolation of EVs, iespecially relevant for for clinical translation. TFF functions by allowing plant-derived EV-containing fluid to flow tangentially across the filter membrane's surface, enabling the separation of EVs based on size while preventing clogging and minimizing shear damage [29]. This method is highly scalable and efficiently process large volumes, making it suitable for both clinical and industrial applications [30]. For instance, Kim et al. demonstrated that TFF effectively isolates small extracellular vesicles from Aloe vera peels, yielding promising results for wound healing applications due to their antioxidant properties [31]. Additionally, Sukreet et al. emphasized the efficacy of TFF in isolating extracellular vesicles from cheesemaking byproducts, resulting in heterogeneous fractions of nanoparticles that may be beneficial for various biomedical applications [29].

2.6. Emerging techniques

In addition to traditional methods, several emerging techniques, including ultrafiltration and field-flow fractionation (AF4), are being utilized to isolate EVs from plants [32,33]. Microfluidic technology and nanoparticle-assisted isolation are also under investigation to improve the extraction of plant-derived EVs. Microfluidic devices facilitate high-efficiency, low-volume separation of EVs with high precise discrimination based on size and charge [34]. Nanoparticle-assisted methods enhance the purity and specificity of isolated EVs, resulting in improved yield and functionality, as evidenced by recent studies [35]. It is important to note that no single technique is flawless, and a combination of methods may be necessary [36]. In conclusion, the optimal method should be selected according to specific requirements. Integrating multiple techniques, such as combining differential centrifugation with ultrafiltration, can further enhance the purity and yield of plant-derived EVs.

3. Characterization and storage of plant-derived EVs

3.1. Characterization of plant-derived EVs

The characterization of plant-derived EVs, encompassing the identification of morphology, proteins, nucleic acids, and other small molecules, is essential to confirm their successful isolation and maintain their structural and functional integrity. Standardized characterization of plant-derived EVs is crucial for advancing further research and applications (Fig. 1).

3.1.1. Morphological characterization

Transmission electron microscopy (TEM) is the most widely employed technique for the morphological characterization of plant-derived EVs [37]. Typically, plant-derived EVs exhibit round and saucer-shaped morphologies [12]. In contrast, cryo-electron microscopy (Cryo-EM), which eliminates the need for sample fixation and staining, preserves the nearly native hydrated state of membrane vesicles [12,38]. Furthermore, Cryo-EM offers high-resolution imaging to examine the structure and biophysical properties of EVs, including size, shape, and membrane remodeling [39]. This facilitates a more accurate classification and understanding of the mechanisms underlying the formation of plant-derived EVs. In addition to direct microscopic observation, the morphological characteristics of plant-derived EVs can be assessed using nanoparticle tracking analysis (NTA) and tunable resistive pulse sensing (TRPS). NTA measures the size distribution and quantity of plant-derived EVs, while TRPS evaluates the size, zeta potential, and concentration [40,41]. Recent advancements in these imaging technologies have markedly improved the resolution and depth of morphological analysis, offering clearer insights into the structural integrity and functional capabilities of plant-derived EVs.

3.1.2. Protein characterization

The detection of proteins in mammalian EVs using immunoblotting assays is widely accepted [42]. In contrast, plant-derived EVs typically exhibit low protein abundance. Moreover, the protein composition may vary among different plant-derived EVs. As a result, a comprehensive protein database for the identification is currently lacking. Nonetheless, plant-derived EVs contain certain membrane proteins and intracellular proteins, including actin and proteolytic enzymes [43,44]. A previous study reported that 56.7 % of the proteins in lemon-derived EVs matched those found in mammalian EVs according to a comparison of the ExoCarta database, highlighting the potential for future identification of proteins in plant-derived EVs [45]. The advent of advanced mass spectrometry techniques has enabled researchers to identify novel proteins and lipids in plant-derived EVs that were previously undetectable [46]. This includes the discovery of unique surface markers that may be pivotal in targeted drug delivery and intercellular communication [47]. These findings are significant as they not only expand the known proteome and lipidome of plant-derived EVs but also indicate

new functional roles and therapeutic potentials.

However, the characterization of plant-derived EVs remains in its infancy compared to that of mammalian EVs, primarily due to the absence of well-defined marker proteins. Recent studies have identified potential marker proteins for plant-derived EVs, including heat shock proteins, aquaporins, and certain glycoproteins, which can facilitate more precise characterization and isolation of these vesicles [6,44]. Moreover, advancements in proteomic and lipidomic technologies continue to reveal unique components within plant-derived EVs, enhancing our comprehensive understanding of their molecular composition and potential markers [48]. These developments are crucial for establishing standardized protocols for the characterization of plant EVs, akin to those currently available for mammalian EVs [49].

3.1.3. Nucleic acid characterization

Plant-derived EVs carry a substantial amount of nucleic acids. These genetic messages are delivered to the recipient cells via EVs, facilitating intercellular communication [50,51]. Notably, an increasing body of research indicates that miRNAs in plant-derived EVs may influence the progression of various human diseases [52,53]. For instance, miRNAs from buckwheat tartar-derived EVs can target functional genes in *Escherichia coli* and *Lactobacillus rhamnosus* (LGG), thereby enriching the diversity of the gut microbiome and enhancing intestinal health [54]. Similarly, mdo-miR7267-3p derived from ginger-derived EVs directly regulates the monooxygenase ycnE in LGG, promoting the production of IL-22 mediated by indole-3-carboxaldehyde (I3A). The expression of IL-22 has been shown to ameliorates colitis [55]. Collectively, the identification of nucleic acids in plant-derived EVs is of significant importance for their application, particularly as therapeutic agents and drug carriers.

3.1.4. Small molecule characterization

In addition to proteins and nucleic acids, plant-derived EVs encompass a diverse array of small molecules, including lipids and metabolites, that play crucial roles in their biological functions and therapeutic potential [56,57]. Recent studies underscore the importance of characterizing these small molecules to fully understand the capabilities of plant-derived EVs. For example, specific lipid profiles can significantly influence the biodistribution of EVs within the body. Plant-derived EVs enriched in phosphatidic acid (PA) preferentially localize in the intestine, presenting potential for targeted gastrointestinal therapies [56,57]. Conversely, those rich in phosphatidylcholine (PC) exhibit strong hepatic accumulation, indicating their suitability for liver-targeted drug delivery systems [20,55]. Furthermore, metabolites present in plant-derived EVs—including flavonoids, terpenoids, and alkaloids—contribute to their therapeutic effects [58]. The identification and quantification of these small molecules are essential for optimizing the application of plant-derived EVs in clinical settings. Understanding the small molecule composition of plant-derived EVs not only elucidates their functional mechanisms but also paves the way for their efficient application in medical practice.

3.2. Storage of plant-derived EVs

Drawing from experiences in storing mammalian-derived EVs, the preservation of plant-derived EVs primarily relies on freezing methods [59]. For short-term storage, plant-derived EVs can be kept at 4 °C or −20 °C, while −80 °C is recommended for long-term preservation. However, the activity and concentration of cryopreserved EVs decline with increasing storage time [60,61]. Protective agents, including glycerol, DMSO, and alginate, appear to mitigate this negative effect to some extent [62] (Table 1). Moreover, freeze-drying and spray-drying represent promising storage methods for plant-derived EVs [63,64] (Fig. 1). The integration of cryopreservation with protectants and optimized lyophilization technology may significantly enhance the viability and functionality of plant-derived EVs in the future.

Table 1
Recommended storage conditions for plant-derived EVs.

Storage Condition	Recommended Storage Time	Remarks
4 °C	Short-term (days to weeks)	Suitable for short-term storage, activity may decrease over time [59].
−20 °C	Short-term (weeks to months)	Better than 4 °C for slightly longer storage, still not ideal for long-term [59].
−80 °C	Long-term (months to years)	Most suitable for long-term storage, but activity and concentration decrease with time. Addition of trehalose can improve stability [60,61].
With protective agents (e.g., glycerol, DMSO, alginate)	Extended storage	Protective agents can help maintain activity and concentration during storage. Glycerol and DMSO effective for cryopreservation. Alginate for extended preservation [59,62].
Freeze drying/Spray drying	Long-term	Potential methods for long-term storage, though optimization is needed. Lyophilization with stabilizers like trehalose shows promise. Spray drying still under research [63,64].

Table 2
Recent advances in the use of plant-derived EVs in disease treatment.

Therapeutic Effect	Disease Type	Source of Plant EVs	Mechanism of Action	Related Research
Anti-inflammatory	Colitis	Grape	Induces Lgr5⁺ intestinal stem cells, enhances intestinal tissue renewal, targets intestinal stem cells through GELN lipids, and modulates β-catenin-mediated signaling pathways to protect against DSS-induced colitis.	Ju et al., 2013 [65]
Anti-inflammatory	Colitis	Grapefruit	Selectively taken up by intestinal macrophages, upregulates heme oxygenase-1 (HO-1), inhibits IL-1β and TNF-α production, and enhances therapeutic effects of Methotrexate (MTX) while reducing its toxicity.	Wang et al., 2014 [106]
Anti-inflammatory	Colitis	Ginger	Reduces pro-inflammatory cytokines (e.g., TNF-α, IL-6, and IL-1β), increases anti-inflammatory cytokines (e.g., IL-10 and IL-22) to restore immune balance in the gut, alleviates acute colitis symptoms, prevents chronic colitis and cancer development, and targets the colon to be efficiently absorbed by intestinal epithelial cells and macrophages, exerting significant local anti-inflammatory effects.	Zhang et al., 2016 [67]
Anti-inflammatory	Colitis	Broccoli	Activates AMP-activated protein kinase (AMPK) in dendritic cells (DCs), prevents DC activation, induces DC tolerance, and protects against DSS-induced colitis through sulforaphane (SFN)-mediated activation of AMPK.	Deng et al., 2017 [19]
Anti-inflammatory	Ulcerative Colitis	Ginger	Targets colon tissues specifically, reduces CD98 expression, and delivers siRNA efficiently with high biocompatibility, overcoming limitations of synthetic nanoparticles.	Zhang et al., 2017 [107]
Anti-inflammatory	Colitis	Mulberry bark	Activates AhR signaling through HSPA8, induces COPS8 expression in intestinal epithelial cells, and promotes the production of anti-microbial peptides, providing protection against DSS-induced colitis.	Sriwastva et al., 2022 [10]
Anti-inflammatory, Antioxidant	Intestinal Diseases	Ginger, Grapefruit, Carrot	Induces expression of heme oxygenase-1 and IL-10 in macrophages, promotes activation of nuclear factor (erythroid-derived 2), and activates Wnt signaling in intestinal crypts, leading to improved intestinal homeostasis.	Mu et al., 2014 [20]
Anti-inflammatory, Anti-cancer	Inflammatory Bowel Disease, Colitis-associated Colon Cancer	Tea Leaves	Mediates specific internalization by macrophages via galactose receptor-mediated endocytosis, reduces reactive oxygen species, inhibits pro-inflammatory cytokines, increases anti-inflammatory IL-10 secretion, restores colonic barriers, and enhances gut microbiota diversity and abundance.	Zu et al., 2021 [66]
Anti-inflammatory, Antiviral	COVID-19-induced Lung Inflammation	Ginger	Inhibits expression of Nsp12 and spike genes through miRNA aly-miR396a-5p and rlcv-miR-rL1-28–3p, reduces TNF-α, IL-6, and IL-1β secretion, and prevents apoptosis of lung epithelial cells by suppressing NF-κB activation.	Teng et al., 2021 [100]
Antioxidant	Alcohol-induced Liver Damage	Ginger	Activates Nrf2 pathway, induces expression of liver detoxifying and antioxidant genes, inhibits reactive oxygen species production, and mediates effects through shogaol in a TLR4/TRIF-dependent manner.	Zhuang et al., 2015 [76]
Antioxidant	Oxidative Stress	Strawberry	Contains anthocyanins, folic acid, flavonols, and vitamin C, prevents oxidative stress in human MSCs in a dose-dependent manner, and maintains cell viability through vitamin C-mediated antioxidative effects.	Perut et al., 2021 [11]
Antioxidant	Alzheimer's disease	Lemon	Crosses the blood-brain barrier (BBB), exhibits antioxidant activity comparable to ascorbic acid, and maintains over 80 % cell viability in SH-SY5Y cells, suggesting potential for combating oxidative stress in neurodegenerative diseases.	Dolma et al., 2024 [108]
Antioxidant, Pro-differentiative	Oxidative Stress, Bone Health	Lemon	Delivers and preserves micronutrients such as vitamin C and citrate, protects against oxidative stress, and promotes mesenchymal stromal cell differentiation towards the osteogenic lineage.	Baldini et al., 2018 [73]
Antioxidant, Anti-apoptotic	Myocardial Infarction, Parkinson's Disease	Carrot	Inhibits reactive oxygen species (ROS) generation and apoptosis, prevents reduction of antioxidative molecules (Nrf-2, HO-1, NQO-1), and shows low cytotoxicity in cardiomyoblasts and neuroblastoma cells.	Kim et al., 2021 [74]
Anticancer	Colitis-associated colorectal cancer (CAC)	Ginger	Inhibits the progression of CAC by targeting intestinal epithelial cells (IECs) and macrophages, promoting IEC survival and proliferation, repairing damaged tissues, modulating tumor-suppressive molecular targets, and preventing the transition from chronic inflammation to cancer.	Zhang et al., 2016 [67]

(*continued on next page*)

Table 2 (*continued*)

Therapeutic Effect	Disease Type	Source of Plant EVs	Mechanism of Action	Related Research
Anticancer	Melanoma	Ginseng (Panax ginseng C.A. Mey.)	Promotes M2-to-M1 macrophage polarization, increases ROS, induces melanoma cell apoptosis, and inhibits tumor growth via TLR4 and MyD88 signaling pathway activation.	Cao et al., 2019 [87]
Anticancer	Gastric Cancer	Lemon	Induces cell cycle S-phase arrest and apoptosis in gastric cancer cells, and mediates anticancer activities through the generation of ROS.	Yang et al., 2020 [83]
Anticancer	Colon Cancer	Ginger	Serves as a delivery platform for Doxorubicin (Dox), targets colon cancer cells efficiently, exhibits high biocompatibility, demonstrates superior pH-dependent drug-release profile, and enhances tumor inhibition through folic acid modification	Zhang et al., 2016 [109]; Zhang,.et al., 2024 [110]
Anticancer	Breast Cancer	Tea Flowers	Generates ROS, triggers mitochondrial damage, arrests cell cycle, and modulates gut microbiota, leading to inhibition of breast cancer growth and lung metastasis.	Chen et al., 2022 [84]
Intestinal Remodeling	Inflammatory Bowel Disease, Colorectal Cancer	Grape	Increases Lgr5 gene expression in intestinal stem cells, modulates stem cell microenvironment, and resists degradation in the gastrointestinal tract, enabling efficient oral delivery.	Rahimi et al., 2018 [111]
Antibacterial, Anti-pathogenic	Periodontitis	Ginger	Selectively taken up by Porphyromonas gingivalis via phosphatidic acid (PA) interactions with HBP35 protein, inhibits bacterial pathogenic mechanisms through PA and miRs cargo molecules, and reduces multiple pathogenic factors in *P. gingivalis*.	Sundaram et al., 2019 [94]
Wound Healing	Skin Wounds	Wheat (*Triticum aestivum*)	Promotes cell viability and migration of endothelial, epithelial, and dermal fibroblast cells, increases collagen type I mRNA expression, reduces apoptotic cell number, and enhances tube-like structure formation in endothelial cells.	Şahin et al., 2019 [102]
Anti-fibrotic	Liver Fibrosis	Tea Leaves	Inhibits hepatic stellate cell (HSC) activation, reduces collagen deposition and lipid droplets, lowers serum AST and ALT levels, and suppresses TGF-β1 signaling and miR-44 expression.	Gong et al., 2022 [112]

4. Application of plant-derived EVs as therapeutic agents and drug carriers

4.1. Therapeutic agents (Fig. 2, Table 2)

4.1.1. Anti-inflammatory effects

Plant-derived EVs exhibit significant anti-inflammatory properties, positioning them as promising candidates for treatment of inflammation-related diseases. Recent research has deepened our understanding of how these EVs can modulate various biological pathways to elicit therapeutic outcomes. For instance, grape-derived EVs can modulate the Wnt/β-catenin pathway, alleviating intestinal inflammation in mice by activating $Lgr5^{+}$ intestinal stem cells [65]. Phase I clinical trials have been conducted to evaluate oral grape-derived EVs for the treatment of oral mucositis induced by chemotherapy for neck and head cancers (www.clinicaltrials.gov/ct2/show/NCT01668849). Moreover, broccoli-derived EVs have been shown to significantly reduce dextran sodium sulfate (DSS)-induced colitis by maintaining intestinal immune homeostasis through AMPK induction in dendritic cells [19]. Similarly, Zu et al. demonstrated that the surface of green tea-derived EVs is enriched with galactose moieties, facilitating their entry into macrophages via the endocytic pathway [66]. Consequently, macrophages secreted substantial amounts of the anti-inflammatory factor IL-10, effectively preventing or inhibiting colitis and inflammation-associated colon cancer. Interestingly, green tea-derived EVs have also been shown to enhance the diversity of the intestinal microbiota and contribute to the restoration of the intestinal mucosal barrier [66].

Consistent with these findings, ginger-derived EVs not only directly induced the upregulation of anti-inflammatory factors in colon-26 and RAW264.7 cells but also reduced the secretion of pro-inflammatory factors, thereby alleviating DSS-induced colitis. Additionally, these EVs modulated the secretion of indole-3-carboxaldehyde (I3A) from *Lactobacillus rhamnoides* (LGG), further stimulating IL-22 production and enhancing the intestinal microflora [67]. Furthermore, exosome-like nanoparticles from mulberry bark have been shown to prevent DSS-induced colitis through the AhR/COPS8 pathway, underscoring another novel mechanism by which plant-derived nanoparticles can mitigate inflammation [10]. Exosome-like nanovesicles derived from pueraria lobata have been demonstrated to significantly ameliorate lung inflammation associated with DSS-induced colitis by modulating macrophage polarization, emphasizing their potential in treating both intestinal and related lung inflammation [68]. Nanovesicles derived from tomato fruit, enriched with curcumin, have shown significant anti-inflammatory effects [69]. Additionally, oral administration of exosome-like nanovesicles has proven effective in treating colitis in mice by exhibiting both anti-inflammatory and pro-resolving properties. These nanovesicles primarily alleviate symptoms of colitis by inhibiting the NF-κB pathway, which regulates the production of pro-inflammatory cytokines including TNF-α, IL-6, and IL-1β. Moreover, they enhance the expression of the antioxidant

gene HO-1, thereby contributing to their therapeutic effect [70]. This underscores the substantial potential of plant-derived EVs in the treatment of inflammatory-related diseases.

4.1.2. Antioxidant effects

Numerous studies have investigated the antioxidant effects of specific components derived from fruits and vegetables [71,72]. Plant-derived EVs, which can effectively protect these antioxidants, may be utilized in the prevention and treatment of various oxidative stress-related diseases. Lemon-derived EVs are rich in ascorbic acid (vitamin C) and citrate, which protect mesenchymal stem cells (MSCs) from oxidative stress [73], suggesting that these natural EVs may contribute to tissue regeneration and positively influence bone development and repair. Similarly, strawberry-derived EVs, which are naturally rich in anthocyanins, folic acid, flavonols, and vitamin C, have demonstrated significant antioxidant capacity in MSCs [11]. Carrot-derived exosome-like nanoparticles, referred to as Carex, exhibited significant antioxidant activity in models of myocardial infarction and Parkinson's disease [74]. Carex induces the expression of several antioxidant proteins, including Nrf-2, HO-1 and NQO-1, thereby inhibiting excessive reactive oxygen species (ROS) production in H9C2 cardiomyocytes and SH-SY5Y neuroblastoma cells [74]. Extracellular vesicles derived from Citrus reticulata Blanco cv. 'Dahongpao' have demonstrated significant antioxidant activity and potential for drug delivery [75]. Moreover, ginger-derived EVs may effectively protect against alcohol-induced liver injury by reducing ROS. Mechanistically, shogaol present in ginger-derived EVs activates the key redox-related molecule Nrf2 by regulating the TLR4-TRIF axis, thereby promoting the transcription of various hepatic detoxification and antioxidant genes [76].

4.1.3. Anticancer effects

Despite the remarkable efficacy of many newly developed antineoplastic agents, their associated side effects—including allergic reactions, immune system dysfunction, and impaired hepatic function—pose significant clinical challenges [77–79]. Recent intensified research on plant-derived EVs has progressively unveiled their potent antitumor activity. These EVs, exhibit minimal side effects and low immunogenicity, thereby offering promising prospects for antitumor applications [80–82]. Yang and colleagues demonstrated that lemon-derived EVs significantly induced cell cycle arrest and apoptosis in gastric cancer cells through the accumulation of ROS [83]. Similarly, lemon-derived EVs have been shown to induce apoptosis in chronic myeloid leukemia cells by activating TRAIL-mediated pathway [45]. Moreover, recent studys have demonstrated the efficacy of tea flower-derived EVs in treating metastatic breast cancer. Specifically, tea flower-derived EVs induced oxidative stress in breast cancer cells, leading to mitochondrial damage and subsequent cell cycle arrest. Further in vivo experiments revealed that the accumulation of tea flower-derived EVs at primary tumor and lung metastases significantly inhibited breast cancer growth and metastasis. Interestingly, these EVs also found to modulate and improve gut microbiota composition [84]. Ginger-derived EVs have been shown to effectively prevent and treat colitis-associated colorectal cancer. In a mouse model of colorectal carcinogenesis induced by azoxymethane and DSS, ginger-derived EVs inhibited the proliferation of intestinal epithelial cells by downregulating the expression of cyclin D1 and various cytokines, thereby suppressing the development and progression of colorectal cancer [67]. Additionally, ginger-derived EVs upregulated the expression of PKG and transferrin, both of which are associated with a favorable prognosis in colitis-associated colorectal cancer [85,86].

Notably, ginseng-derived EVs have shown significant efficacy in enhancing melanoma by modulating macrophage polarization. Mechanistically, ginseng-derived EVs activated the TLR4/MyD88 axis, promoting macrophage polarization from an M2 to M1 phenotype and inducing oxidative stress-mediated apoptosis in murine melanoma cells. Furthermore, ceramide lipids and proteins within ginseng-derived EVs may play a pivotal role in driving macrophage polarization [87]. EVs from the leaves and stems of *Dendropanax morbifera* serve as effective anti-melanogenic agents, inhibiting melanogenesis-related proteins such as MITF, TYR, TRP-1, and TRP-2, in both murine melanoma and human epidermal models [88]. Notably, these EVs exhibited superior anti-melanogenic effects compared to arbutin, without exhibiting any overt cytotoxicity [88]. In a murine lung cancer model, artemisinin-derived nanovesicles (ADNVs) suppressed tumor growth and enhanced anti-tumor immunity by remodeling the tumor microenvironment and reprogramming tumor-associated macrophages (TAMs) [89]. Mitochondrial DNA from artemisinin can be internalized into TAMs via these vesicles, subsequently activating the cGAS-STING pathway. This activation shifts tumor macrophages from an immune-tolerant to a pro-inflammatory phenotype, thereby significantly enhancing the antitumor efficacy of PD-L1 inhibitors in murine models [89]. Moreover, EVs derived from other plants, such as *Asparagus cochinchinensis* and grapefruit, have also exhibited antitumor activity [80,90,91].

4.1.4. Antibacterial and antifungal effects

As previously discussed, the antibacterial and antifungal properties of plant-derived EVs have been demonstrated in various cancer studies, primarily through their modulatory effects on the intestinal microbiota. Nucleic acids within plant-derived EVs can be internalized by bacteria or fungi, thereby modulating their gene expression [52,92,93]. For instance, ginger-derived EVs can prevent or treat chronic periodontitis by inhibiting the growth of the oral pathogen *Porphyromonas gingivalis(P. gingivalis)*. Mechanistically, phosphatidic acid (34:2) present in ginger-derived EVs interacts with HBP35 on the surface of *P. gingivalis*, facilitating its selective uptake. MiRNAs encapsulated in ginger-derived EVs inhibit the expression of T9SS family genes in *P. gingivalis*, thereby preventing its attachment to and invasion of oral epithelial cells [94]. Additionally, coconut water-derived EVs can regulate the growth of *Escherichia coli* K-12 MG1655 by modulating gene expression [95]. This regulatory effect underscores the versatility of plant-derived EVs in targeting diverse bacterial species, thereby broadening their potential application in antibacterial therapies.

4.1.5. Antiviral effects

The ongoing COVID-19 pandemic continues to pose a significant threat to both human health and economic development [96,97]. Plant-derived EVs, abundant in small RNAs (sRNAs), have shown potential for the treatment and prevention of novel coronavirus infections [98,99]. Teng and colleagues reported that ginger-derived EVs significantly alleviated pneumonia mediated by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Mechanistically, rlcv-miR-rL1-28–3p present in ginger-derived EVs suppresses the expression of spike gene. Additionally, ally-miR396a-5p derived from ginger-derived EVs prevents lung macrophage apoptosis by downregulating NF-κB-mediated expression of inflammatory cytokines [100]. Similarly, miRNAs targeting SARS-CoV-2 genomic sequences were identified in grapefruit-derived EVs through predictions using RNA hybridization software [101].

4.1.6. Other therapeutic effects

Plant-derived EVs exhibit a diverse range of biological effects. Wheat-derived EVs have been shown to accelerate wound healing. One study demonstrated that wheat-derived EVs promote the proliferation and migration of endothelial cells, epithelial cells, and dermal fibroblasts. Consistently, the expression of type I collagen was transcriptionally upregulated in cells treated with wheat-derived EVs [102]. Grape-derived nanovesicles protect against LPS/D-GalN-induced acute liver failure, exhibiting pronounced hepatoprotective effects [103]. Garlic-derived exosome-like nanovesicles significantly alleviate acute liver failure by inhibiting CCR2/CCR5 signaling and reducing inflammation, highlighting their potential as hepatoprotective agents [104]. Moreover, ginger-derived EVs have been found to improve glucose tolerance and insulin response, suggesting promising potential for the treatment of obesity. In a high-fat diet-induced insulin resistance animal model, Kumar et al. demonstrated that ginger-derived EVs inhibited AhR by inducing miR-375 and VAMP7 expression [105]. Although a clinical trial investigating ginger-derived EVs for treating insulin resistance and chronic inflammation in patients with polycystic ovary syndrome (POS) was withdrawn due to non-recruitment (www.clinicaltrials.gov/ct2/show/NCT03493984), these findings underscore the potential of plant-derived EVs as therapeutic agents. The therapeutic applications of plant-derived EVs extend beyond the aforementioned effects, demonstrating their

Table 3
Advantages and disadvantages of drug loading methods for plant-derived EVs.

Method	Advantages	Disadvantages	References
Passive loading			
Simple Incubation	● Simple and cost-effective process. ● Minimal equipment and technical expertise required. ● Preserves the structural integrity and biological functions of EVs.	● Low drug loading efficiency, especially for hydrophilic drugs. ● Limited to drugs that can naturally diffuse into EVs. ● Remainders of transfection reagents may influence the encapsulation process and the behaviour of the modified Plant-Derived EVs.	Kooijmans et al., 2013 [121]; Farheen et al., 2024 [122]; Ishida et al., 2023 [123]; Mammadova al., 2023 [69]; Kürtösi et al., 2024 [17].
Active loading			
Sonication	● Increased drug loading efficiency compared to simple incubation. ● Suitable for both hydrophobic and hydrophilic drugs. ● Can temporarily permeabilize EV membranes to facilitate drug entry.	● Requires parameter optimization, potential damage to EVs ● Possible aggregation of EVs and loss of functionality.	Li et al., 2022 [115]; Chen et al., 2022 [116]; Mammadova al., 2023 [69].
Electroporation	● High drug loading efficiency, particularly for nucleic acids and large molecules. ● Suitable for both hydrophilic and hydrophobic drugs, including RNA, proteins, and small molecule drugs.	● Structural Damage: This method can significantly damage the structure of EVs, causing vesicle aggregation and morphological changes. ● Variable Loading Efficiency: The loading efficiency varies widely across different studies, depending on the type of exogenous cargo. ● Need for Optimization: This method requires careful optimization to balance drug loading efficiency with the structural integrity of EVs.	Kooijmans et al., 2013 [121]; Zuppone et al., 2024 [117].
Freeze-Thaw	● Simple and widely used technique. ● Minimal technical expertise required. ● High drug loading efficiency for certain types of drugs. ● Good Stability: maintain physical stability, including size, particle dispersion, and surface charge.	● Multiple freeze-thaw cycles significantly affect the stability of EVs, leading to increased particle size and reduced negative charge. ● Lipid Degradation: At higher temperatures (such as room temperature and 4 °C), lipids in EVs degrade quickly, affecting membrane integrity. ● Increased Heterogeneity: Although particle size and dispersion remain stable at low temperatures, the heterogeneity of PEVs increases with more freeze-thaw cycles, and the surface charge changes.	Nemidkanam et al., 2023 [118].
Coextrusion	● Uniform size distribution of drug-loaded EVs. ● High drug loading efficiency, especially for hydrophobic drugs. ● Preserves the biological functions of EVs.	● Requires specialized equipment and technical expertise. ● Potential for loss of EVs during the extrusion process.	Chen et al., 2022 [116]; Mammadova al., 2023 [69]; Fernandes al., 2020 [119].

versatility across various medical fields.

4.2. Drug carriers (Fig. 2)

4.2.1. Methods for drug loading

Plant-derived EVs serve as exceptional drug delivery carriers due to their lipid bilayer structure, enabling the encapsulate both hydrophobic and hydrophilic drugs [113]. Several drug-loading techniques have been developed, each presenting distinct advantages and limitations (Table 3). These methods include passive loading, such as simple incubation, and active loading techniques, including sonication, electroporation, freeze-thaw cycles, and coextrusion [69,114–119].

Simple Incubation: This method involves directly incubating the drug with plant-derived EVs at a controlled temperature. This approach is straightforward and relatively effective for drug loading. For instance, Xiao et al. encapsulated doxorubicin into lemon-derived EVs via coincubation, which preserved the drug's antitumor activity while significantly reducing its side effects [120]. However, as drug encapsulation in this method relies solely on passive diffusion, the loading efficiency of coincubation tends to be low [121–123].

Sonication: Sonication rapidly alters the lipid bilayer structure of plant-derived EVs, facilitating drug penetration [69]. This method utilizes ultrasonic waves to induce cavitation in the liquid medium, temporarily disrupting the vesicle membrane to permit drug entry. Sonication is one of the most widely used approaches for drug loading due to its relative simplicity and high efficiency [115,116]. Studies have shown that plant-derived EVs retain their properties post-sonication with minimal alterations to their lipid and protein contents [6]. Furthermore, sonication has been shown to enhance the encapsulation efficiency of hydrophobic drugs; however, prolonged exposure to ultrasonic waves may lead to degradation of both vesicles and drug molecules [115,116]. In contrast, mammalian cell-derived EVs exhibit distinct behavior. Post-sonication, mammalian EVs often fail to fully recover their original properties and may experience more pronounced structural and functional impairments [124]. Therefore, the recovery and stability of EVs post-sonication can differ significantly between plant-derived and mammalian cell-derived EVs, underscoring the necessity of optimizing sonication parameters for each EV source.

Electroporation: Electroporation employs electric fields to generate transient pores in the vesicle membrane, enabling the entry of drug molecules. This technique is particularly effective for loading large or charged molecules. However, it may induce drug precipitation and compromise the vesicle's structural integrity and function [117,121].

Freeze-Thaw Cycles: This method involves freezing a mixture of EVs and the drug, followed by thawing, which induces pore in the vesicle membranes to facilitate drug entry. The freeze-thaw cycles are typically repeated multiple times to enhance the loading efficiency. However, this process may lead to vesicle aggregation and induce structural alterations in the EVs [118].

Coextrusion: Coextrusion is another effective method for encapsulating drugs into plant-derived EVs. In this method, the drug is passed through a filter with a defined pore size along with larger plant-derived EVs, resulting in successful encapsulation [125]. However, coextrusion requires specialized equipment and technical expertise, and there is a risk of EVs loss during the extrusion process [69,116,119].

In summary, each drug loading method presents distinct advantages and limitations, and thus the selection of an appropriate method should be guided by the specific objectives and requirements of the study. In certain cases, combining multiple methods may be necessary to achieve optimal loading efficiency.

4.2.2. Distribution and targeting

Plant-derived EVs encapsulated with therapeutic agents exhibit multiorgan distribution upon systemic administration, primarily targeting the gastrointestinal tissues, liver, and spleen [20,76,87]. The distribution is influenced by several factors, including the route of administration and surface composition of the EVs. From a drug administration perspective, plant-derived EVs administered orally primarily accumulate in the gastrointestinal tissues. In contrast, plant-derived EVs administered via intraperitoneal or intravenous routes are predominantly enriched in the liver and spleen. As drug carriers, plant-derived EVs should ideally avoid accumulation in non-target tissues or organs, making this a crucial consideration for researchers. Notably, surface proteins and polysaccharides of

Table 4
Application of plant-derived EVs as drug carriers.

Type of Drug Carrier	Disease Type	Source of Plant EVs	Application Example	Related Research
Methotrexate	Inflammation	Grapefruit	Methotrexate in grapefruit-derived EVs improved anti-inflammatory effects and reduced toxicity	Wang et al., 2014 [106]
Paclitaxel	Colon Cancer	Grapefruit	Paclitaxel in grapefruit-derived EVs inhibited tumor growth and reduced chemotherapy-induced toxicity	Wang et al., 2015 [129]
Stat3 Inhibitor	Glioma	Grapefruit	Stat3 inhibitor JSI-124 in grapefruit-derived EVs crossed the blood-brain barrier and inhibited glioma tumor growth	Wang et al., 2015 [129]
Doxorubicin	Colorectal Cancer	Cabbage/Red cabbage	Doxorubicin in cabbage-derived EVs showed stronger antitumor activity in colorectal cancer	You et al., 2021 [130]
Sorafenib	Various Cancers	Kiwi	Kiwi-derived EVs significantly improved oral bioavailability and therapeutic efficacy of sorafenib	Fang et al., 2023 [131]
Curcumin	Colon Cancer	Various plants	Clinical trials investigating curcumin-loaded plant-derived EVs for modulating immune system and glucose metabolism in colon cancer patients	ClinicalTrials.gov

Table 5
Application of plant-derived EVs in gene therapy.

Gene Therapy Approach	Source of Plant EVs	Application Example	Related Research
miRNA Delivery	Broccoli	Broccoli-derived EVs loaded with miRNAs inhibited tumor cell viability in colorectal cancer	Del Pozo-Acebo et al., 2022 [136]
siRNA Delivery	Grapefruit	Grapefruit-derived EVs used for targeted gene silencing in human immortalized epidermal cells	Itakura et al., 2023 [137]
siRNA Delivery	Ginger	Ginger-derived EVs loaded with siRNA-CD98 reduced CD98 expression associated with colitis and colitis-associated cancers	Zhang et al., 2017 [107]
siRNA Delivery	Ginger	Ginger-derived EVs encapsulated with siRNA-BIRC5 prevented tumor progression and demonstrated safety in treated mice	Li et al., 2018 [138]

plant-derived EVs facilitate cellular entry by binding to specific receptors on target cells [17,126,127]. One study identified over 100 homologous proteins potentially involved in the vesicle transport process [44].

4.2.3. Applications in drug delivery (Table 4)

Enhanced Drug Properties: Plant-derived EVs have the capability to encapsulate a broad range of small molecule drugs, enhancing their bioavailability, stability, and solubility while mitigating side effects. For instance, encapsulating methotrexate within grapefruit-derived EVs significantly enhanced its anti-inflammatory efficacy and reduced associated toxicity [106]. Grapefruit-derived EVs can also be used to encapsulate antitumor drugs. In a tumor xenograft model using mouse CT26 and human SW620 colon cancer cells, paclitaxel encapsulated within grapefruit-derived EVs significantly inhibited tumor growth and alleviated chemotherapy-induced toxic side effects [128]. Similarly, in a GL26 cell-derived murine glioma model, grapefruit-derived EVs encapsulating the Stat3 inhibitor JSI-124 successfully crossed the blood–brain barrier and suppressed tumor growth, markedly extending the survival of mice. Notably, grapefruit-derived EVs do not cross the placental barrier, as evidenced by the lack of a fluorescent signal in the placenta after administration of fluorescently labeled EVs to pregnant mice [128]. This finding suggests that grapefruit-derived EVs may serve as promising drug carriers for therapeutic applications in pregnant women.

Advanced Delivery Systems: Researchers have further enhanced grapefruit-derived EVs by incorporating activated leukocyte plasma membranes to facilitate the targeted delivery of therapeutic agents to inflamed tumor sites [129]. Cabbage and red cabbage-derived EVs have been employed to deliver doxorubicin for colorectal cancer treatment, demonstrating enhanced antitumor activity compared to doxorubicin alone [130]. Another significant advancement is the use of kiwi-derived EVs for the oral delivery of sorafenib, which markedly increased its oral bioavailability, thereby enhancing therapeutic efficacy and reducing systemic toxicity [131]. Excitingly, clinical trials are currently recruiting participants to evaluate the use of plant-derived EVs as drug carriers (www.clinicaltrials.gov/ct2/show/NCT01294072). In this clinical study, curcumin—a natural chemopreventive agent for colon cancer—will be encapsulated into plant-derived EVs to modulate immune function and glucose metabolism in postoperative colon cancer patients [132].

4.2.4. Gene therapy (Table 5)

Gene-based therapeutics are usually less stable and often exhibit high toxicity and immunogenicity [133,134]. Plant-derived EVs serve as ideal carriers to protect various gene therapeutics, such as miRNAs and siRNAs, from degradation [113,135]. A recent study revealed that broccoli-derived EVs can be efficiently loaded with exogenous miRNAs. These therapeutic miRNAs, when encapsulated within broccoli-derived EVs, exhibited enhanced cellular uptake and were protected from RNase degradation and gastrointestinal digestion. When broccoli-derived EVs loaded with ath-miR159a, ath-miR159b-3p, ath-miR166b-3p, and ath-miR403–3p were incubated with colorectal cancer Caco-2 cells, tumor cell viability was significantly inhibited [136]. However, whether these miRNA-loaded EVs affect the viability of normal cells remains to be further elucidated. Grapefruit-derived EVs have been engineered for targeted delivery via surface functionalization. These EVs effectively silenced genes in human immortalized epidermal cells using a microfluidic device [137]. Ginger-derived EVs can be loaded with therapeutic siRNAs for the treatment of ulcerative colitis. In colon-26 and RAW 264.7 cells, ginger-derived EVs loaded with siRNA-CD98 significantly reduced the expression levels of CD98, which are strongly associated with colitis and colitis-associated cancers. Notably, ginger-derived EVs alone did not induce apoptosis in RAW 264.7 cells [107]. Additionally, ginger-derived EVs encapsulated with siRNA-BIRC5 (survivin) inhibited tumor progression. No significant changes in body weight were observed in treated mice, indicating the safety of this drug delivery system [138].

5. Conclusion and future perspectives

Due to their lack of mobility, plants have evolved sophisticated intercellular communication mechanisms to maintain homeostasis under challenging environmental conditions. Plant-derived EVs, which encapsulate diverse proteins, lipids, and genetic material, serve as pivotal mediators of intercellular communication [139,140]. Given the historical importance of natural products in drug development, plant-derived EVs, enriched with the essence of plant constituents have garnered substantial attention. Extensive research has highlighted several advantages of these vesicles, including enhanced stability, ease of accessibility, low immunogenicity, and diverse bioactivities, making them promising candidates for disease treatment and drug delivery [141,142]. However, significant challenges remain in comprehensively understanding the therapeutic potential of plant-derived EVs and successfully translating these findings

into clinical applications.

The limited understanding of the biological properties of plant-derived EVs currently hampers their broader therapeutic applications. Plant-derived EVs, which originate from various plant species, may carry a wide range of natural bioactive components. Therefore, the use of plant-derived EVs as therapeutic agents or drug carriers should be approached cautiously until their key bioactive components are thoroughly characterized. For instance, using plant-derived EVs with known wound-healing properties in cancer treatment, or as vehicles for delivering anticancer drugs to tumors, could lead to unintended and counterproductive outcomes. Recent advancements in multiomics technologies and bioinformatics have enabled the rapid and comprehensive characterization of plant-derived EVs, providing valuable insights for effective drug-loading strategies and synergistic therapeutic approaches [143,144].

The targeting mechanisms of plant-derived EVs remain incompletely understood. However, it is evident that membrane proteins or other bioactive components present on these EVs play pivotal roles in their targeting capabilities. Thus, modifying the membranes of these natural EVs, such as by incorporating folic acid, provides a viable strategy to overcome poor selectivity caused by interspecies differences [145,146]. Additionally, employing membrane fusion technology can endow plant-derived EVs with diverse properties, thereby partially mitigating this issue. It's also noteworthy that membrane fusion technology can enhance the cargo-loading capacity of plant-derived EVs [147].

In addition, the potential adverse effects of plant-derived EVs on homeostasis or disease progression should be considered. Although plant-derived EVs hold significant therapeutic promise, high-dose applications or specific conditions could lead to adverse effects. Studies on other types of extracellular vesicles, such as milk-derived EVs, have demonstrated that high-dose food-derived EVs may cause adverse effects, underscoring the importance of careful dose management and a comprehensive understanding of their molecular effectors [148,149].

The primary objective of researching plant-derived EVs is to facilitate their practical application. Although methods such as ultracentrifugation and density gradient centrifugation are commonly employed for isolation, they are suitable only for laboratory research and fail to meet the requirements for commercial or therapeutic translation [11]. Notably, the lack of standardized management and regulatory protocols for the clinical use of plant-derived EVs remains an unresolved issue [150]. This presents a significant barrier to their successful clinical translation.

Future research on plant-derived EVs should prioritize several key areas of investigation. Firstly, in-depth studies on the biogenesis and release mechanisms of plant-derived EVs are essential to optimize large-scale production strategies [139,140,151]. Efforts in this direction can leverage insights from the scalable and reproducible EV isolation protocols that have been successfully developed for MSCs. Pioneering work by Bernd Giebel and colleagues have demonstrated the successful large-scale production of MSC-derived EVs, offering a roadmap for analogous advancements in the large-scale production of plant-derived EVs [152–154].

Secondly, exploring the therapeutic potential of plant-derived EVs in treating neurodegenerative diseases, autoimmune disorders, and metabolic conditions could unveil new therapeutic avenues [155,156]. Additionally, enhancing the targeting and delivery efficiency of plant-derived EVs through bioengineering techniques, such as surface modification with ligands or antibodies and the application of nanotechnology, could significantly improve their specificity and therapeutic efficacy [157]. Integrating plant-derived EVs with other therapeutic modalities, such as chemotherapy, immunotherapy, and gene therapy, could yield synergistic effects and enhance patient outcomes [158].

Furthermore, critical considerations for developing potency assays and ensuring the clinical translation of human-derived EVs, discussed by researchers such as Warnecke A [159] and Mario Gimona [160], highlight the necessity of rigorous characterization and stringent manufacturing standards. These considerations are equally applicable to plant-derived EVs and can inform the development of standardized protocols for their isolation, characterization, and clinical application. Collaboration among researchers, industry stakeholders, and regulatory bodies is crucial for achieving these goals. By prioritizing these directions, the field of plant-derived EVs will continue to expand and make substantial contributions to medicine and healthcare.

This review provides an overview of the isolation methods for plant-derived EVs and explores their unique biological characteristics. Additionally, it summarizes and discusses the current state of research on the use of plant-derived EVs as therapeutic agents or drug delivery vehicles. Our aim is to engage biomedical researchers with diverse interests and provide foundational insights into this rapidly evolving field.

CRediT authorship contribution statement

Li Lv: Writing – original draft, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Zhenkun Li:** Data curation, Conceptualization. **Xin Liu:** Software, Formal analysis, Data curation. **Wenhui Zhang:** Resources, Methodology. **Yi Zhang:** Methodology, Investigation, Formal analysis, Data curation. **Ying Liang:** Software, Resources, Project administration, Methodology. **Zhixian Zhang:** Visualization, Validation. **Yueqiao Li:** Visualization, Validation. **Mingxia Ding:** Writing – review & editing, Supervision, Conceptualization. **Rongqing Li:** Writing – original draft, Supervision, Funding acquisition, Conceptualization. **Jie Lin:** Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition, Conceptualization.

Data availability statement

The data associated with this study are fully presented and referenced within the article. The research data is publicly available in the Zenodo repository and can be accessed through the following link: DOI 10.5281/zenodo.13269863.

Ethics statement

This review article does not involve any experimental studies with human participants or animals conducted by the authors. Therefore, no ethics approval or informed consent was required.

Declaration of competing interest

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