Received: 31 August 2022 | Revised: 27 October 2022 | Accepted: 2 November 2022

DOI: 10.1002/jev2.12283

REVIEW ARTICLE

Plant-derived extracellular vesicles: Recent advancements and current challenges on their use for biomedical applications

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Funding information
This work was supported by the National University of Singapore (Nano Nash Program A-0004336-00-00 & A-0008504-00-00). G.P. would also like to thank the Industry Alignment Fund—Pre-Positioning (IAF-PP) grant(A20G1a0046 and R-148-000-307-305/A-0004345-00-00); Nanyang Technological University, Grant/Award Number: 001487-00001

Abstract

Extracellular vesicles (EVs) represent a diverse class of lipid bilayer membrane vesicles released by both animal and plant cells. These ubiquitous vesicles are involved in intercellular communication and transport of various biological cargos, including proteins, lipids, and nucleic acids. In recent years, interest in plant-derived EVs has increased tremendously, as they serve as a scalable and sustainable alternative to EVs derived from mammalian sources. In vitro and in vivo findings have demonstrated that these plant-derived vesicles (PDVs) possess intrinsic therapeutic activities that can potentially treat diseases and improve human health. In addition, PDVs can also act as efficient and biocompatible drug carriers. While preclinical studies have shown promising results, there are still several challenges and knowledge gaps that have to be addressed for the successful translation of PDVs into clinical applications, especially in view of the lack of standardised protocols for material handling and PDV isolation from various plant sources. This review provides the readers with a quick overview of the current understanding and research on PDVs, critically analysing the current challenges and highlighting the immense potential of PDVs as a novel class of therapeutics to treat human diseases. It is expected that this work will guide scientists to address the knowledge gaps currently associated with PDVs and promote new advances in plant-based therapeutic solutions.

KEYWORDS

anticancer, anti-inflammatory, antioxidant, drug delivery system, plant-derived extracellular vesicles, plant-derived nanoparticles, plant-derived nanovesicles, therapeutics

1 | INTRODUCTION

Extracellular vesicles (EVs) are defined as non-viable lipid bilayer particles naturally released from cells (Abels & Breakefield, 2016; Raposo & Stoorvogel, 2013; Théry et al., 2018; van Niel et al., 2018). The term EVs includes, but is not limited to, exosomes,

Meredith Qikai Lian and Wei Heng Chng contributed equally to this work.

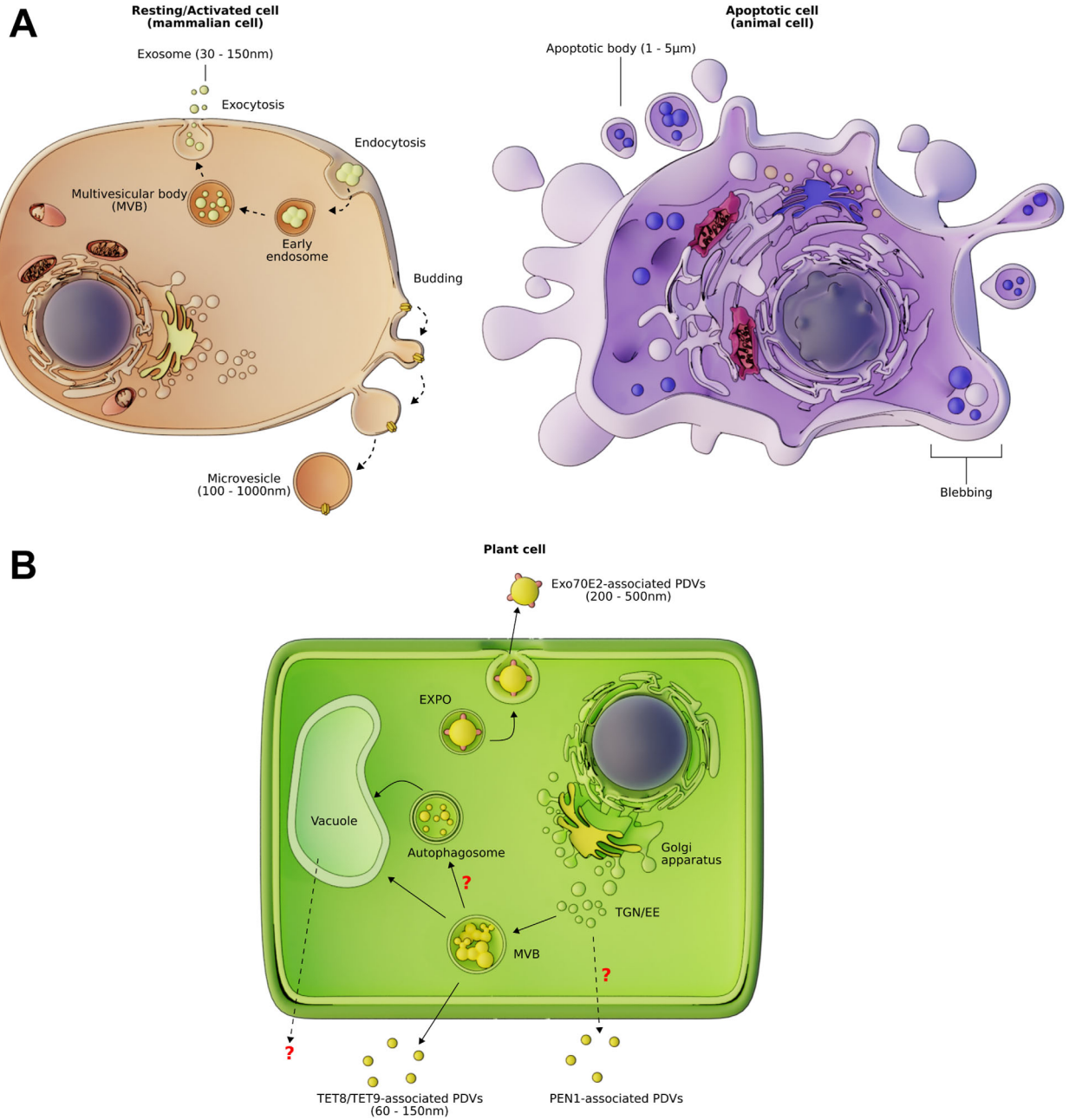

FIGURE 1 (A) Biogenesis of extracellular vesicles (EVs). The formation of small EVs/exosomes (30–150 nm) begins when the plasma membrane undergoes endocytosis to form an early endosome. The inward budding of the endosomal membrane forms intraluminal vesicles (ILVs), generating a multivesicular body (MVB). The MVB fuses with the plasma membrane to release the ILVs as exosomes. Microvesicles (100–1000 nm) are formed directly by budding from the plasma membrane. Apoptotic bodies (usually > 1000 nm) are formed when the membrane of an apoptotic cell bulge outwards (blebbing). (B) Biogenesis pathway of PDVs. Similar to the biogenesis pathway of exosomes, PDVs associated with tetraspanin 8/tetraspanin 9 (TET8/TET9) are secreted from multivesicular bodies (MVBs). The formation of PDVs associated with penetration 1 (PEN1) has not been determined. Another subpopulation of PDVs is derived from exocyst-positive organelles (EXPO). There might be other subpopulations of PDVs derived from the vacuoles and/or autophagosomes.

microvesicles and apoptotic bodies (Raposo & Stoorvogel, 2013) (Figure 1A). EVs from mammalian sources can be isolated from cell culture media and different body fluids such as blood, urine, saliva and breast milk (Taylor & Gercel-Taylor, 2013; Yáñez-Mó et al., 2015). While EVs were initially thought to be involved in the removal of cellular debris and unwanted materials, research in the recent decade has revealed that these vesicles play an important role in intercellular communication and transportation of bioactive molecules (Couch et al., 2021; Meldolesi, 2018; Woith et al., 2019). These bioactive molecules include proteins, nucleic acids and other metabolites that function as cellular signals (O'Brien et al., 2020; van Niel et al., 2018). By facilitating the intercellular transfer of bioactive molecules, EVs serve as cellular messengers that can regulate physiological processes in multicellular organisms (Raposo & Stoorvogel, 2013; Théry et al., 2018).

In the last decade, EVs have propelled themselves as a novel class of therapeutics for the treatment of several conditions (György et al., 2015; Zhang et al., 2016). Numerous studies have demonstrated that mammalian-derived EVs can directly improve the outcomes of several diseased states, ranging from various cancers to neurological and musculoskeletal-related disorders (Wiklander et al., 2019). For example, an intra-articular injection of murine bone marrow mesenchymal stem cells (BMMSCs)-derived EVs at the knees can protect mice from joint damage in an osteoarthritis model (Cosenza et al., 2017). Furthermore, the nanoscale dimensions of small EVs (<200 nm) and their ability to encapsulate therapeutic molecules have been exploited to design novel drug delivery systems (DDSs) for targeted delivery of bioactive molecules towards diseased sites. The use of EVs as DDSs can overcome some limitations of drugs, such as poor bioavailability and off-target effects (Elsharkasy et al., 2020; Meng et al., 2020). However, challenges such as scalability, immunogenicity, and potential risk of infection by zoonotic or human pathogens with mammalian-derived EVs limit their translation into clinical use (Dad et al., 2021). To circumvent these limitations, scientists in recent years have either engineered some cell-derived nanovesicles (Goh et al., 2017; Jang et al., 2013; Neupane et al., 2021) or non-immunogenic biohybrids (Goh et al., 2018; Ou et al., 2021; Ou et al., 2022) that work as EV biomimetics, or enhance their isolation (Busatto et al., 2018; Jiang et al., 2021; Wang et al., 2020) or begun to turn to alternative sources of EVs such as plants (Woith et al., 2019).

Plant cells have been observed to secrete vesicles that are biogenically and morphologically comparable to mammalian-derived EVs (An et al., 2007). Plant-derived EVs, plant exosome-like nanovesicles, plant-derived nanovesicles and edible plant-derived nanovesicles are the common terms used in different literature to describe nano and micro-sized vesicles (50–1000 nm) isolated from plants. Throughout this review, we will refer to these vesicles as plant-derived vesicles (PDVs). PDVs have been shown to be vital in the regulation of physiological processes via intercellular transport of bioactive cargos (Cui et al., 2020; Woith et al., 2019). For instance, proteomic studies have demonstrated that PDVs are abundant in enzymes that are essential for cell wall remodelling as well as proteins with antifungal and antimicrobial properties (de la Canal & Pinedo, 2018; Liu et al., 2021; Regente et al., 2017; Rutter & Innes, 2018). In addition, PDVs have also been found to transfer small non-coding RNAs (sRNAs) between cells, suggesting a role in gene regulation that can potentially alter cell proliferation and differentiation (Cui et al., 2020; Urzì et al., 2021). Although the biogenesis of PDVs is not well-studied, evidence showed that there are at least three different pathways (Cai et al., 2019) (Figure 1B; Nemati et al., 2022).

By virtue of their natural origins, physicochemical and biophysical features, PDVs could represent a new class of therapeutics. Indeed, PDVs are biocompatible and remain stable in the human body even when administered orally (Wang et al., 2014; Yang et al., 2018; Zhang et al., 2016). Furthermore, an increasing amount of evidence indicates that PDVs possess intrinsic therapeutic activities that benefit human health (Alfieri et al., 2021; Yu et al., 2020). They can be internalised by mammalian cells and modulate cellular processes of the host cell (Song et al., 2020; Urzì et al., 2021). In addition, it has been postulated that the contents of PDVs contribute to their intrinsic therapeutic activities as well.

Apart from being a promising new class of therapeutics, PDVs could also be developed as novel biologically-derived drug carriers with additional benefits over current DDSs (Dad et al., 2021). PDVs are capable of protecting and delivering hydrophilic or hydrophobic payloads to target sites (Sarvarian et al., 2021). As most reported PDVs are isolated from edible plants, they are expected to be safe for human consumption. In vitro and in vivo studies have also demonstrated the non-toxicity of PDVs (Raimondo et al., 2015). This makes them highly attractive candidates for the development of next-generation DDSs. They may also exhibit intrinsic targeting by delivering encapsulated molecules to the target sites while minimising cytotoxicity and undesirable off-target effects (Wang et al., 2014; Wang et al., 2013; Zhang et al., 2016). Of note, the targeting specificity of PDVs can be further enhanced through surface membrane engineering with appropriate targeting ligands (Li et al., 2018). Additionally, therapeutic drugs loaded into PDVs can function synergistically with the intrinsic therapeutic activities of the PDVs (Yang et al., 2021). This improvement in targeting and enhancement of efficacy has led to an overall improvement of the therapeutic outcomes (Wang et al., 2014; Yang et al., 2021). Finally, as plants are the source of PDVs, PDVs are seen as an environmentally friendly and sustainable resource with large-scale production capabilities (Adamo et al., 2021) (Figure 2). In other words, plants can serve as 'nanofactories' for the production of medical PDVs, which could offer a novel approach to current therapies (Iravani & Varma, 2019).

Despite the growing interest, research into PDVs is still in its early phases; currently, there is a lack of standardised protocols for PDVs isolation, and the precise biological functions of PDVs are still not well understood. In this review, we aim to describe the current methods that have been employed to isolate PDVs and the current understanding of their biological functions. We further discuss the recent developments of PDVs as therapeutic agents and DDSs in various diseased states, and the potential of PDVs in the development of novel classes of therapeutics.

2 | ISOLATION OF PDVs

The isolation of PDVs takes place in two main stages: (1) sample preparation followed by (2) isolation and purification (Figure 3). In this section, we introduce the existing methods utilised for PDVs isolation, as well as describe the benefits and drawbacks of each approach.

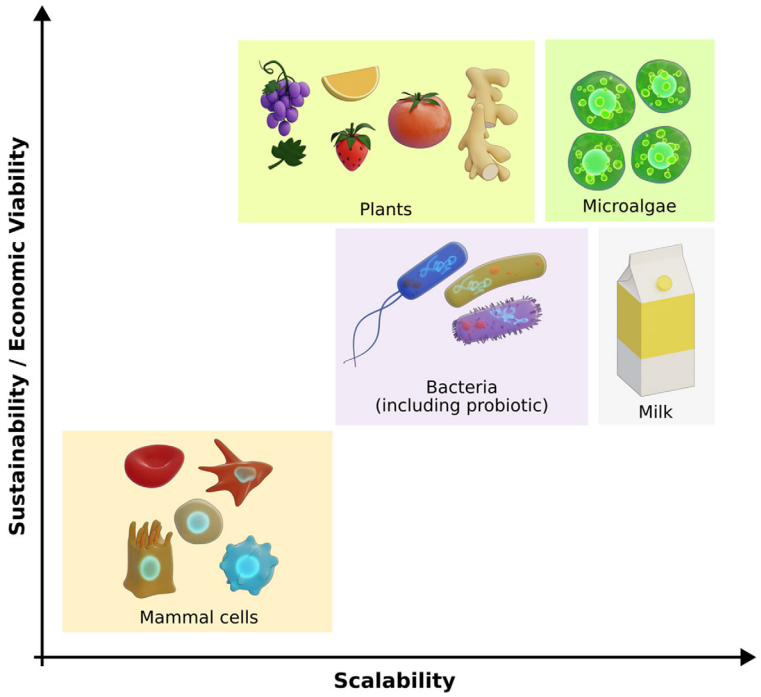

FIGURE 2 The scalability and sustainability of vesicles derived from different sources for clinical use. Figure modified from Adamo et al. (2021). Besides isolating vesicles from plants, researchers have also isolated nanovesicles from bacteria (such as probiotic-derived EVs), microalgae (known as nanoalgosomes) and milk (Adamo et al., 2021; Morishita et al., 2021; Tong et al., 2021).

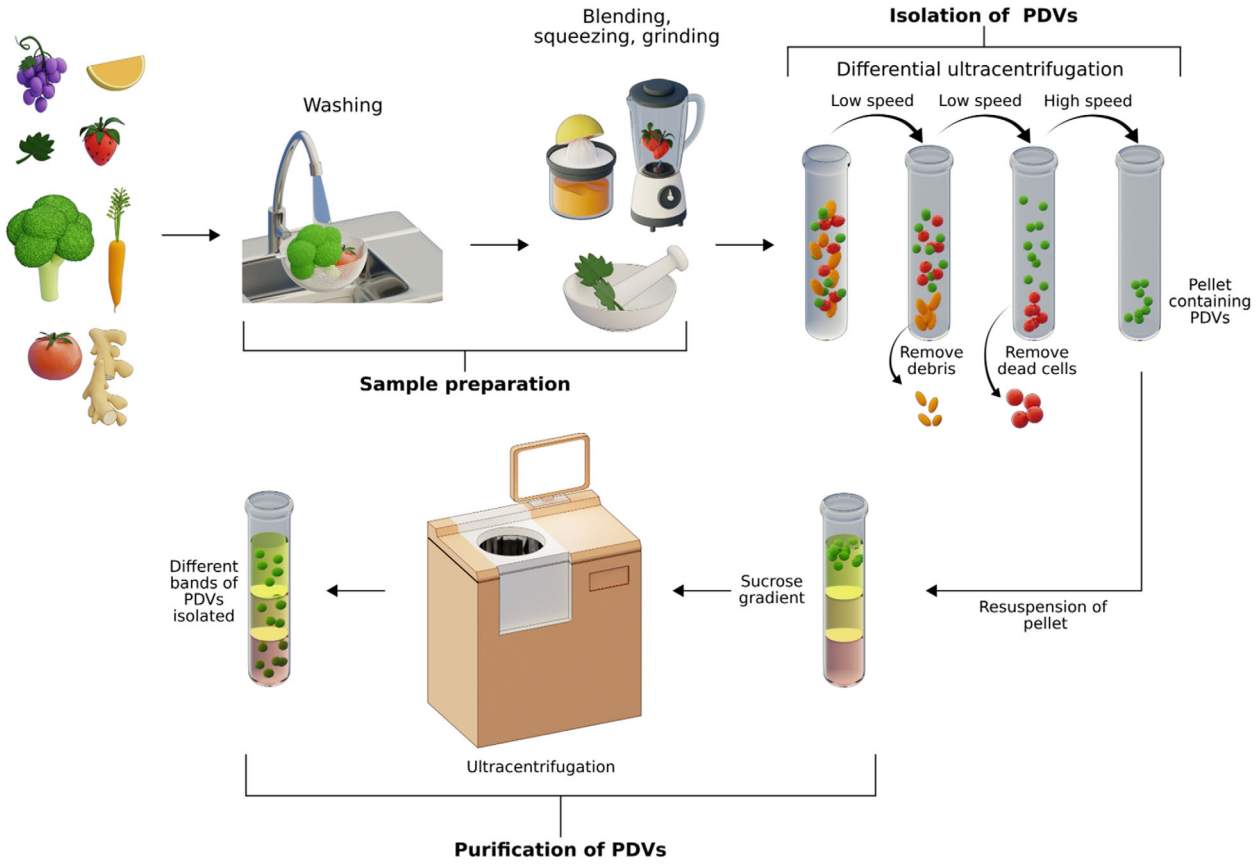

FIGURE 3 Isolation process of PDVs: The plant organ (such as fruit, leaves, or roots) will first undergo a sample preparation step (such as blending, squeezing, or grinding). This will be followed by the isolation and purification of PDVs (such as by differential ultracentrifugation and sucrose gradient ultracentrifugation). Image modified from Cong et al. (2022).

2.1 | Sample preparation

Prior to formal isolation, various physical methods can be employed to disrupt the plant tissues (also known as soft tissue disruption) and extract the juice from different plant parts such as fruits and stems. These physical methods include grinding, squeezing and blending (Cao et al., 2019; Lee et al., 2020; You et al., 2021). While there are currently no studies on the difference in PDVs obtained from different juicing methods, it has been reported that juicing methods have an effect on the phytochemical profiles and antioxidant activities in common vegetables juice (Wang et al., 2021). In this context, it may be possible that the juicing method may affect the content and bioactivity of isolated PDVs as well.

The juicing process to break down plant tissue is usually destructive. As a result, the plant extracellular matrix may contaminate the extracted juice. Pretreatment of the plant juice before formal isolation can improve the yield and purity of the PDVs obtained. For instance, Tris-HCl is usually added to the citrus plant-derived juice to remove co-purifying pectin (Pocsfalvi et al., 2018; Stanly et al., 2020). There may also be a need for other pretreatment protocols to eliminate other plant extracellular matrix.

Besides extracting the juice from plants' parts with abundant juice, the apoplast washing fluid can also be extracted by vacuum infiltration (Chen et al., 2022; Rutter & Innes, 2017). The apoplast is the space outside of the cell plasma membrane and comprises of the intercellular space and cell wall. Infiltration fluid is first introduced into the plant part (usually leaves or roots) by pressure difference (manual pressure). The infiltrated plant parts are then centrifuged at low speeds between 700 and 5000 *g* to extract the apoplast washing fluid.

Interestingly, it has also been reported that root exudates of hydroponically grown plants can also be collected for PDVs isolation (De Palma et al., 2020). The nutrient solution is replaced with ultrapure water to collect the exudates. The solution is then collected to isolate the PDVs.

As research into PDVs progresses, researchers can develop less intrusive juicing methods for PDVs isolation. This will help improve the purity and yield of the PDVs isolated and minimise the presence of contaminants.

2.2 | Methods to isolate and purify PDVs

In the last few decades, the increased research interest in mammalian-derived EVs has led to the development of numerous EVs isolation methods, such as ultracentrifugation, size-exclusion chromatography (SEC), ultrafiltration, precipitation and immunoaffinity capture (Brennan et al., 2020; Konoshenko et al., 2018). Briefly, these methods exploit the physical properties of EVs, such as size, density, charge, solubility and surface-exposed proteins, to isolate these vesicles from other biological contaminants (Konoshenko et al., 2018). Amongst these methods, differential ultracentrifugation (DUC) is presently the method adopted for the isolation of mammalian-derived EVs. Given the similar morphology of PDVs to mammalian-derived EVs, the methods developed for isolating mammalian-derived EVs should also be applicable to that of PDVs. Indeed, present DUC protocols reported in PDVs research are highly similar to those employed in mammalian-derived EVs isolation (Dad et al., 2021). Table 1 summarises the current PDVs isolation methods. Table 2 summarises the isolation yields of PDVs by different isolation methods.

2.2.1 | Ultracentrifugation-based (DUC and GUC)

DUC protocols for PDVs isolation and purification are generally adapted from those of mammalian-derived EVs (Rutter & Innes, 2020). Briefly, plant juice/extracts containing the PDVs are subjected to several rounds of centrifugation with increasing speeds, through which particles of different sizes and sedimentation rates can be separated between each round. For example, plant juice is first centrifuged at low speeds (500–10,000 *g*) to remove large, bulky matter such as fruit pulp and debris. The resultant supernatant is then centrifuged at increasingly higher speeds (40,000–100,000 *g*) for longer durations to remove smaller-sized contaminants, after which the PDVs can be collected as a pure pellet (Konoshenko et al., 2018). Presently, there are no standardised DUC protocols for PDVs isolation, with different studies utilising varying centrifugation steps, speeds and durations (Rutter & Innes, 2020).

One drawback of DUC, however, is that higher centrifugation speeds in later steps may lead to the co-sedimentation of biological contaminants such as proteins complexes and aggregates (Talebjedi et al., 2021). In view of this drawback, several researchers have also combined DUC with gradient ultracentrifugation (GUC) to isolate PDVs with higher purity (Bokka et al., 2020; Li et al., 2018; Wang et al., 2014). Briefly, GUC involves establishing a density gradient by layering liquids of varying densities, such as sucrose or iodixanol solutions of varying concentrations. Subsequent centrifugation with the density gradient can then separate the particles according to their buoyant densities (Gandham et al., 2020). While the purity of PDVs isolated with DUC coupled with GUC is generally higher, the final yield obtained is also relatively lower.

TABLE 1 Current PDVs isolation methods: Benefits and drawbacks and isolation yields

Isolation methods	Principles	Benefits	Drawbacks	Refs.
Ultracentrifugation-based • Differential ultracentrifugation (DUC) • Gradient ultracentrifugation (GUC)	• DUC – Differences in sedimentation rates of PDVs and other biological contaminants • GUC – Differences in the buoyant densities of PDVs and other biological contaminants across a density gradient	• Low cost • Large sample capacity • Low contamination risk with extra isolation reagents	• Risk of damaging PDVs • Co-sediments contaminants into the PDVs • Lengthy process • Difficulty in scaling up production by gradient ultracentrifugation • Requires specialised equipment	(Talebjedi et al., 2021; Yang et al., 2020)
Ultrafiltration (UF)	• Difference in size between PDVs and other biological contaminants	• Preserves vesicle structure • Minimises co-isolation of contaminants • Allows for concurrent processing of different samples	• Membrane fouling leads to low yields • Large sample size can be time consuming	(You et al., 2021)
Size-exclusion chromatography (SEC)	• Differences in hydrodynamic radius of PDVs and other biological contaminants	• Preserves vesicle structure • Minimise co-isolation of contaminants	• Requires specialised equipment • Lengthy process • Difficult to scale up	(Gámez-Valero et al., 2016)
Polymer-based precipitation	• Altering solubility of PDVs with polymers to significantly increase sedimentation rate	• Fast method • User-friendly operation • Low cost • No need for special equipment • Able to process samples of large volumes	• Co-precipitation of other biological contaminants • Difficulty in purification	(Konoshenko et al., 2018; Suresh et al., 2021)
Electrophoresis coupled dialysis (ELD)	• Differences in size between PDVs and other biological contaminants across an applied electric field and membrane	• Fast method • Does not require specialised equipment	• Membrane fouling leads to low yields • Manual process • May cause aggregation of vesicles	(Yang et al., 2020)
Aqueous Two-Phase System (ATPS)	• Differences in partition coefficient of PDVs and other biological contaminants between two immiscible solvents	• High efficiency and purity • Less time • No specialised equipment	• Presence of dextran which may interfere in common analytical techniques, affect cell viability, and increase viscosity of samples	(Kırbaş et al., 2019; Savcı et al., 2021)

Despite DUC/GUC being the most used isolation methods for PDVs, researchers are still divided on the ideal conditions for pelleting PDVs (Rutter & Innes, 2020). In DUC/GUC, repeated pelleting of PDVs under high centrifugal forces (usually above 100,000 *g*) may compromise the structural integrity of the PDVs and cause agglomeration. Furthermore, subjecting samples to high centrifugal forces for an extended duration can result in the co-precipitation or co-isolation of non-PDVs metabolites. This can negatively influence the reproducibility and downstream analysis of PDVs and results in inaccurate reports.

Moreover, unlike mammalian-derived EVs, which can be easily isolated from cell cultures and physiological fluids, PDV sources are more diverse. Since different plants are unique in terms of structure and composition, PDVs derived from various plant sources using the same isolation method may differ significantly. For instance, the viscosity of fluid obtained from different PDV sources may vary and influence ultracentrifugation, creating more complexity in PDVs isolation (Cvjetkovic et al., 2014; Rutter & Innes, 2020). Researchers will need to assess the different parameters of ultracentrifugation protocols for each unique plant to produce more consistent results. Future developments in isolation protocols should take into consideration these differences.

2.2.2 | Ultrafiltration (UF)

Apart from DUC, UF has also been explored as an alternative method to isolate PDVs. Briefly, UF utilises a membrane filter to trap particles above a certain size threshold while allowing smaller particles to flow through. For instance, Lee et al. demonstrated the successful isolation of PDVs from the leaves and stems of *Dendropanax morbifera* using a 100 kDa molecular weight cut-off centrifugal filter unit (Lee et al., 2020). Separately, You et al. reported the utilisation of UF and SEC for the isolation of cabbage

TABLE 2 Reported yields of PDVs

Plant source	Isolation method	Yield	Refs.
Grapefruit	DUC	$5.7 \pm 0.7 \times 10^{13}$ particles/ml	(Garaeva et al., 2021)
Strawberry	DUC	18 ± 3 μg/250 ml juice	(Perut et al., 2021)
Ginger	DUC	0.5 to 2×10^{11} vesicles/g ginger	(Chen et al., 2019)
Ginger	DUC	0.72×10^{10} particles/ml juice 1.78 μg protein/ml juice	(Li et al., 2018)
Ginger	DUC/cushion	1.85×10^{10} particles/ml juice 5.76 μg protein/ml juice	
Ginger	DUC/GUC	~50 mg/kg of ginger	(Zhang et al., 2016)
Ginger	DUC/GUC	4.2×10^{9} particles/g ginger	(Teng et al., 2018)
Arabidopsis thaliana	DUC/GUC	7.2×10^{10} particles/g leaf 1.0×10^{8} particles/g leaf (apoplast)	(Liu et al., 2020)
Ginseng	DUC/GUC	~500 mg/kg ginseng	(Cao et al., 2019)
Tomato	DUC/GUC	3.8×10^{16} particles/kg tomato 26 ± 11 mg/kg tomato	(Bokka et al., 2020)
Tomato	DUC/SEC	2.6×10^{15} particles/kg tomato 6.9 ± 1.5 mg/kg tomato	(Berger et al., 2020)
Orange	DUC/SEC	1 mg protein/350 ml juice	
Dendropanax morbifera sap	UF	~10 mg protein/10 g sap	(Kim et al., 2020)
Pinus densiflora sap	UF	~1.5 mg protein/10 g sap	
Dendropanax morbifera	UF	1.53×10^{9} particles/g (from leaf) 4.98×10^{8} particles/g (from stem)	(Lee et al., 2020)
Cabbage	UF/SEC	1.504×10^{11} particles/ml	(You et al., 2021)
Red Cabbage	UF/SEC	1.098×10^{11} particles/ml	
Cucumber	UF/SEC	0.263×10^{11} particles/ml	
Pepper	UF/SEC	0.135×10^{11} particles/ml	
Tomato	UF/SEC	0.119×10^{11} particles/ml	
Carrot	UF/SEC	3.2×10^{11} particles/g carrot	(Kim & Rhee, 2021)
Ginger	Polymer-based precipitation	2–3.8 g/kg ginger	(Kalarikkal et al., 2020)
Ginger	Polymer-based precipitation	~20 g/kg ginger (at pH 4) ~4 g/kg ginger (at pH 7)	(Suresh et al., 2021)
Grapefruit	ATPS	$\sim 1.70 \times 10^{11}$ particles/ml	(Savcı et al., 2021)

and red cabbage derived PDVs. Specifically, cabbage and red cabbage juice were first concentrated using ultrafiltration, after which the concentrate was subjected to SEC. Analysis of the SEC fractions revealed that the PDVs were collected in the earlier fractions with minimal protein impurities, suggesting that the coupling of UF with SEC was successful at isolating PDVs (You et al., 2021).

2.2.3 | Polymer-based precipitation

Polymer-based precipitation methods have also been employed to successfully isolate PDVs. In this method, polymers such as polyethylene glycol (PEG) are employed as crowding agents. Specifically, PEG serves as a crowding agent that efficiently traps the PDVs in a mesh-like net. This reduces the solubility of these vesicles, allowing them to be isolated conveniently with low-speed centrifugation (Konoshenko et al., 2018; Savcı et al., 2021; Suresh et al., 2021). To illustrate the feasibility of this method, Kalarikkal et al. evaluated the yield and biochemical properties of the ginger-derived PDVs that were isolated via PEG-based precipitation against those from DUC (Kalarikkal et al., 2020). The findings from this study revealed that the yield of PDVs obtained from PEG-based precipitation (3.8 g/kg ginger) was comparable to that of DUC (4 g/kg ginger). Furthermore, the PDVs obtained from the two methods had no significant differences in size, charge, and uptake efficiency. In a subsequent study, the authors optimised the PEG-precipitation method by reducing the pH of the precipitation step (Suresh et al., 2021). This increased the yield to 30 g/kg ginger. Besides an increase in isolation yield, the researchers also observed an increase in protein

content, suggesting the possibility of co-precipitating other biological contaminants (Konoshenko et al., 2018; Talebjedi et al., 2021).

2.2.4 | Electrophoresis with dialysis (ELD)

Besides conventional methods, other innovative techniques have also been developed for PDVs isolation. For example, Yang et al. reported a novel method based on the coupling of electrophoresis with dialysis (ELD) to isolate lemon-derived PDVs (Yang et al., 2020). Specifically, lemon juice within a 300 kDa cut-off dialysis bag was subjected to an electric current of 300 mA, resulting in the migration of biological contaminants (such as proteins and nucleic acids) out of the dialysis bag under the electric field generated. Analysis of the PDVs obtained indicated that purification via ELD was successful, where the PDVs size and particle concentration were similar to those obtained from DUC. Moreover, transmission electron microscopy (TEM) images showed that the PDVs isolated by ELD remained intact. The feasibility of ELD was further verified by the same authors in a separate study, where they reported the successful isolation of bitter melon-derived PDVs via ELD (Yang et al., 2021).

2.2.5 | Aqueous Two-Phase System (ATPS)

ATPS can be formed by mixing two polymers or polymers with salt solutions to form an immiscible mixture. Savci et al. demonstrated the use of ATPS to isolate grapefruit-derived PDVs (Savcı et al., 2021). After removing larger contaminants by centrifugation and filtration, the grapefruit juice was then mixed with PEG/dextran (PEG/DEX) (7.7:3.3 (w/w)) and centrifuged at 1000 *g* for 10 min to separate the two phases. The upper phase (PEG phase) was then removed and replaced with PEG/DEX solution. The process was repeated, and the PDVs in the lower phase (DEX phase) were collected for further analysis. While no further characterisation was done on the purity, an earlier study by the research group showed that isolation by ATPS can remove protein contaminants and non-protein contaminants such as ascorbic acid and lipids (Kırbaş et al., 2019).

2.3 | Physical characterisation of PDVs

The size distribution of PDVs isolated from various plant sources is summarised in Figure 4A. The reported sizes of the PDVs are below 500 nm. This is verified by imaging such as atomic force microscopy (AFM), TEM and scanning electron microscopy (SEM). Figure 4B–D show examples of AFM, SEM and TEM images of ginger-derived PDVs, respectively. Under electron microscopy, PDVs confirmed to have a spherical, oval or cup-shaped morphology (Karamanidou & Tsouknidas, 2022). The zeta potentials of PDVs isolated from different plant sources are negative, ranging between –1.5 and –49.2 mV (Figure 4E).

3 | COMPOSITION AND CLASSIFICATION OF PDVs

Multiomics play an important role in the identification and characterisation of EVs and PDVs contents. Several methods including immunoblotting, mass spectrometry, ELISA and gel electrophoresis have been used to study the biochemical composition of these vesicles. While not fully investigated, there is a possibility that the same plant harvested from different origins (e.g., ginger harvested from Korea vs. ginger harvested from India) may affect the contents carried by PDVs. It has been reported that environmental factors, such as soil condition, can alter a plant's chemical composition (Yang et al., 2018). This, in turn, may affect the composition of the PDVs. In fact, a study by Logozzi et al. showed that PDVs isolated from organic agriculture exhibited a higher total antioxidant capacity as compared to PDVs isolated from conventional agriculture (Logozzi et al., 2021).

In-depth analyses of PDVs contents have been reported only for a few plant species (Pinedo et al., 2021). As such, further omics efforts should be encouraged to fully understand the biologically active contents delivered by PDVs and harness their potential for clinical use. Four major components have been identified in PDVs: lipids, proteins, nucleic acids, and secondary metabolites - all of which may play a crucial role in the function and stability of PDVs (Cai et al., 2019, 2021) (Figure 5; Martínez-Ballesta et al., 2018; Regente et al., 2017).

3.1 | Lipids

The bilayer membrane of PDVs is mainly made up of lipids, which have been found to be important for maintaining stability of PDVs. Lipidomic analyses have revealed that the major classes of lipid species present in PDVs include phosphatidic acid (PA), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) (Karamanidou & Tsouknidas, 2022). In contrast,

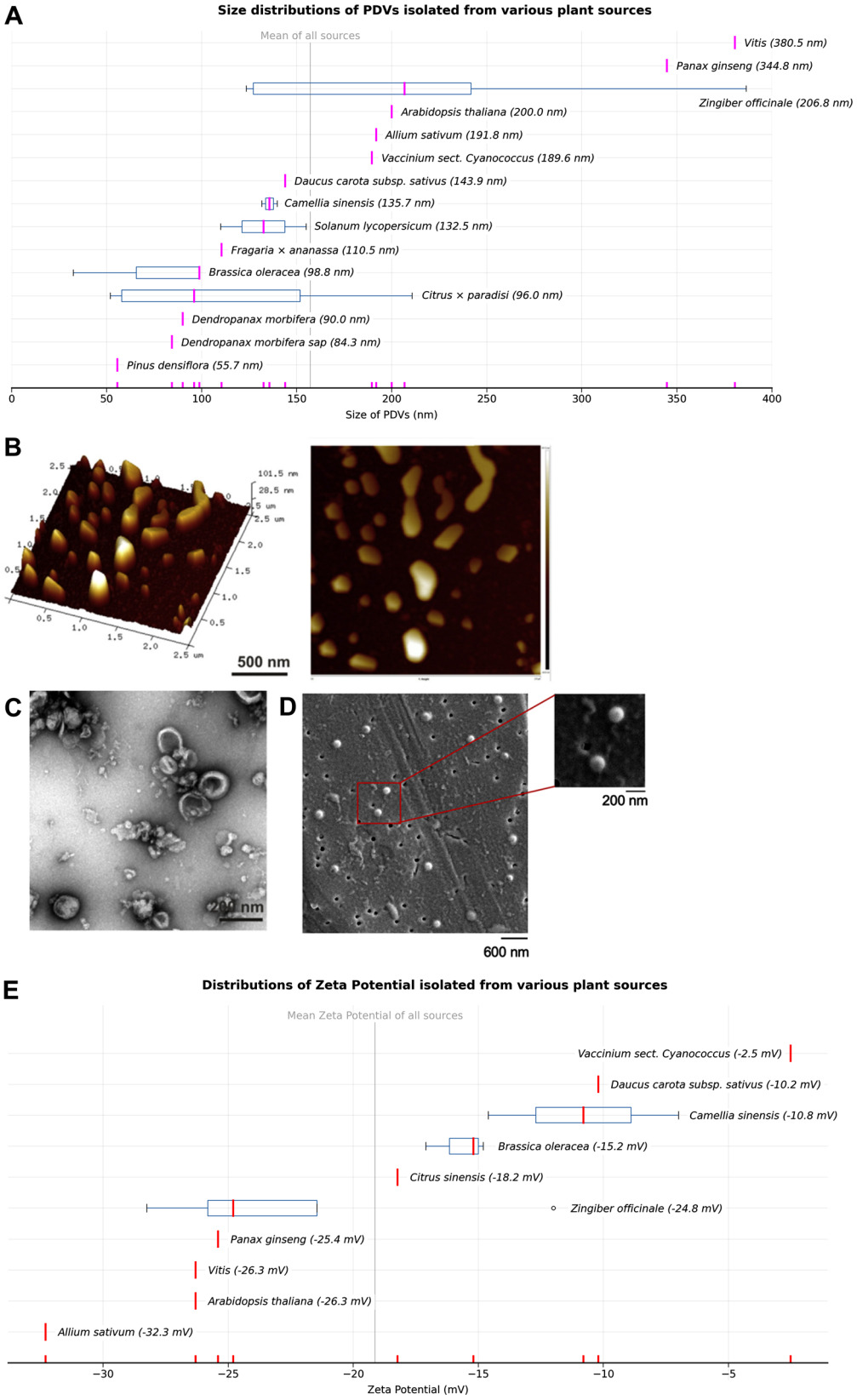

FIGURE 4 (A) The size distributions of PDVs isolated from various plant sources. (B) AFM image of ginger-derived PDVs (image from Zhang et al. (2016), reproduced with permission from Biomaterials, https://doi.org/10.1016/j.biomaterials.2016.06.018). (C) TEM image of ginger-derived PDVs (image from Zhang et al. (2016), reproduced with permission from Biomaterials, https://doi.org/10.1016/j.biomaterials.2016.06.018). (D) SEM image of ginger-derived PDVs (image from Chen et al. (2019), reproduced with permission from Molecular Pharmaceutics, https://pubs.acs.org/doi/10.1021/acs.molpharmaceut.9b00246). Any further permission related to the material excerpted should be directed to Elsevier or ACS respectively. (E) The zeta potential distributions of PDVs isolated from various plant sources.

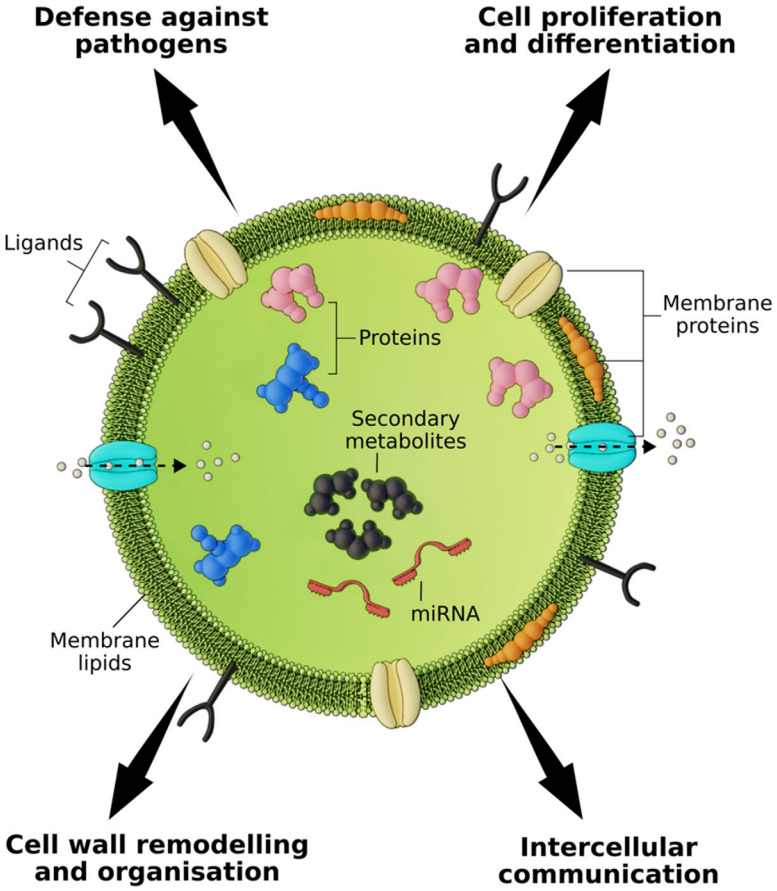

FIGURE 5 Schematic representation of PDVs contents and their relation to PDVs functions. The composition of PDVs include lipids, proteins, nucleic acids and secondary metabolites.

mammalian-derived EVs lipid profile are mainly made up of ceramides, cholesterol and sphingomyelin (Iravani & Varma, 2019). The lipid composition varies between PDVs derived from different sources. For instance, PA was described to be a major component in ginger-derived PDVs and grape-derived PDVs, while grapefruit-derived PDVs and garlic-derived PDVs were reported to be enriched with PE and PC (Wang et al., 2014; Zhang et al., 2016).

Interestingly, differences in the lipid composition of PDVs affect the uptake of these vesicles by specific gut bacteria (Teng et al., 2018). Specifically, PA-enriched ginger-derived PDVs were preferentially taken up by *Lactobacillus rhamnosus*, while PC-enriched grapefruit-derived PDVs were preferentially taken up by intestinal *Ruminococcaceae* (Teng et al., 2018). This suggests that specific lipids and lipid compositions could serve as a signal for preferential uptake by different recipient cells, giving rise to targeted therapeutic activities as reported in other works (Deng et al., 2017; Ju et al., 2013; Wang et al., 2014). For instance, PA was also observed to be highly fusogenic in the presence of calcium and has been postulated to induce inter-vesicular fusion (Record, 2013; Wiczer Brian & Thomas, 2012; Zhang et al., 2016). Despite so, the driving mechanisms behind the uptake and internalisation of PDVs by target cells are currently still not well defined.

In addition to being engaged in the uptake and internalisation of PDVs, lipids may also contribute to PDVs' intrinsic therapeutic activities. For instance, grapefruit-derived PDVs are enriched with both PE and PC which may contribute to the antioxidant and anti-inflammatory properties (Eros et al., 2009; Treede et al., 2007; Wang et al., 2014). Extensive lipidomic analyses should be performed to have a complete understanding on the role of the main lipid components in PDVs.

3.2 | Proteins

Proteins identified in PDVs are considered to have an integral role in plant physiology. For instance, membrane proteins may have a role in facilitating the uptake and internalisation of PDVs by mammalian cells, similarly to the above-mentioned lipids. In an interesting study, garlic-derived PDVs were treated with trypsin to remove all surface proteins (Song et al., 2020). It was observed that trypsin-treated garlic-derived PDVs showed lower uptake compared to their untreated counterpart, indicating that membrane proteins may be involved in the internalisation of PDVs. In addition, another study showed that aquaporins present on broccoli-derived PDVs help to maintain membrane stability (Martínez-Ballesta et al., 2018).

Interestingly, several studies have reported that the cytosolic proteins in PDVs are associated with cell wall remodelling and defence against invading pathogens (De Palma et al., 2020; Liu et al., 2021). *Arabidopsis thaliana*-derived PDVs were shown to be abundant in proteins involved in the plant immune responses (Rutter & Innes, 2017). Several proteins identified in *Arabidopsis thaliana*-derived PDVs include RPM1-interacting protein 4 and remorin, which are highly expressed in response to stress.

It has also been observed that the protein content of PDVs differs among species. Unlike *A. thaliana*-derived PDVs, ginger-derived PDVs were found to have a low protein content (Zhang et al., 2016). A comparison of proteomes of PDVs isolated from apoplast washing fluid also reveals that there are three common protein families – heat shock protein 70, S-adenosyl-homocysteinase and glyceraldehyde 3-phosphate dehydrogenase (Pinedo et al., 2021). The protein profile of PDVs is also specific to their origin in terms of secretory pathways and matrices (Urzì et al., 2021). Moreover, PDVs contain significantly lower protein content compared to mammalian-derived EVs (Iravani & Varma, 2019).

Although different studies have reported the protein composition of PDVs, there are no investigations demonstrating an unequivocal relationship between PDVs proteins and their intrinsic therapeutic activities. Hence, further proteomic analyses should be performed to uncover their biological functions.

3.3 | Nucleic acids

The ability of PDVs to modulate biological functions in plants can be attributed to their nucleic acid content, specifically RNAs, which are encapsulated within these vesicles. PDVs carry a significant number of small RNAs (sRNAs), such as microRNAs (miRNAs) (Xiao et al., 2018; Zhao et al., 2018). In addition, 'tiny RNAs' that are 10–17 nucleotides long are also detected in PDVs, although their function is still currently unknown (Baldrich et al., 2019). Several studies have verified the presence of RNAs in PDVs, with the ability to regulate gene expression and recipient cells' functions via cross species interactions (Chin et al., 2016; Xiao et al., 2018; Zhang et al., 2012; Zhao et al., 2018). Furthermore, it has been reported that plant miRNAs are naturally modified at their 3′-ends with a 2′-O-methylation, which protects them from degradation, thus conferring them greater stability (Chin et al., 2016). Plant miRNAs have also been reported to be able to prevent and treat human diseases (Li et al., 2021; Saiyed et al., 2022). For instance, miRNA-156a from cabbage, spinach and lettuce have protective effects against atherosclerosis (Hou et al., 2018). Additionally, plant miRNAs can also alter the composition and localisation of the gut microbiota (Teng et al., 2018). As such, RNAs in PDVs may represent a new class of cross-kingdom modulators, mediating animal-plant interactions at the molecular level. For instance, Xiao et al. reported the presence of miRNAs in the PDVs derived from 11 different plant species. In addition, sRNAs sequencing results demonstrated that most of the miRNAs were 20–22 nucleotides in length, with a varying number of miRNAs types present among species (Xiao et al., 2018). Moreover, further evaluation with target gene prediction using TargetScan indicated that miRNAs isolated from PDVs can potentially target and theoretically regulate mammalian genes associated with inflammatory and cancer-related response. This result is consistent with recent evidence suggesting that plant-derived miRNAs possess immunomodulatory and anticancer properties (De Robertis et al., 2020; Kim et al., 2020). With the advancement in bioinformatics, these transcriptomics analyses of PDVs may reveal novel pharmacological activities of PDVs.

3.4 | Metabolites

It is well known that plants produce a plethora of secondary metabolites that can influence human health when consumed (Russell & Duthie, 2011; Teoh, 2015). Multiple studies have focused on the bioactivity and nutritional relevance of several classes of phytochemicals such as carotenoids, flavonoids, saponins and glucosinolates (Marzocchella et al., 2011; Mena & Angelino, 2020) . As such, it is possible that these secondary metabolites can be encapsulated within PDVs and contribute to PDVs' intrinsic therapeutic activities. For instance, Zhang et al. demonstrated that the active constituents of ginger, 6-gingerol and 6-shogaol, were present within ginger-derived PDVs (Zhang et al., 2016). Furthermore, the anti-inflammatory activities of ginger-derived PDVs can be attributed to both 6-gingerol and 6-shogaol (Bischoff-Kont & Fürst, 2021; De Lima et al., 2018; Hong et al., 2020). In a separate study, Wang et al. demonstrated that naringin, a major flavonoid found in grapefruits, as well as its functional component naringenin, were present in grapefruit-derived PDVs (Wang et al., 2014). Given that naringenin has a wide range of pharmacological properties, the bioactive metabolites present in grapefruit-derived PDVs might have contributed to their pharmacological activities as well (Zeng et al., 2018).

On the other hand, it is also observed that not all secondary metabolites produced by the plant will be packaged into PDVs. Although vitamin C and naringenin can be found in orange juice, orange-derived PDVs were shown to not contain these bioactive metabolites (Berger et al., 2020). This indicates that there is still uncertainty on how the contents of PDVs are packaged into PDVs during biogenesis. Furthermore, there is a limited number of studies which have included a metabolomic analysis of PDVs. Given that PDVs may represent a valuable source of bioactive phytochemicals, metabolomic analyses of PDVs should be included to expand the current bank of metabolomic data and improve our understanding of their roles in PDVs.

3.5 | Classification of PDVs

Current knowledge on PDVs is insufficient to establish a proper classification system. The variety of PDVs populations isolated, along with difficulty of distinguishing them by current isolation and characterisation approaches, has led to considerable confusion over the specific classification of PDVs. In this section, we propose that PDVs may be classified based on their protein markers and buoyant densities.

3.5.1 | Protein markers

The membrane proteins of PDVs may be derived during their biogenesis. Although the biogenesis of PDVs is not well-studied, there as some suggestions that there exist at least three different pathways (Liu et al., 2021) (Figure 1B).

For instance, the two major subpopulations of PDVs isolated from *A. thaliana* can be separated by varying the ultracentrifugation speeds. Penetration 1 (PEN1)-positive PDVs are isolated when pelleted at 40,000 *g* while Tetraspanin 8 (TET8)-positive PDVs are isolated when pelleted at 100,000 *g* (Cai et al., 2019; Mu et al., 2014). More proteomic analysis of PDVs may be required to elucidate other subpopulations of PDVs and to ensure that this classification is consistent and reproducible. Moreover, the isolation method employed should distinguish PDVs with or without the protein markers to ensure a homogenous subpopulation. One possible isolation method is immunoprecipitation, where PDVs expressing a specific protein can be separated and isolated. However, there are limited studies on the use of immunoprecipitation to isolate PDVs, mainly attributed to the current lack of understanding of surface-exposed membrane proteins on PDVs and the availability of appropriate antibodies (Chen et al., 2022; He et al., 2021; Huang et al., 2021). Currently, the immuno-isolation of TET8-positive *A. thaliana* PDVs is conducted by incubation with protein A beads coupled to rabbit polyclonal anti-*Arabidopsis* TET8 antibodies homemade by the laboratory.

3.5.2 | Density

The DUC/GUC isolation method separates the particles present within the sample according to their sizes and densities. In this context, it is plausible that different PDVs subpopulations with different buoyant densities can be separated with this method. Zhang et al. reported that ginger-derived PDVs collected in different bands of the sucrose gradient displayed different pharmacological activities. Ginger-derived PDVs collected from band 2 (30/45%) exerted anti-inflammatory effects, but not those collected from band 1 (8/30%) (Zhang et al., 2016). Further analysis with HPLC/MS revealed that band 2 PDVs contained a higher concentration of 6-gingerol and 6-shogoal than band 1 PDVs. A similar phenomenon was also reported by Wang *et al. f*or grapefruit-derived PDVs (Wang et al., 2014). Moreover, grapefruit-derived PDVs isolated in different bands also had different lipid profiles (Wang et al., 2013).

Additionally, it has been reported that PDVs from different biogenesis pathways may also have differences in densities. TET8-positive PDVs were enriched in the fraction of densities between 1.120 and 1.190 g/ml while PEN-1-positive PDVs were enriched in the fraction of densities between 1.029 and 1.056 g/ml (He et al., 2021; Liu et al., 2021; Rutter & Innes, 2017). Collectively, these results suggest that PDVs isolated in different bands possess different composition and bioactivities. As such, it may be plausible to classify PDVs by their size and buoyant densities.

Taking into consideration the overlapping size and buoyant density of PDVs derived from different biogenesis pathways, it will be nearly impossible to have a single homogenous subpopulation isolated by DUC/GUC. With DUC/GUC as the most common isolation methods of PDVs, classifying PDVs by densities may appear to be apt. However, each density band may contain PDVs from different biogenesis pathways, and the subpopulations may have different biochemical compositions. This would be similar to the recommendations under the MISEV2018, where researchers are encouraged to use operational terms for the different EV subtypes (Théry et al., 2018).

4 | THERAPEUTIC ACTIVITIES OF PDV

The bioactive cargos present within PDVs have been shown to play a role in interspecies communication (Mu et al., 2014). Mu et al. demonstrated that PDVs could modulate the gene expression of mammalian cells. As such, it is postulated that these cargos give rise to the intrinsic effects reported for PDVs and they are potentially capable of alleviating pathological conditions in humans (Cao et al., 2019; Mu et al., 2014). The main attributes associated with PDVs include anticancer, anti-inflammatory, antioxidant, and regenerative properties (Table 3).

TABLE 3 Different sources of PDVs and their intrinsic activity in different disease models

Therapeutic activity	Source of PDVs	Remarks	Refs.
Anticancer	Bitter melon	•Bitter melon-derived PDVs displayed cytotoxicity against CAL27 and WSU-HN6 cells •Functions synergistically with 5-FU to enhance anticancer activity in vivo	(Yang et al., 2021)
Anticancer	Citrus (Grapefruit, lemon, oranges, and bitter oranges)	• Citrus-derived PDVs displayed selective cytotoxicity against MCF7, A375 and A549 cells, amongst which grapefruit-derived PDVs displayed superior activity • Grapefruit-derived PDVs arrested the cell cycle of A375 cells at the G2/M checkpoint • Lemon-derived PDVs displayed cytotoxicity against A549, SW480 and LAMA84 cells • Lemon-derived PDVs inhibited LAMA84 tumour growth and SGC-7901 tumour growth in vivo • Lemon-derived PDVs induced cell cycle arrest at the S-phase in gastric cancer cell lines, attributed to the upregulation of the GADD45A gene	(Raimondo et al., 2015; Stanly et al., 2020; Yang et al., 2020)
Anticancer	*Dendropanax morbifera* *Pinus densiflora*	• PDVs displayed cytotoxicity against MDA-MB-231, MCF7 and A431 cells • PDVs derived from both plant sources exhibit synergistic cytotoxic effects when utilised together	(Kim et al., 2020)
Anticancer	Ginseng	• Ginseng-derived EVs can promote polarisation of macrophages from M2 to M1 phenotype • Polarisation is dependent on Toll-like receptor (TLR)-4 and myeloid differentiation antigen 88 (MyD88)-mediated signaling • Alteration of M2 polarisation in vivo contributes to antitumour response against B16F10 tumour in vivo	(Cao et al., 2019)
Anticancer	*Moringa oleifera*	• PDVs displayed selective cytotoxicity against Jurkat and HeLa cells • Target gene prediction detected 12 miRNAs present in PDVs that can potentially bind to human mRNAs involved in antiapoptotic mechanisms	(Potestã et al., 2020)
Anticancer	Tea flower	• Tea flower-derived PDVs exhibited strong cytotoxcity against breast cancer cell lines • Displayed anticancer activity against breast cancer tumour in vivo • Reduced migration capacity of breast cancer cell lines, suggesting anti-metastatic activity • Exhibited anti-lung metastasis effect in vivo	(Chen et al., 2022)
Anti-inflammatory	Broccoli	• Broccoli-derived PDVs exhibited protective effects against dextran sulfate sodium (DSS)-induced colitis in vivo • Protective effects ascribed to decreased expressions of pro-inflammatory cytokines (TNF-α, IL-17A, IFN-γ) and increased expression of the anti-inflammatory cytokine IL-10 • PDVs also inhibited activation of intestinal dendritic cells	(Deng et al., 2017)
Anti-inflammatory	Cabbage Red cabbage	• PDVs derived from both sources protected RAW264.7 cells against LPS-induced inflammation • Protective effects attributed to reductions in pro-inflammatory cytokines (IL-6 and IL-1β) and COX-2	(You et al., 2021)
Anti-inflammatory	Garlic	• Garlic-derived PDVs protected HepG2 cells against LPS-induced inflammation	(Song et al., 2020)
Antioxidant	Strawberry	• Strawberry-derived PDVs protected adipose-derived mesenchymal stem cells against oxidative stress	(Perut et al., 2021)
Anti-inflammatory/ Antioxidant	Blueberry	• Blueberry-derived PDVs protected EA.hy926 cell lines against TNF-α-induced cytotoxicity and oxidative stress • Blueberry-derived PDVs attenuated mitochondrial oxidative stress in nonalcoholic fatty liver disease models	(De Robertis et al., 2020; Zhao et al., 2022)
Anti-inflammatory/ Antioxidant	Carrot	• Carrot-derived PDVs pro tected H9C2 cells against induced-oxidative stress by inhibiting the decrease in Nrf-2 expression • Similar antioxidative effects were observed with SH-SY5Y cells induced with intracellular oxidative stress • Also shown to increase expression of IL-10 and nuclear translocation of Nrf-2 in RAW264.7 cells	(Kim & Rhee, 2021; Mu et al., 2014)

(Continues)

TABLE 3 (Continued)

Therapeutic activity	Source of PDVs	Remarks	Refs.
Anti-inflammatory/ Antioxidant	Ginger	• Ginger-derived PDVs inhibited progression of DSS-induced colitis in vivo • Increases the expression of heme oxygenase-1 (HO-1), IL-10, and nuclear translocation of Nrf-2 in RAW264.7 cells • Demonstrated protective effect against alcohol-induced liver damage in vivo • Protective effect is attributed to the activation of Nrf-2, possibly by 6-shogaol present in the GPDVs • NLRP3 inflammasome assembly and activation	(Chen et al., 2019; Mu et al., 2014; Zhang et al., 2016; Zhuang et al., 2015)
Anti-inflammatory/ Antioxidant	Grape	• Grape-derived PDVs increased nuclear translocation of Nrf-2 in RAW264.7 cells	(Ju et al., 2013)
Anti-inflammatory/ Antioxidant	Grapefruit	• Grapefruit-derived PDVs exhibited protective effects against DSS-induced colitis in vivo • Protective effects attributed to suppression of pro-inflammatory cytokines and chemokines (IL-6 and IL-1β) • Increased expression of anti-inflammatory mediators HO-1 and IL-10 in colonic macrophages • Increased nuclear translocation of Nrf-2 in RAW264.7 cells	(Mu et al., 2014; Wang et al., 2014)
Anti-inflammatory/ Antioxidant	Tea	• Tea leaf-derived PDVs displayed preferential uptake by macrophages, attributed to galactose-mediated endocytosis • Displayed anti-inflammatory activity In vitro by upregulating HO-1 expression levels and increasing expression of IL-10 in macrophages • Demonstrated to be able to maintain homeostasis of gut microbiome • Exhibited capacity to treat irritable bowel disease and inhibit the progression of colitis-associated cancer	(Zu et al., 2021)
Regenerative	Grape	• Grape-derived PDVs exhibited protective effects against DSS-induced colitis in vivo by promoting intestinal stem cell proliferation	(Ju et al., 2013)
Regenerative	Grapefruit	• Grapefruit-derived PDVs promoted cell viability and migration of HaCaT cells In vitro • Enhanced migration attributed to upregulation of wound-healing genes (COL1A1, fibronectin (FBN), vimentin, laminin) • Also increased tube formation capacities of HUVEC cells In vitro	(Savcı et al., 2021)
Regenerative	Wheat	• Wheat-derived PDVs promoted cell viability and migration of HaCaT, HUVEC and human dermal fibroblast (HDF) cells In vitro • Also increased tube formation capacity of HUVEC cells In vitro	(Sahin et al., 2019)
Regenerative	Apple	• Apple-derived PDVs promoted cell proliferation and increased COL1A1 and FBN1 expression in HDF cells In vitro • Also promoted collagen biosynthesis in UVA-irradiated HDF cells	(Seo et al., 2021)
Anti-obesity	Orange	• Orange-derived PDVs accumulated in intestinal region involved in dietary lipid absorption • Increased villi size and modulated the expression of genes involved in lipid absorption	(Berger et al., 2020)

4.1 | Anticancer activities of PDVs

Cancer is one of the top causes of death globally, accounting for nearly 10 million deaths in 2020 (Ferlay et al., 2021). Conventional cancer treatments, such as chemotherapy or radiotherapy, eliminate tumour cells by inhibiting cell proliferation or by inducing apoptosis (Basak et al., 2021; America, A.C.o.R.a.t.R.S.o.N). However, these treatments are also commonly associated with high treatment failure rates and intolerable side effects as a result of drug resistance and non-selective targeting (Chakraborty & Rahman, 2012). The poor outcomes of current treatments warrant the development of novel cancer treatment options. In recent years, mammalian-derived EVs have been investigated for their anticancer properties and have progressed to clinical trials (Zhang et al., 2022). Besides mammalian-derived EVs, there is also an increased interest on the use of PDVs as anticancer therapeutics. Several studies have demonstrated that PDVs have anti-proliferative or pro-apoptotic activities, which give rise to their anticancer properties. Some of these activities are summarised in Figure 6.

For example, PDVs isolated from *Citrus limon* juice (size range: 50–70 nm) inhibited the proliferation of tumour cell lines without affecting the cell viability of healthy cells In vitro (Raimondo et al., 2015). Specifically, it was shown that lemon-derived PDVs induced TNF-related apoptosis-inducing ligand (TRAIL)-mediated apoptosis through the TRAIL/Dr5 pathway.

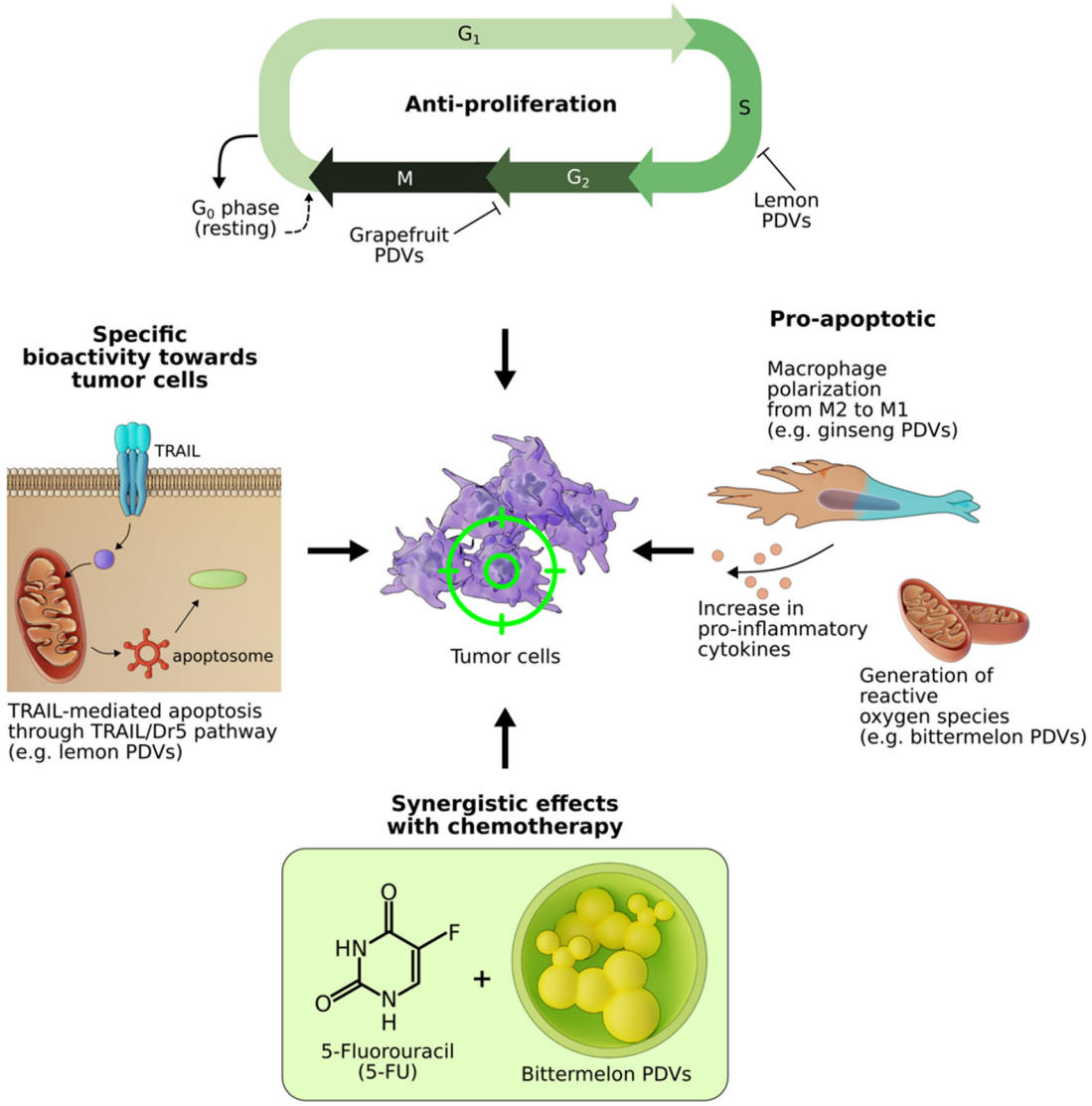

FIGURE 6 Various anticancer mechanisms exhibited by PDVs. The anticancer activity of PDVs can be attributed to pro-apoptosis and anti-proliferative effects in target cancer cells

TRAIL-mediated apoptosis has been shown to selectively induce apoptosis in cancerous cells while sparing healthy cells (Ishibashi & Ohtsuki, 2008; Koornstra et al., 2003; Yagita et al., 2004). The lemon-derived PDVs were also shown to significantly reduce tumour growth in vivo (Raimondo et al., 2015). PDVs isolated from other citrus species also exhibited similar selective anticancer properties (Stanly et al., 2020). This targeted anti-proliferative and pro-apoptotic activities towards cancerous cells can minimise side effects resulting from non-specific interactions with healthy tissues and improve the safety profile of the treatment regimen.

On top of that, PDVs also exhibit synergistic effects with current anticancer treatments. The use of chemotherapy may lead to chemotherapy-induced inflammation associated with drug resistance and metastasis (Vyas et al., 2014). For instance, the use of 5-fluorouracil (5-FU) in oral squamous cell carcinoma (OSCC) inadvertently upregulated NLR family pyrin domain containing 3 (NLRP3) expression. Concomitantly, this led to resistance against 5-FU, which is often associated with tumour growth and metastasis in OSCC (Feng et al., 2017; Wang et al., 2018). The co-administration of bitter melon-derived PDVs with 5-FU (combination therapy) was able to improve treatment outcomes in OSCC, by inducing a synergistic anti-proliferative and cytotoxic effect (Yang et al., 2021). Compared to 5-FU or bitter melon derived-PDV treatments, tumours treated with the combination therapy were significantly inhibited and showed an increase in apoptotic OSCC cells. Furthermore, it was demonstrated that the combination therapy downregulates the protein expression of NLRP3 and IL-1β. Given the role of NLRP3 in 5-FU resistance, these findings suggest that co-administration of bitter melon-derived EVs and 5-FU can prevent resistance of 5-FU and improve the outcomes of OSCC treatment. While there was no evidence that PDVs might also reduce drug resistance by altering intracellular localisation, it would be worthwhile to investigate whether the internalisation of PDVs and cytoplasmic drug release could reduce efflux of 5-FU.

4.2 | Anti-inflammatory activities of PDVs

Inflammation is a physiological response of the body to damaging stimuli or conditions and is associated with diseases such as inflammatory bowel disease (IBD) (Furman et al., 2019; Medzhitov, 2008). Chronic inflammatory diseases are usually managed by steroidal drugs and immunosuppressants (Lamb et al., 2019). However, similar to cancer treatments, the therapeutic efficacy of these anti-inflammatory treatments is severely limited due to their non-specific targeting and off-target toxicity (Wang et al., 2014). In vitro and in vivo studies have demonstrated that mammalian EVs derived from mesenchymal stem cells and probiotic EVs such as those derived from *Lactobacillus* have anti-inflammatory properties that can be used to treat inflammatory diseases such as arthritis and chronic kidney disease (Cosenza et al., 2017; Cosenza et al., 2018; Eirin et al., 2017; Hao et al., 2021; Kim et al., 2018; Nassar et al., 2016).

PDVs have shown immunomodulatory effects on gastrointestinal homeostasis. For instance, Wang et al. and Zhang et al. demonstrated that grapefruit-derived PDVs and ginger-derived PDVs, respectively, were able to increase the resistance of treated mice against DSS-induced colitis significantly with no detectable toxicity (Wang et al., 2014; Zhang et al., 2016). Specifically, In vitro studies revealed that grapefruit-derived PDVs were able to upregulate the expression of heme-oxygenese-1 (HO-1) and IL-10 in intestinal macrophages which, in turn, decreased the expression of proinflammatory cytokines and chemokines, such as IL-6, IL-1β and TNF-α (Wang et al., 2014). Moreover, it was also observed that grapefruit-derived PDVs were able to modulate the expression of E-cadherin on intestinal epithelial cells, a major protein that mediates intercellular adhesion, where impaired expression is associated with disturbed intestinal barrier function and homeostasis (Schneider et al., 2010). Hence, grapefruit-derived PDVs are likely able to enhance the anti-inflammatory capacity of intestinal macrophages and maintain the expression of E-cadherin in intestinal epithelial cells, thus conferring protective effects in vivo. Similarly, ginger-derived PDVs exerted anti-inflammatory activity by downregulating the expression of pro-inflammatory cytokines while upregulating pro-healing anti-inflammatory cytokines. Furthermore, ginger-derived PDVs were also able to enhance the survival and proliferation of intestinal epithelial cells (Zhang et al., 2016).

Anti-inflammatory activities have also been reported in similar studies performed on PDVs derived from broccoli, grapes, and carrots (Deng et al., 2017; Mu et al., 2014). Currently, grape-derived PDVs are being studied in a phase 1 clinical trial (NCT01668849) investigating their ability to prevent oral mucositis associated with chemotherapy and radiation treatment in head and neck cancer (Edible Plant Exosome).

4.3 | Antioxidant activities of PDVs

Reactive oxygen species (ROS) are typically produced in cells during aerobic respiration, but also in response to xenobiotics, cytokines, and bacterial invasion (Ray et al., 2012). ROS behave like a double-edged sword: while they play an important role in cell survival and proliferation, an imbalance between ROS production and the cellular antioxidant response can change ROS from a beneficial to a harmful species (Bardaweel et al., 2018; Liu et al., 2018). Overproduction of ROS leads to oxidative stress, which causes oxidative damage to biomolecules and results in the pathogenesis of several diseases such as neurodegenerative diseases and heart diseases (Lugrin et al., 2014). Treatment against oxidative stress involves antioxidants, which have the capability to scavenge ROS and ward off the noxious effects of oxidative stress. Several small molecules such as antioxidant enzyme mimics and nuclear factor-erythroid factor 2-related factor 2 (Nrf-2) activators have been studied as antioxidants. Although they exhibited promising results in preclinical studies, clinical trial results have not been outstanding (Forman & Zhang, 2021).

Mammalian-derived EVs have been shown to carry antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), and can regulate oxidative stress (Bodega et al., 2019). For instance, numerous studies have demonstrated the antioxidant activity of mesenchymal stem cell-derived EVs in different disease models (Bodart-Santos et al., 2019; Xia et al., 2019; Yan et al., 2017). Given that numerous fruits and vegetables are rich in antioxidants, researchers have explored the antioxidant activities of PDVs derived from various sources (Carlsen et al., 2010).

In a recent study, strawberry-derived PDVs were shown to exert antioxidant activity In vitro, protecting adipose-derived mesenchymal stem cells (ADMSCs) against oxidative stress without exerting any significant toxicity (Perut et al., 2021). Specifically, strawberry-derived PDVs improved the survival of oxidative stress-induced ADMSCs in a dose dependent manner and were able to decrease the intracellular ROS production. Vitamin C, a known free radical scavenger, was also detected in these vesicles, which may contribute to its antioxidant activity (Liu et al., 2018; Perut et al., 2021). Separately, carrot-derived PDVs were also shown to exhibit antioxidant activity. Specifically, these carrot-derived PDVs were able to protect H9C2 cardiomyoblasts and human neuroblastoma SH-SY5Y cells from oxidative stress by inhibiting the downregulation of Nrf-2 expression, a transcription factor that regulates the cellular responses against oxidative insults (Kim & Rhee, 2021). A separate study also further corroborated that carrot-derived PDVs increased nuclear translocation of Nrf2 (Mu et al., 2014). Similarly, another study showed that ginger-derived PDVs could also stimulate the expression of Nrf2 in hepatocytes and protect the cells against alcohol-induced liver damage in mice alcoholic liver model. The study further revealed that 6-shogaol, a bioactive constituent in ginger-derived

PDVs, plays a role in the antioxidant activity of ginger-derived PDVs. Specifically, 6-shogaol was demonstrated to activate Nrf-2, which led to an increased expression of liver detoxifying and antioxidant genes, hence suppressing ROS production, and contributing to hepatoprotection (Zhuang et al., 2015). While PDVs reduce oxidative stress, future work could investigate whether these PDVs have the ability to repair oxidative damage such as detoxifying lipid peroxides.

4.4 | Regenerative properties of PDVs

Wound healing is an intricate, multi-stage process that involves a plethora of biological mediators (Velnar et al., 2009). Due to its complex nature, intercellular communication is vital to preserve tissue homeostasis and ensure that wound heals in a coordinated manner (Rodrigues et al., 2019).

The use of plants for the treatment of wounds dates back to prehistoric times; for example, our ancestors utilised medicinal plants such as turmeric and liquorice to treat wounds (Sharma et al., 2021). With the modernisation of medicinal chemistry, scientists have in recent decades discovered that the therapeutic effects of such plants stem from certain natural bioactive compounds found in them (Tanideh et al., 2014; Tejada et al., 2016). With the same rationale, it has been postulated that PDVs may also harbour these bioactive compounds and mediate wound healing through cross-kingdom intercellular communication.

In two separate studies, wheat-derived PDVs and grapefruit-derived PDVs were shown to induce skin regeneration by eliciting proliferation and migration of human epidermal keratinocytes (HaCaT) in a dose dependent manner (Sahin et al., 2019; Savcı et al., 2021). Both PDVs were also able to increase tube-like structure formation in human umbilical vein endothelial cells (HUVEC), suggesting that these PDVs were proangiogenic and had the ability to induce vascular formation during wound healing. Moreover, both studies also reported that the wheat-derived and grapefruit-derived PDVs were able to increase the mRNA levels of collagen type I (COL1A1) in human dermal fibroblast (HDF) and HaCaT cells. Further investigations also showed that the grapefruit-derived PDVs induced the upregulation of the expression of various wound-healing factors, including laminin, fibronectin, vimentin and epidermal growth factor. Collectively, these results suggest that both wheat-derived and grapefruit-derived PDVs promote skin regeneration by enhancing both wound healing and wound closure (Sahin et al., 2019; Savcı et al., 2021). Despite proven evidence *in vitro, in vivo* experiments should be conducted to evaluate the efficacy of PDVs in wound repair. Wound healing is a complex process involving inflammation, proliferation and remodelling. Moreover, it would also be interesting to investigate if these PDVs have any regenerative effects on other injured organs such as cardiac regeneration after myocardial infarction.

4.5 | PDVs to treat gut-related diseases

The application of PDVs from edible plants for the treatment of gut-related diseases also represents an attractive approach, especially since the human gut is constantly exposed to PDVs when the plants are consumed. As such, the effects of PDVs against intestinal disorders, such as colitis, have also been widely investigated. As mentioned in the earlier section, grapefruit and ginger-derived PDVs are capable of exerting anti-inflammatory activities and protect mice from DSS-induced colitis. In a separate study utilising the DSS-induced colitis mice model, grape-derived PDVs were shown induce the proliferation of $Lgr5^{+}$ intestinal stem cells by Wnt/β-catenin pathway under both physiological and pathological conditions. The increased proliferation of intestinal stem cells accelerated the regeneration of intestinal epithelial tissue and restored the intestinal architecture. Since Lgr5 expression is not exclusive to intestinal stem cells, the application of grape-derived PDVs to induce stem cells proliferation could possibly be extended to other parts of the body to promote tissue regeneration in other ailments (Ju et al., 2013). Moreover, miRNAs in PDVs have been shown to affect bacterial genes and modulate commensal microbiota, indirectly influencing human health. For instance, ginger-derived PDVs can improve DSS-induced colitis in a mouse model by targeting gut bacteria *Lactobacillus rhamnosus* (LGG) (Teng et al., 2018). miRNA in ginger-derived PDVs targets the LGGycnE gene in LGG genome, leading to an increased production of indole-3-carboxaldehyde (I3A). Excess of I3A induces the secretion of interleukin-22 (IL-22), a cytokine that enhances gut barrier function and ameliorates colitis.

4.6 | Other therapeutic activities

On top of the current therapeutic applications, PDVs have the potential to be repurposed for the treatment of other human diseases, including previously untreatable diseases and new medical conditions in the future. As mentioned in the previous section, PDVs contain a variety of bioactive miRNAs that can target different genes and regulate cell functions (Xiao et al., 2018). Using the miRNA sequences database of PDVs coupled with bioinformatics, researchers can identify potentially novel uses of PDVs through *in silico* approaches (Witkos et al., 2011). This method was applied in the recent COVID-19 pandemic. Kalarikkal et al. demonstrated that miRNAs from PDVs can target different regions within the SARS-CoV-2 genome through *in*

silico target prediction analysis (Kalarikkal & Sundaram, 2021; Xiao et al., 2018). The expression of these SARS-CoV-2 targeting miRNAs in grapefruit- and ginger-derived PDVs were further verified by qRT-PCR (Kalarikkal & Sundaram, 2021; Xiao et al., 2018). In addition, another study by Teng et al. also demonstrated that ginger-derived PDVs were able to downregulate the gene expressions of SARS-CoV-2 S and viral Nsp12 In vitro and contributed to the inhibition of lung inflammation in vivo, due to the miRNAs present in ginger-derived PDVs (Teng et al., 2021). This case study emphasises the need for a comprehensive omics database of PDVs to accelerate the discovery of new therapeutic applications of PDVs. Future therapeutic applications of PDVs could be discovered through computational analysis.

5 | PDVs AS NOVEL DRUG DELIVERY SYSTEMS

Non-specific accumulation leading to drug toxicity and poor bioavailability are problems faced by several drugs. To address these challenges, researchers have developed DDSs that serve as carriers to deliver the active agent where it is most needed. These DDSs are aimed at improving the pharmacokinetic and pharmacodynamic profiles of the drug molecule in the body. Synthetic nanoparticles, such as liposomes and lipid nanoparticles, have been utilised as drug carriers both in research and in the clinical setting, and have shown promising results (Alavi et al., 2017; Lila & Ishida, 2017). However, issues with toxicity and immunogenicity, poor biodistribution and costly up-scaling production still represent unsolved issues (Sercombe et al., 2015; Shah et al., 2020). Therefore, the development of alternative DDSs to overcome the limitations of synthetic nanoparticles is very timely.

Not surprisingly, the interest in the use of natural carriers such as EVs for drug delivery has been growing expeditiously (Elsharkasy et al., 2020; Raimondo et al., 2019; Vader et al., 2016). EVs are lipid-bounded structures capable of encapsulating hydrophilic drugs within the aqueous core while accommodating hydrophobic drugs in the lipid bilayer. Furthermore, they are able to ferry bioactive molecules between neighbouring cells or to distant sites, thereby modulating the physiological processes of the recipient cells via efficient intercellular communication (Raposo & Stoorvogel, 2013). Compared to conventional synthetic nanoparticles like liposomes, EVs are more biocompatible and show minimal cytotoxicity when used in vivo (Stremersch et al., 2016; Zhang et al., 2016). As such, researchers have identified EVs as carriers that could potentially replace synthetic nanoparticles in drug delivery.

Several studies and ongoing clinical trials have supported mammalian-derived EVs as potential DDSs (iExosomes in 2021; Sun et al., 2010). However, EVs derived from bovine and murine sources, and even human sources still carry the risk of immunogenicity. Although this issue can be addressed by using mammalian-derived EVs in an autologous manner, several hurdles still limit this strategy. For instance, the utilisation of tumour-derived EVs to deliver therapeutics to tumour tissues in cancer patients poses the risk of transmitting pro-cancerous traits to normal cells (Al-Nedawi et al., 2008; Balaj et al., 2011; Peinado et al., 2012). In addition, EVs derived from infected cells that may be used to treat infectious diseases bear the risk of pathogen propagation as EVs derived from these sources have been reported to deliver functional viral RNA (Aline et al., 2004; Kalamvoki et al., 2014; Pegtel et al., 2010; Roy et al., 2011; Wiklander et al., 2019).

PDVs represent an attractive alternative to mammalian-derived EVs as novel drug carriers. Similar to EVs, PDVs are composed of a lipid bilayer and have the ability to transport both hydrophobic and hydrophilic payloads. Moreover, there are several reasons that could render PDVs a better DDS compared to synthetic nanoparticles and mammalian-derived EVs. Firstly, plants do not harbour zoonotic or human pathogens (Dad et al., 2021). In contrast to mammalian-derived EVs, PDVs reduce the risk of unintended transfer of pathogenic genes or proteins. Second, the natural provenance of PDVs makes them less likely to cause adverse reactions. In addition, PDVs have demonstrated excellent cellular uptake, stability in gastrointestinal tract and specific target ability (Wang et al., 2014; Zhang et al., 2016). Finally, as natural 'nanofactories', plants present themselves as a cost-effective, sustainable and renewable resource for PDVs compared to mammalian sources or artificially synthesised nanoparticles (Iravani & Varma, 2019; You et al., 2021). In view of their unique cross species transport capabilities and intrinsic ability to cross physical barriers, researchers have explored the idea of using PDVs as novel drug carriers (Xian et al., 2021).

Currently, PDVs can be used as DDSs in two different ways. They can either be directly used for drug loading and delivery post isolation or be modified into plant-derived nanovectors (PDNVs) derived from their lipids (Figure 7).

5.1 | PDVs as carriers

Multiple studies have demonstrated that PDVs are useful drug delivery vehicles when used directly for drug loading and delivery (Table 4).

For instance, You et al. treated SW480 colon cancer cells with PDVs derived from cabbage and red cabbage loaded with the chemotherapy drug doxorubicin (Dox) (You et al., 2021). The isolated PDVs were incubated with Dox for 4 h at 37°C to obtain Dox-loaded PDVs. After 72 h, it was reported that Dox-loaded cabbage-derived PDVs were able to reduce cell viability of SW480 cells to 57.5%, comparable to the 61.0% with Dox only (You et al., 2021). This indicates that the PDVs were internalised by the

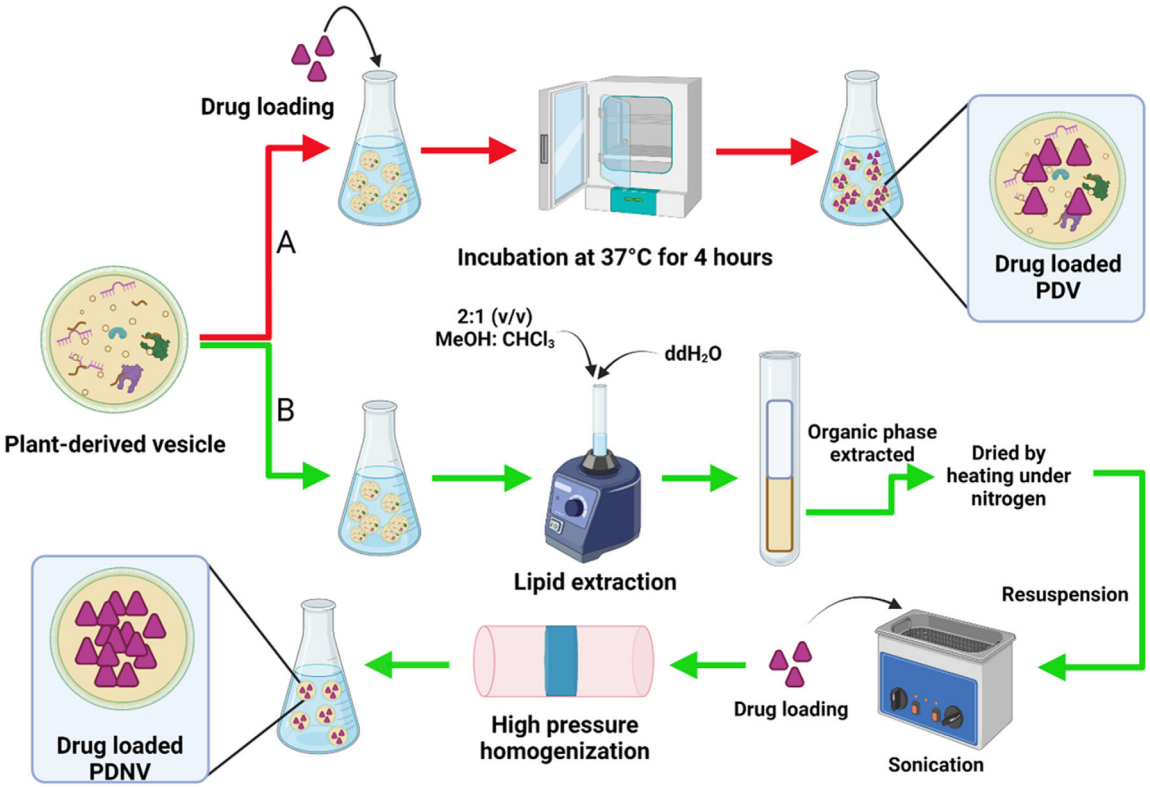

FIGURE 7 PDVs as drug delivery carriers. Drugs can either be loaded directly into unmodified PDVs (A, in red arrows) or into modified PDNVs (B, in green arrows). Figure modified from Dad et al. (2021). Created with BioRender.com

TABLE 4 PDVs as drug carriers

Source of PDVs	Target	Therapeutic loaded	Drug loading method	Refs.
Grapefruit	Mouse intestinal macrophages	Methotrexate (MTX)	Conjugation of MTX to PDVs	(Wang et al., 2014)
	LN229 and U87 glioma cells	Doxorubicin (Dox)	Co-inbuation of Dox-loaded herapin-based nanoparticles with PDVs	(Niu et al., 2021)
Ginger	KB cells	Survivin siRNA	Transfection into PDVs	(Li et al., 2018)
Bitter melon	OSCC cells	Fluorouracil (5-FU)	Co-sonication of 5-FU with PDVs	(Yang et al., 2021)
Cabbage/Red cabbage	Human keratinocyte & human dermal fibroblast	miR-184	Transfection into PDVs	(You et al., 2021)
	SW480 colon cancer cells	Dox	Co-incubation of Dox with PDVs	

cancer cells and successfully delivered active doses of Dox. In addition, the authors also loaded cabbage-derived PDVs with miR-184, demonstrating the capability of PDVs to deliver nucleic acids (You et al., 2021).

Apart from delivering therapeutic molecules, PDVs have also been observed to reduce the side effects of the encapsulated drug while improving its therapeutic efficacy. For example, Wang et al. successfully conjugated grapefruit-derived PDVs with methotrexate (MTX), an immunosuppressant and anti-inflammatory agent, and treated colitis in mice. The MTX-loaded grapefruit derived PDVs were observed to lower the expression of pro-inflammatory cytokines in macrophages to a greater extent compared to MTX alone. The synergistic effect of MTX and intrinsic anti-inflammatory activity of grapefruit-derived PDVs resulted in an enhanced therapeutic effect. Furthermore, orally administered grapefruit-derived PDVs remained stable in the gastrointestinal tract and were selectively uptaken by the macrophages in the lamina propria. (Wang et al., 2014). However, it is currently unknown why grapefruit-derived PDVs exhibit selective targeting towards macrophages. Overall, the use of PDVs as DDSs led to an improvement in therapeutic efficacy in terms of an enhanced anti-inflammatory effect with fewer MTX-induced side effects.

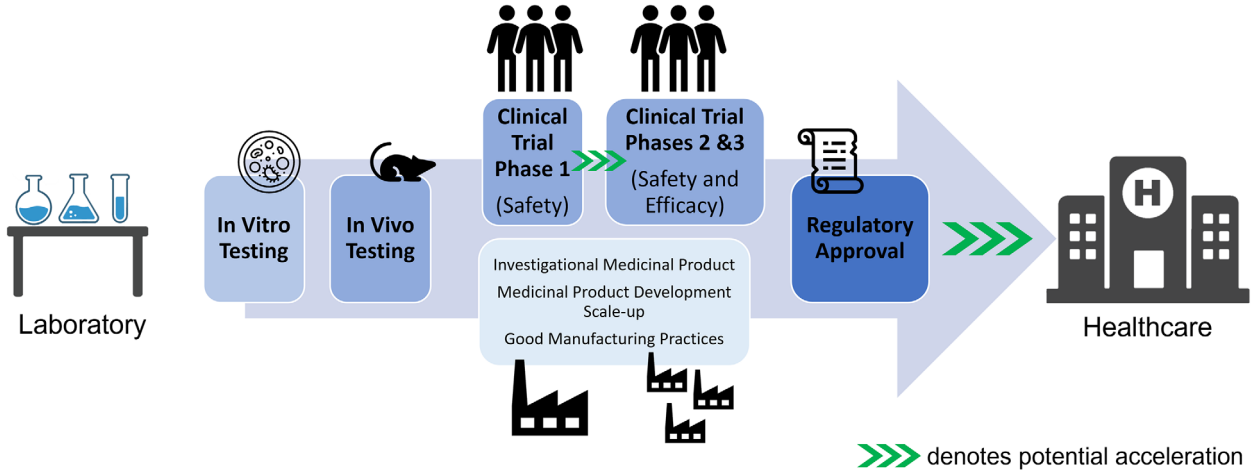

FIGURE 8 Translating PDV research from benchtop to bedside. PDVs isolated from edible plants should be safe for consumption and could move to later phases of clinical trials faster than conventional investigational new drug. The isolation and purification of PDVs (manufacturing process) needs to be scaled-up and meet current good manufacturing practices (GMP). The regulatory approval could also be accelerated if it is approved as a dietary supplement

These data suggest that the direct use of PDVs as drug carriers is an attractive and convenient option. However, the heterogenous sizes of PDVs isolated using current methods may affect the drug loading efficacy and the pharmacokinetic profile of PDVs in the body. Furthermore, it is still unknown how the bioactive components of PDVs from different plant sources interact with the loaded drugs. Hence, more systematic characterisations of PDVs will be required to assess whether the combination of loaded drugs and payload of the PDVs produce synergistic or counteractive effects.

5.2 | PDVs lipids re-engineered as plant-derived nanovectors (PDNVs)

Other than directly using PDVs for drug delivery, scientists have also utilised lipids extracted from PDVs to design nanovesicles known as PDNVs. Currently, researchers extract total lipids from the PDVs by the Bligh and Dyer method (Figure 8, path B). Briefly, the lipids of PDVs are extracted using 2:1 (v/v) methanol:chloroform. Chloroform and water are then added, and the mixture is centrifuged to separate out the organic phase. The organic phase is dried under nitrogen. The dried lipids are then resuspended in buffer and sonicated before extrusion to form PDNVs. The PDNVs produced are more uniform in size and appear spherical under the electron microscope (Wang et al., 2013). Similar to PDVs, PDNVs are also capable of delivering therapeutic molecules to different types of cells in a safe manner (Table 5). In addition, the lipid bilayer of the PDNVs can be further modified to achieve enhanced tumour targeting (Li et al., 2018; Zhuang et al., 2016).

In an interesting study, Zhuang et al. demonstrated that grapefruit-derived nanovectors (GDNVs) were able to deliver therapeutic microRNA (miRNA) to the brain tumour of mice via intranasal route with no detectable toxicity (Zhuang et al., 2016). DIR-dye labelled GDNVs administered intranasally to mice were detected in the brain after 12 hours. In contrast, DOTAP liposomes used for gene transfer could not be detected in the brain. This suggests that, unlike liposomes, GDNVs have the ability to cross biological barriers such as the blood brain barrier and can potentially be used for treatment of central nervous system diseases. GDNVs were also capable of protecting therapeutic RNA from degradation. Furthermore, polyethylenimine (PEI)/RNA complexed with GDNVs were less toxic and did not induce any immune response as compared to PEI/RNA only. The PEI-GDNVs were also coated with folic acid (FA-pGDNVs) to enhance the targeting of folate receptor-positive GL-26 brain tumour. Evaluation of the brain tumours also indicated that FA-pGDNVs were selectively taken up by the GL-26 tumour cells and miR17 present also inhibited the gene expression of MHC-1 in GL-26 cells. This led to a prolonged survival of mice in the mouse brain tumour model. These results demonstrate the capability of GDNVs to deliver therapeutics and be modified to enhance targeting (Zhuang et al., 2016). Safety-wise, Wang *et al.* also demonstrated that GDNVs were unable to cross the placenta barrier in pregnant mice, suggesting that GDNVs can potentially be used as drug carriers in pregnant women without risking pregnancy complications (Wang et al., 2013).

5.3 | Comparison between PDVs and PDNVs

It is evident that PDVs and PDNVs have immense potential as drug carriers, and both offer exclusive advantages. For instance, one advantage of PDNVs is that the size of PDNVs can be manipulated by using a high-pressure homogeniser to formulate

TABLE 5 PDNVs as drug carriers

Source of PDNVs	Target	Therapeutic(s) loaded	Drug loading method	Refs.
Grapefruit	4TI breast cancer cells A549 lung carcinoma cells GL26 brain tumour cells CT26 colon cancer cells SW620 colon cancer cells	Luciferase reporter gene siRNA Anti-Stat3 inhibitor (JSI-124) Paclitaxel Curcumin	Thin-film formation and hydration of PDV lipids with individual therapeutics, followed by co-sonication	(Wang et al., 2013)
	CT26 colon cancer cells 4T1 breast cancer cells	Doxorubicin (Dox) Curcumin	Thin-film hydration of PDV lipids with drug and co-extrusion	(Wang et al., 2015)
	GL26 brain tumour cells	miR-17	Formation of polyethylenimine (PEI)/RNA complex and co-sonication with PDV lipids	(Zhuang et al., 2016)
	Liver Kupffer cells	miR-18a	Co-UV irradiation and co-sonication of PDV lipids and RNA mixture	(Teng et al., 2016)
Ginger	Colon 26 colon cancer cells Caco2-BBE cells HT-29 colon cancer cells	Doxorubicin	Thin-film formation and hydration of PDV lipids with Dox, followed by co-sonication and co-extrusion	(Zhang et al., 2016)
	Caco2-BBE cells Colon 26 colon cancer cells RAW 264.7 macrophages	siRNA-CD98	Thin-film hydration of PDV lipids with siRNA-CD98, followed by co-sonication and co-extrusion	(Zhang et al., 2017)

uniform-sized nanoparticles. The size of a nanoparticle affects its clearance from the body and may enhance accumulation in the tumour (Dreaden et al., 2012; Qi et al., 2012). Given that the size of PDNVs can be tailored, it is possible to extend the retention of PDNVs in circulation and increase the chances of PDNVs targeting the diseased site, enhancing the treatment outcomes.

However, PDVs may have additional benefits derived from the synergistic effect between their content and the loaded drug molecules. As mentioned previously, it has been observed that the synergistic effect leads to better outcomes while minimising side effects of the drug molecule (Wang et al., 2014; Yang et al., 2021). In addition, PDVs also offer the advantage of minimising the resources and time required during formulation. Formulation of drug-loaded PDVs requires less equipment and steps compared to PDNVs. Furthermore, PDVs have demonstrated their intrinsic targeting abilities without any membrane manipulation. Currently, it is very challenging to isolate uniform-sized PDVs. However, further optimisation and refinements of current isolation methods may enable uniform-sized PDVs as drug carriers in the near future.

All these studies have shown that PDVs can be a novel way to deliver small molecule drugs to target sites. PDVs, when used directly or modified into nanovectors, are able to deliver therapeutics in a safe manner and can be produced economically on a large scale. Furthermore, intranasal and oral administration options provide practical, non-invasive methods to deliver therapeutic agents to a patient. Given the advantages that PDVs offer over mammalian-derived EVs and current drug carriers, PDVs may reasonably represent the next generation DDSs.

6 | IN VIVO BIODISTRIBUTION PROFILES OF PDVs AND PDNVs

With the potential for PDVs and PDNVs to be used as therapeutics and drug delivery carriers to treat disease, it will be essential to determine the distribution of these nanovesicles in vivo. The biodistribution profile of PDVs and PDNVs can be affected by the route of administration and the surface ligands.

6.1 | Route of administration

PDVs and PDNVs have been delivered in several in vivo models through various routes, such as peroral or intranasal administration, or intravenous and intraperitoneal injections. Depending on the route of administration, the biodistribution of PDVs and PDNVs may differ. For instance, intranasal administration is often used for delivery to the brain (Wang et al., 2013; Zhuang et al., 2016). Similar to other forms of nanoparticles, PDVs and PDNVs administered via intravenous or intraperitoneal route preferentially accumulate in the liver and spleen (Cao et al., 2019; Raimondo et al., 2015).

The stability in the gastrointestinal (GI) tract provides the option for oral and intragastric administration. Such stability can be tested by incubating the nanovesicles in pepsin solution (stomach-like conditions), followed by pancreatin and bile extract (intestine-like conditions) at 37°C (Mu et al., 2014; Wang et al., 2014; Zhang et al., 2016; Zhuang et al., 2015). Following oral or intragastric administration, PDVs and PDNVs preferentially accumulate in the GI organs and liver within several hours, which

TABLE 6 Membrane functionalisation of PDVs and PDNVs to improve targeting specificity in vivo

Source of PDVs/PDNVs	Type of membrane functionalisation	Targeting ligand	Target cells/organs	Refs.
PDVs				
Ginger PDVs	Hydrophobic interaction (3WJ aptamer)	Folic acid	KB cells	(Li et al., 2018)
Grapefruit PDVs	Chemical conjugation (EDC/NHS coupling)	cRGD peptide	LN229 glioma cells	(Niu et al., 2021)
PDNVs				
Grapefruit PDNVs	Coating with activated leukocyte derived plasma membrane	LFA-1	Inflammatory sites	(Wang et al., 2015)
	Hydrophobic interaction	Folic acid	GL-26 brain tumour cells	(Zhuang et al., 2016)
Ginger PDNVs	Hydrophobic interaction	Folic acid	Colon 26 colon cancer cells	(Zhang et al., 2016)

is particularly beneficial when treating GI-associated diseases such as colitis and gastric cancer (Berger et al., 2020; Ju et al., 2013; Wang et al., 2014; Zhang et al., 2017; Zhao et al., 2022; Zhuang et al., 2015; Zu et al., 2021). Moreover, oral administration is associated with other advantages such as convenience, improved patient compliance, non-invasiveness, and lower toxicity (Alqahtani et al., 2021).

Interestingly, there are several reports that compare the in vivo biodistribution of PDVs with different routes of administration. For example, ginseng-derived PDVs accumulated mainly in the liver and spleen when administered intravenously but accumulated in the stomach and gut when administered intragastrically (Cao et al., 2019). Furthermore, these PDVs were found in the liver, spleen, gut, and stomach when administered intraperitoneally (Cao et al., 2019).

Likewise, edible tea flower-derived PDVs accumulated mainly in the liver and lungs when administered intravenously (Chen et al., 2022). This biodistribution profile closely resembled that of the oral route of administration, albeit the distribution in the GI tract was not investigated. Intravenous administration resulted in an earlier and higher accumulation at the tumour site, which likely resulted in stronger pharmacological effects. However, higher levels of complement C3, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were also detected in mice administered intravenously with these PDVs as compared to the oral route, suggesting higher toxicity in the liver associated with intravenous administration (Chen et al., 2022).

With the option of oral delivery of PDVs and PDNVs, future work should continue to investigate and evaluate the efficacy and toxicity of PDVs and PDNVs when administered using different routes. The biodistribution profiles can also be compared at different timepoints to gain a more in depth understanding of the pharmacokinetic profiles of PDVs and PDNVs.

6.2 | Surface membrane engineering of PDVs and PDNVs

Besides changing the administration route, the biodistribution of PDVs and PDNVs can also be modulated by changing their surface membrane. Similar to mammalian derived EVs, the surface membrane of PDVs and PDNVs can also be engineered to include targeting ligands, which is mainly achieved through chemical ligations and hydrophobic interactions. However, applying bioengineering strategies, such as genetically fusing ligands with surface proteins, to modify the surfaces of PDVs and PDNVs remains difficult. The inclusion of targeting ligands on the surface of the PDVs greatly improves the bioavailability at the intended site of action and reduces any potential off-target effects. For instance, the chemical conjugation of cRGD peptide-conjugated herapin-based nanoparticles on grapefruit-derived PDVs resulted in an increased accumulation of PDVs in the brain tumour (Niu et al., 2021). Separately, cholesterol-conjugated three-way junction of the bacteriophage phi29 motor packing RNA (RNA-3WJ-Chol) aptamer has also been used to display targeting ligands on PDVs (Li et al., 2018). The incorporation of folic acid-conjugated aptamer on ginger-derived PDVs also improved the delivery of siRNA to KB cells-derived xenograft and reduced tumour growth.

The targeting specificity for PDNVs can also be engineered. For instance, the incorporation of folic acid onto PDNVs by mixing the lipids with folic acid prior to PDNVs production led to higher accumulations in tumours (brain and colon in separate studies) (Zhang et al., 2016; Zhuang et al., 2016). Furthermore, the membrane of PDNVs can also be coated with another membrane with intrinsic targeting properties, via the so-called membrane cloaking. This includes the coating of grapefruit-derived PDNVs with activated leukocyte-derived plasma membrane, which improved the accumulation of the PDNVs at inflammatory sites (Wang et al., 2015). Table 6 provides a summary of the examples of membrane functionalisation of PDVs and PDNVs to improve targeting specificity in vivo.

7 | TRANSLATING PDVs FROM BENCH TO BEDSIDE

Today, a variety of EV preparations are explored for therapeutic purposes. PDVs exhibit outstanding potential in translational research. They can be used to treat various disease conditions due to their intrinsic therapeutic nature or as DDSs. With promising In vitro and in vivo studies, it seems worth exploring the possible translation of PDVs from bench to bedside. The isolation and purification of PDVs would need to meet current good manufacturing practices (GMP) guidelines, and the use of PDVs would necessitate approval by regulatory authorities. Figure 8 shows the expected process for translating PDV research from benchtop to bedside.

7.1 | Good manufacturing practices for PDVs

For the use of PDVs in clinical settings, the isolation of PDVs would need to meet current good manufacturing practices (cGMP). Adopting the existing standards for the production of other types of EVs, the workflow can be broadly classified into the steps of upstream processing, downstream processing and characterisation (Chen et al., 2020; Exosomes Development).

The upstream processing of PDVs includes the source and type of plants used for the extraction (e.g., environmental conditions, time of harvest, age of the plants) as well as the medium harvest (such as the extraction of apoplast washing fluid from different parts of the plants) (Kameli et al., 2021). These factors may ultimately affect the composition of PDVs isolated, including their stability and efficacy. The downstream processing of PDVs involves isolation and purification and ensures the absence of unwanted debris (Chen et al., 2020). It often balances process yield, purity and scalability (Chen et al., 2020). Ultrafiltration, for example, offers the advantage of a shorter extraction time and a high recovery rate during purification, but the process may also destabilise the protein fraction of the extract. Whenever these proteins are pharmacologically active, this may affect the efficacy as well (Chen et al., 2020). Without knowing the exact composition of the therapeutic entities, the manufacturing process determines the product's identity. Hence, from a regulatory perspective, every change in important process parameters will be considered as a change in the drug product. This would require careful monitoring and, in some cases, even the implementation of additional clinical testing (Whitford & Guterstam, 2019). A detailed characterisation of the composition of the PDVs, as well as of the main interim products (e.g., after pre-extraction steps) with suitable characterisation methods, often gives the manufacturer more flexibility regarding process changes because they may serve as a confirmation of the product's identity.

The characterisation of PDVs can take reference from that of EVs, where the physicochemical and biological properties of PDVs are measured (Chen et al., 2020). Physicochemical properties of PDVs include size, particle concentration, and morphology (e.g., through transmission electron microscopy) (Chen et al., 2020). This is often supported by chemical analysis of PDVs' protein markers and quantification of the total protein and nucleic acid content (Chen et al., 2020). Whenever one or more of the therapeutic entities are known, these would often serve as 'active markers.' Their role will be explained in more detail in the later sections. A biological characterisation for the purpose of quality control (which would be carried out for every batch of the drug product) may include toxicological and pharmacological assays.

These characterisation methods and the related critical quality attributes can be used to detect batch variations and inform the manufacturer about the impact of process changes (Exosomes Development). Moreover, pharmacopeial standards would also apply to ensure the quality and safety of PDVs in the market. Critical quality attributes would also need to be developed to ensure storage conditions would not cause any significant loss in quality (Karamanidou & Tsouknidas, 2022).

7.2 | Pharmaceutical regulation of PDVs

When translating PDVs from bench to bedside, the regulatory pathway ensures that the efficacy, safety and quality requirements are met by the (investigational) drug products during and after the first clinical trials were performed. Although a variety of new technologies are applied to produce new drug products, the regulatory pathways often remain very similar. These include distinct frameworks for food, cosmetics, medical devices, dietary supplements as well as medicinal or drug products (Marques et al., 2019; Wacker, 2014).

With an emphasis on pharmaceutical applications, in the European Union (EU), PDVs can be classified as herbal medicinal products which are defined as '*any medicinal product, exclusively containing as active ingredients one or more herbal substances or one or more herbal preparations, or one or more such herbal substances in combination with one or more such herbal preparations*'. Although the United States of America (USA) applies a very similar definition, the respective guidelines use different terminology and refer to botanical products (Botanical Drug Development: Guidance for Industry, 2016).

Generally, herbal medicines fulfil the same requirements applied to any other medicinal product but, additionally, there are specific rules in production and quality control due to their complex character (Steinhoff, 2019). PDVs are multimolecular assemblies and represent a physicochemical rather than a chemical entity. They are obtained in multi-step extraction processes and

come with similar challenges as observed for other herbal preparations (Kameli et al., 2021). As compared to purified drugs, the exact composition, for instance, varies between batches, and a more detailed characterisation would be extremely challenging. Also, in many cases, using analytical methods with higher resolution does not represent an economically viable option.

Although similar flaws apply to many biotechnological drugs that often exhibit small differences in their chemical structure (e.g., glycosylation pattern), setting target specifications for a purified therapeutic entity would at least require a well-defined pharmacophore. The pharmacological action of PDVs is often achieved through an unknown combination of active and inactive ingredients. The composition is mainly controlled by monitoring the extraction process. These overwhelming similarities to other herbal medicines are likely to determine the regulatory pathway.

Alternatively, regulatory authorities could follow the current framework on mammalian-derived EVs (Muthu et al., 2021). Similarities in morphology and function would support this idea (Cong et al., 2022). However, when applying this approach, In vitro and in vivo data on the structure-effect relationships of PDVs could become the bottleneck limiting their market entry. Currently, as indicated by the application of dietary supplement regulations, the food-like composition seems to offer unique advantages with regard to the accessible regulatory pathways. Accordingly, the current regulations on herbal medicinal products may become the most suitable framework for all PDV-based therapeutics.

In the EU, a shared scientific guideline was issued by the Committee on Herbal Medicinal Products (HMPC), the Committee for Medicinal Products for Human Use (CHMP), and the Committee for Veterinary Medicinal Products (CVMP) and summarises the most important specifications to be applied to herbal medicines (Guideline on specifications, 2022). They include a wide variety of quality considerations such as physicochemical characterisation steps, analytical fingerprinting, and the selection of suitable markers to determine the quality levels from batch to batch (Guideline on specifications, 2022). These include active markers and analytical markers. While active markers represent constituents with known therapeutic activity, analytical markers are substances that are consistently found in the extract and may indicate quality changes in the extraction or purification process (Reflection Paper on Markers, 2008). The abovementioned membrane proteins found in many PDVs could, for example, qualify as analytical markers as long as they do not play a role in the pharmacological activity.

When exploring the landscape of clinical research involving PDVs, a wider circle of regulatory pathways must be taken into consideration. Many ongoing investigations involve dietary supplements. They fall under the Dietary Supplement Health and Education Act of 1994 and are intended for ingestion (Dietary Supplement Health and Education, 1994). Among other requirements, they must contain a dietary ingredient intended to supplement a diet and do not require market approval by the FDA (Dietary Supplement Health and Education, 1994). Generally, the peroral and the topical routes of administration come with a lowered exposure compared to, for instance, injectable drug products. Currently, phase-I clinical trials on PDVs include 2 dietary supplements (NCT01668849, NCT01294072) and one perorally administered investigational drug product (NCT04879810). A clinical trial exploring a medical application of PDVs in polycystic ovary syndrome was withdrawn (NCT03493984). More details on each trial are provided in Table 7. Besides as therapeutics, PDVs could also have the potential to be used as cosmetics, for which the regulatory pathways are expected to be more straightforward, but not simple (Cho et al., 2022; Lee et al., 2020). Public information on PDVs in cosmetics is very limited but there is a considerable number of products including the FDA-registered ExoDrop product line (South Korea). Of note, the European regulations would make it much more difficult to develop such PDV-based products. In the current regulatory framework, all materials with more than 50% of the particles having a diameter below 100 nm, fall under the European Commission's definition of 'nanomaterials' (Marques et al., 2019; Wacker, 2014; Wacker et al., 2016). This definition is an important part of the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) regulation, which controls market access and would make it more difficult to import or distribute the extracted materials in the EU. Therefore, it is not surprising that most products are registered in the USA or Asia. Just recently, in 2022, the European Commission decided to revise the 'nanomaterial' definition and related regulatory frameworks on chemicals and cosmetics. Still, this revision is unlikely to improve the situation for the import and manufacture of PDVs. However, with a rising interest in this technology, the number of products and clinical trials is expected to increase.

8 | CONCLUSION AND FUTURE PERSPECTIVES

When developing novel therapeutic treatments, it is quintessential to achieve optimal therapeutic efficacy, avoid off-target effects and toxicity, and minimise large-scale production costs. In light of these requirements, it is evident that PDVs present themselves as a viable option for their therapeutic properties. Owing to their natural source, it is possible to isolate PDVs in large volumes in a sustainable manner. Furthermore, preclinical studies have also demonstrated that PDVs are safe and possess intrinsic therapeutic activities that are able to improve pathological conditions in various disease models. Moreover, PDVs also represent promising drug carriers, having shown to deliver different drug molecules to target sites efficiently and effectively. These findings suggest that PDVs can be used to treat human diseases and influence human health positively.

The expanding body of evidence on PDVs' intrinsic therapeutic activities, attributed to their bioactive contents and their ability to ferry active molecules to various diseased sites, has made PDVs research particularly appealing. These complex vesicles offer a range of interesting possible future applications. However, research on PDVs is still in its infancy and the potential of PDVs has

TABLE 7 Summary of PDVs used in clinical trials

Indication	Use	NCT	Month, Year	Clinical trial phase	Patients	Regulatory status/Source of PDVs	Status	Estimated completion date
Colon cancer	Using PDVs as a drug delivered agent to delivered curcumin	NCT01294072	January 2011	Phase I	35	Dietary supplement/ Plant (not specified)	Active, recruiting	December 2022
Radiation-and chemotherapy-induced oral mucositis	Using grape PDVs as therapeutics	NCT01668849	August 2012	Phase I	60	Dietary supplement/ Grape	Active, not recruiting	August 2022
Inflammatory bowel disease	Combination therapy using ginger PDVs and curcumin as therapeutics	NCT04879810	March 2019	N/A	90	IND / Ginger	Recruiting	December 2022
Insulin resistance and chronic inflammation in polycystic ovary syndrome	Using PDVs found in ginger or aloe plant as therapeutics	NCT03493984	May 2018	–	–	IND / Ginger Aloe	Withdrawn as investigator left prior to study approval	-

Abbreviation: IND, investigational drug product.

yet to be fully recognised. To promote more translational applications of PDVs in the future, efforts can be made in the following directions:

1. Presently, PDVs are isolated from only a handful of plants. Given the variety and abundance of medicinal plants available, more PDVs derived from medicinal plants should be considered for research. Medicinal plants may be valuable sources for PDVs that carry specific phytochemicals, proteins and nucleic acids that may provide more treatment options than current synthetic and protein drugs. In addition, current research also does not explicitly demonstrate if PDVs isolated from different parts of the plant differ in terms of their content, intrinsic therapeutic activities, or drug delivery capabilities.
2. With the advancements in analytical technologies, multi-omics approach can deepen our understanding of the biological and pharmacological effect of PDVs contents in the human body. Furthermore, a systematic analysis of PDVs' proteins, RNAs and metabolites can be supported by bioinformatics prediction approaches. This may help researchers identify potential targets in different diseases through *in silico* analysis and develop novel treatments for previously untreatable diseases. Proteomic analyses can also help identify specific markers present in PDVs that can aid in the classification as well as isolation of PDVs through immunoaffinity approaches.
3. The potential interaction between PDVs and the drug loaded should be investigated. Some plants may contain phytochemicals which, when consumed with certain drugs, result in adverse food-drug interactions. For example, flavonoids in grapefruit juice can inhibit cytochrome P450 3A4 (CYP34A), preventing the metabolism of drugs which could lead to toxicity (Kiani & Imam, 2007). In fact, studies have found presence of naringin in grapefruit PDVs (Wang et al., 2014). The PDV-drug interactions will be an area worth investigating.

While there are currently challenges associated with the isolation and characterisation of PDVs, existing knowledge on PDVs highlights their promising future for biomedical applications. New advances in PDV research, if conducted systematically and thoroughly, will be able to revolutionise the current field of PDVs, as well as the attributed roles of vegetables and fruits far beyond healthy diets. Future research is expected to make a substantial contribution to the development of naturally derived remedies. As such, we should invest more effort into PDVs in order to expand our understanding in this field.

Wei Heng Chng: Data curation; Formal analysis; Investigation; Methodology; Visualization; Writing – original draft; Writing – review & editing. Jeremy Liang: Data curation; Methodology; Validation; Writing – review & editing. Hui Qing Yeo: Data curation; Writing – original draft. Choon Keong Keong Lee: Data curation; Investigation; Writing – review & editing. Mona Belaid: Data curation; Formal analysis; Methodology; Writing – original draft. Matteo Tollemeto: Writing – review & editing. Bertrand Czarny: Methodology; Writing – review & editing. Giorgia Pastorin: Conceptualization; Formal analysis; Project administration; Resources; Supervision; Writing – original draft; Writing – review & editing.

ACKNOWLEDGEMENTS

This work was supported by the National University of Singapore (NanoNash Program A-0004336-00-00 & A-0008504-00-00), and Nanyang Technological University, Singapore (grant number 001487-00001). G.P. would also like to thank the Industry Alignment Fund—Pre-Positioning (IAF-PP) grant (A20G1a0046 and R-148-000-307-305/A-0004345-00-00). The authors would also like to thank Dr Anja Wacker for her advice on pharmaceutical regulations, Dr Chammika Udalagama for the plots on Figure 4A,E, and Dr Diego Pitta De Araujo for the preparation of Figures 1, 2, 3, 5 and 6.

CONFLICT OF INTEREST

The authors of this review declare no conflicts of interest.

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How to cite this article: Lian, M. Q., Chng, W. H., Liang, J., Yeo, H. Q., Lee, C. K., Belaid, M., Tollemeto, M., Wacker, M. G., Czarny, B., & Pastorin, G. (2022). Plant-derived extracellular vesicles: Recent advancements and current challenges on their use for biomedical applications. *Journal of Extracellular Vesicles*, *11,* e12283. https://doi.org/10.1002/jev2.12283