

# From Plant Based Therapy to Plant-Derived Vesicle-Like Nanoparticles for Cancer Treatment: Past, Present and Future

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**Abstract:** Cancer stands as a formidable malady profoundly impacting human health. Throughout history, plant-based therapies have remained pivotal in the arsenal against cancer, evolving alongside the epochs. Presently, challenges such as the arduous extraction of active components and potential safety concerns impede the progression of plant-based anticancer therapies. The isolation of plant-derived vesicle-like nanoparticles (PDVLNs), a kind of lipid bilayer capsules isolated from plants, has brought plant-based anticancer therapy into a novel realm and has led to decades of research on PDVLNs. Accumulating evidence indicates that PDVLNs can deliver plant-derived active substances to human cells and regulate cellular functions. Regulating immunity, inducing cell cycle arrest, and promoting apoptosis in cancer cells are the most commonly reported mechanisms of PDVLNs in tumor suppression. Low immunogenicity and lack of tumorigenicity make PDVLNs a good platform for drug delivery. The molecules within the PDVLNs are all from source plants, so the selection of source plants is crucial. In recent years, there has been a clear trend that the source plants have changed from vegetables or fruits to medicinal plants. This review highlights the mechanisms of medicinal plant-based cancer therapies to identify candidate source plants. More importantly, the current research on PDVLN-based cancer therapy and the applications of PDVLNs for drug delivery are systematically discussed.

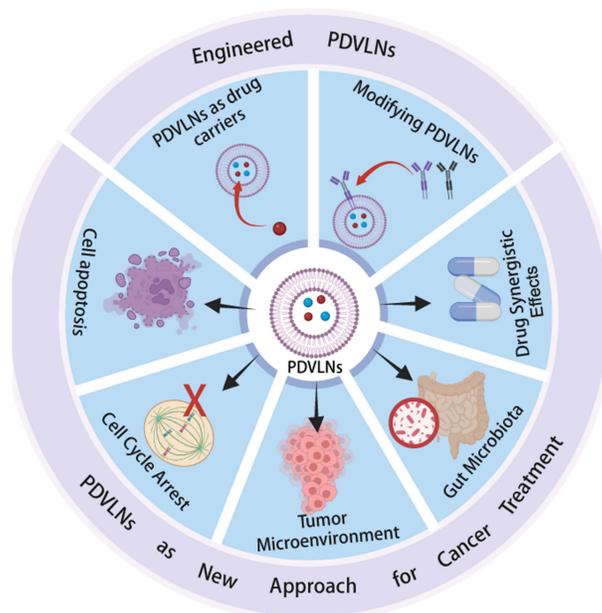
**Keywords:** plant-derived vesicle-like nanoparticles, plant-based anticancer therapy, drug delivery system, anticancer

## Introduction

Extracellular vesicles (EVs) are heterogeneous nano-sized lipid bilayer capsules that are naturally secreted from cells (Figure 1A). Classically, EVs can be divided into three major subgroups according to their biogenesis, biological function, size, and content namely, microvesicles (MVs), exosomes, and apoptotic bodies. In the last decades, mammal-derived EVs have been the focus of EV research, and detailed research has been conducted on their biogenesis and function. Through transporting bioactive molecules among cells, EVs adjust metabolic balance and play an important role in intercellular communications.<sup>1</sup> In addition to widely involved in the regulation of physiological processes, EVs have been found to participate in the development and progression of many diseases.<sup>2</sup> In recent years, EVs have emerged as a new type of therapeutic agent and have been widely applied in the treatment of numerous diseases, not just for understanding and intervening in their own biological functions. For example, mesenchymal stem cells (MSCs) derived exosomes have shown great potential in regenerative medicine because they can repair myocardial infarction and wound healing in mouse models.<sup>3,4</sup> EVs are also widely used in cancer therapy.

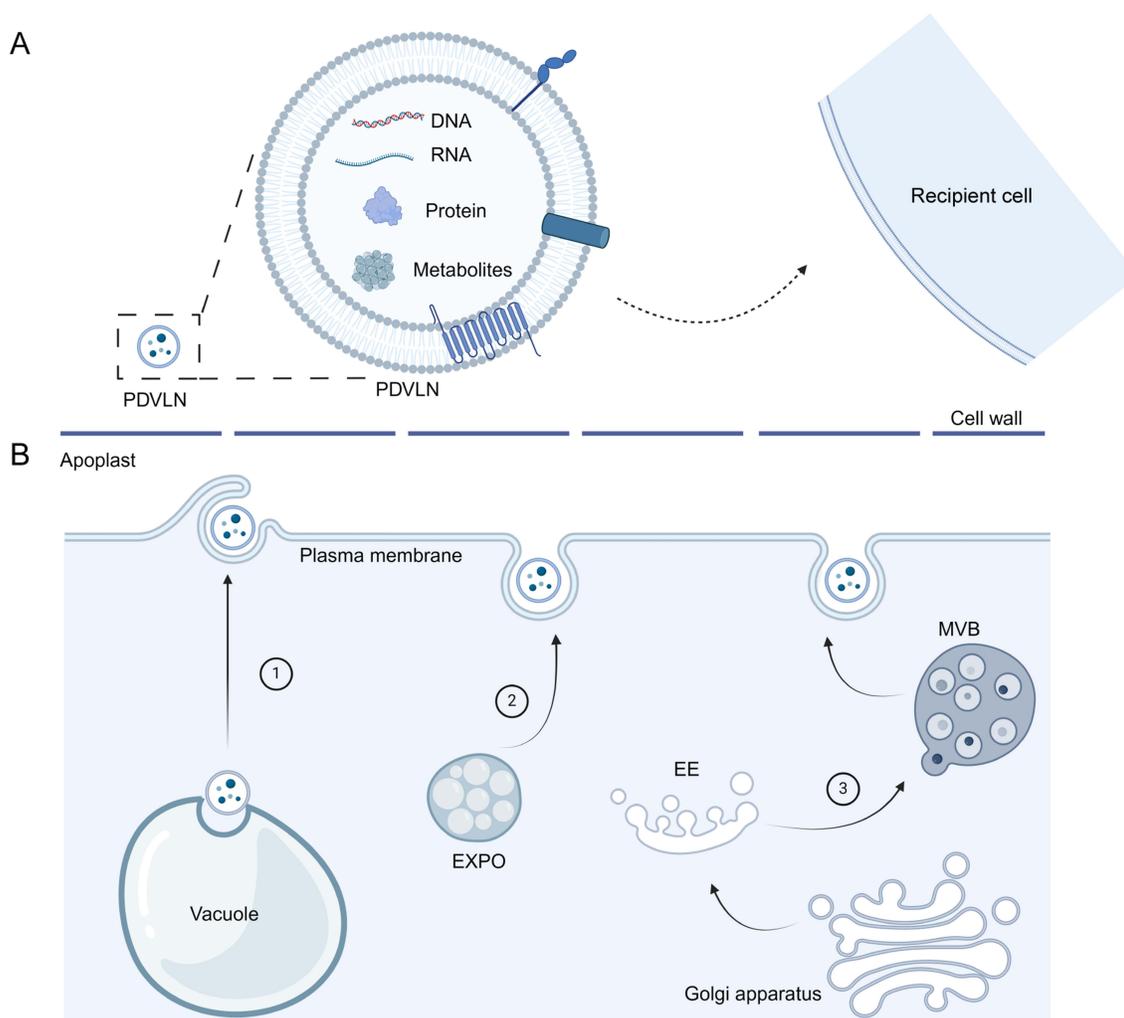
Cancer is a complex disease and the leading cause of death worldwide every year. Although chemotherapy achieves good clinical cancer control, it cannot perfectly target tumor cells and may damage normal cells. Therefore, various new treatments for cancer are currently under development, and EV-based therapies have attracted considerable attention. In addition to the

## Graphical Abstract



early direct application of EVs in cancer treatment, researchers have preferred to use EVs as a treatment tool.<sup>5</sup> One of the most direct applications of EVs is their use as drug delivery platforms. After loading drugs into EVs, EVs can carry drugs through biological barriers, such as the blood-brain barrier, and reach tumor tissues.<sup>6</sup> Interestingly, EVs exhibit some ability to target and accumulate in tumor tissues, which is further enhanced by adding a targeting ligand on the surface of EVs.<sup>7</sup> For example, targeting EVs could be produced by genetically engineering their parental cells. Antibodies or targeting peptide can be linked to membrane proteins on the surface of the EVs (such as Lamp 2b).<sup>8,9</sup> The intrinsic targeting ability of EVs is due to the inherent properties of the EVs, such as different protein components and lipid ratios on the EVS surface and different parent cells. For example, EVs expressing the  $\beta 5$  subunit are predominantly directed towards Kupffer cells in the liver, while those expressing the  $\beta 4$  subunit are preferentially taken up by CD31-positive endothelial cells in the brain.<sup>10</sup> And EVs could acquire better targeting ability, longer retention time, and better treatment efficacy after engineering, all of which make them a promising agent for tumor treatment. However, problems such as potential tumorigenicity and immunogenicity have encouraged researchers to develop new carriers,<sup>11,12</sup> ultimately turning their attention to plant-derived vesicle-like nanoparticles (PDVLNs).

In 2009, Regente et al first isolated PDVLNs from sunflowers, which opened a new era for extracellular vesicles and led to decades of research on PDVLNs.<sup>13</sup> Plant-derived exosome-like nanoparticles, plant-derived EVs, and plant-derived exosome-like nanovesicles are commonly used terms describing PDVLNs in some studies, and in this review, we use PDVLNs to refer to them. PDVLNs are morphologically and biogenically similar to mammal-derived extracellular vesicles.<sup>14</sup> PDVLNs contain various RNA, DNA, proteins, and metabolites, which perform different functions by transporting them to the recipient cell along with PDVLNs (Figure 1A). Different from mammalian EVs that are mainly secreted through membrane budding and the multivesicular body pathway, current research indicates that there are at least three ways in which plants secrete PDVLNs (Figure 1B). To date, numerous studies have demonstrated that PDVLNs can serve as drug carriers and also exert therapeutic effects on their own. *B. javanica*-derived PDVLNs are capable of treating breast cancer, which is ascribed to the microRNAs (miRNAs) within them.<sup>15</sup> The contents of PDVLNs are basically all derived from the plants. For example, *peu*-MIR2916-p3 derived from garlic PDVLNs have been found to promote the growth of *Bacteroides thetaiotaomicron* in the intestines, thereby alleviating colitis.<sup>16</sup> Therefore, the choice of the source plant is crucial.



**Figure 1** (A) PDVLNs delivery active components such as nucleic acids, metabolites and proteins to recipient cells. (B) Biogenesis pathways of PDVLNs, namely multivesicular bodies (MVB) pathway, exocyst positive organelles (EXPO) and vacuole pathways.

There is an apparent trend that the choice of the source plant changes from fruits and vegetables to medicinal plants. The history of using plants as medicines can be traced back to at least 60,000 years during the Middle Paleolithic age.<sup>17</sup> Various civilizations, such as Ancient China, Ancient India, and Ancient Greece, as well as traditional medical systems have utilized plants to treat various diseases, including cancer.<sup>17,18</sup> Traditional Chinese Medicine (TCM) is an important system for plant-based anticancer therapy, with more than 3500 years of medical practice. Various Chinese herbal medicines are important components of TCM.<sup>19</sup> Currently, TCM is widely used in the treatment of various tumors, such as lung cancer,<sup>19,20</sup> triple-negative breast cancer,<sup>21</sup> and esophageal cancer,<sup>22</sup> and can be used in combination with other anticancer therapies, including chemotherapy, radiotherapy, tyrosine kinase inhibitors (TKIs), and immunotherapy, or as adjunctive or maintenance therapy.<sup>20,23</sup> These TCM have also become important candidates for the sources of PDVLNs.

In this review, we summarize the mechanisms of plant-based anticancer therapy to help identify medicinal plants with the potential to serve as new sources of PDVLNs. Next, we comprehensively describe current research on PDVLNs in cancer treatment and summarize their mechanisms. In addition, we discuss the applications of PDVLNs as carriers and their modification methods.

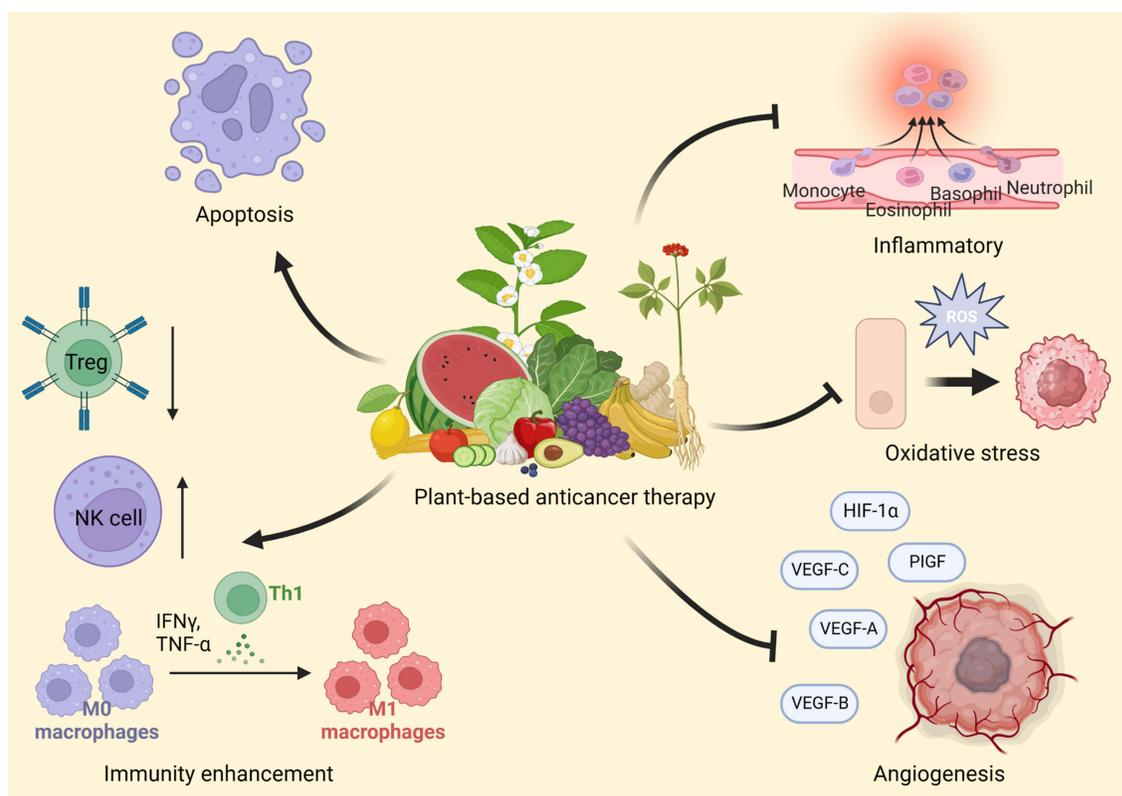
## The Mechanisms of Plant-Based Anticancer Therapies

### Antioxidant Activity and Anti-Inflammatory Activity

Many plants contain abundant antioxidant compounds such as polyphenols, vitamin C, and vitamin E. Polyphenol compounds are characterized by multiple phenolic (aromatic) rings and are abundant in plants. They can be further classified into subclasses such as flavonoids, tannins, lignans, phenolic acids, and stilbenes.<sup>24</sup> Each of them has a unique chemical structure, but almost all have antioxidant and anticancer properties. They can neutralize free radicals, reduce oxidant stress, and prevent DNA damage.<sup>25,26</sup> Chronic inflammation is an important factor in cancer development. They can recruit inflammatory cells, induce cell proliferation, and produce reactive oxygen species (ROS), which can result in DNA damage and abnormal DNA repair, eventually leading to permanent genomic alterations and tumorigenesis.<sup>27</sup> Compounds with antioxidant activity can also fight inflammation by reducing oxidative damage caused by ROS in the inflammatory environment. [Figure 2](#) exhibits the mechanisms of action of some plants for cancer treatment.

### Regulation of Cell Cycle

Certain compounds in plants can regulate the cell cycle and interfere with DNA replication and repair in cancer cells, thus preventing the proliferation and division of cancer cells and halting their growth. One of the most famous examples is taxanes, including paclitaxel (taxol) and docetaxel, which are extracted and purified from the bark of the Pacific yew tree (*Taxus brevifolia*) and have been applied in the treatment of various cancers, such as breast,<sup>28</sup> prostate<sup>29</sup> and many other cancers. Microtubules (MTs), which consist of  $\alpha$ -tubulin and  $\beta$ -tubulin heterodimers, are the main components of the cytoskeleton in eukaryotic cells and play crucial roles in cell division, cell motility, cell shape maintenance and intracellular transport. Taxanes can selectively bind to  $\beta$ -tubulin and promote their polymerization and assembly, thus preventing their disassembly, stabilizing the microtubules, and interfering with the formation of spindles, thereby inhibiting cell division and arresting the cell cycle at the G2/M phase.<sup>30</sup> Other compounds, such as vinblastine and vincristine, can also exert anticancer effects by regulating the cell cycle.<sup>31,32</sup> In addition, different plant-derived compounds can precisely target different stages of the cell cycle, thereby



**Figure 2** Various mechanisms of plant-based anticancer therapy.

achieving precise regulation of the cell cycle. For example, algal sulfated polysaccharide extracted from *Laurencia papillosa* could target G1 phase, and xanthohumol, the major component of *Humulus lupulus* could target S phase.<sup>33</sup>

## Induction of Apoptosis

Apoptosis is a type of programmed cell death (PCD) that can be triggered by multiple internal (release of cytochrome c from mitochondria) and external (binding of death ligands to their corresponding death receptors) signals. A series of signaling events occur once apoptosis is initiated, and all these signaling pathways finally converge on the activation of specific proteases called caspases (caspase-3, 8, and 9, etc.), which cleave essential cellular proteins and initiate the dismantling of the cell.<sup>34</sup> Induction of apoptosis in cancer cells (eg, prostate cancer, breast cancer and colorectal cancer etc). has always been an interesting topic in the field of cancer treatment. Several plant-derived compounds have been shown to target vital molecules in the apoptotic pathways, such as Bcl-2 and caspases. Resveratrol can be extracted from more than 70 plants including grapes, peanuts, and pistachios. It has antioxidant and anti-inflammatory properties, which make it a promising candidate for cancer treatment. In particular, it can suppress the phosphorylation of Src-STAT3 and induce apoptosis in cancer cells to directly kill tumor cells.<sup>35</sup> In addition to small-molecule anticancer agents, proteinaceous anticancer agents derived from plants, such as lectins, can also induce apoptosis to exert anticancer effects. Lectins are a class of glycoproteins which exist ubiquitously in different plant species. They can interact with sugar-binding receptors on the plasma membrane, are internalized through endocytosis, and are finally transported to the mitochondria. ROS are then generated, and cytochrome c is released into the cytoplasm to activate downstream apoptotic pathways.<sup>36</sup> Moreover, some plant-derived compounds, such as terpenoids and flavonoids, can induce other types of PCD, such as autophagy-dependent cell death, ferroptosis and necroptosis.<sup>37,38</sup> For example, betulin, a triterpenoid extracted from *Isatidis Radix*, could induce autophagy-dependent cell death in colorectal cancer cells by inhibiting PI3K/Akt/mTOR signaling pathways.<sup>39</sup> Lou et al found Ginkgetin derived from Ginkgo biloba leaves could increase labile iron pool and lipid peroxidation, thus inducing ferroptosis in EGFR wild-type non-small-cell lung cancer.<sup>40</sup>

## Inhibition of Angiogenesis

Pathological angiogenesis is a hallmark of cancer, and pro-angiogenic members of the vascular endothelial growth factor (VEGF) family (VEGF-A, VEGF-B, VEGF-C, etc). and their corresponding receptors (VEGFR-1, VEGFR-2, and VEGFR-3) play a vital role in this process. Catechins in green tea have been proven to possess antioxidant and anti-inflammatory activities, as well as their ability to inhibit angiogenesis. Epigallocatechin-3-gallate (EGCG) is the main catechin found in green tea. Studies have found that EGCG can reduce the expression of HIF-1  $\alpha$ , which strongly activates the expression of VEGF, and EGCG could also inhibit the binding of VEGF to its corresponding receptor, thus preventing the activation of the VEGF/VEGFR axis.<sup>41</sup> In addition, plant-based anticancer therapies can also improve the anti-angiogenesis efficacy of other treatments. For example, Ao et al found that the extract from coix seed in combination with thalidomide significantly inhibited angiogenesis in liver cancer by inhibiting VEGF and B-FGF. Lin et al also reported that HIF-1 $\alpha$  and VEGF, which are vital for angiogenesis, could be remarkably reduced by astragalus polysaccharide combined with cisplatin and adriamycin in live cancers.<sup>42</sup>

## Enhancement of Immunity

Some plant-derived agents can not only kill tumor cells directly but can also play an anticancer role by enhancing the body's immunity. A good example is Traditional Chinese Medicine.<sup>43-45</sup> TCM can enhance antitumor immunity by influencing both the innate and adaptive immune systems.<sup>45</sup> Macrophages are important components of innate immunity and can primarily be classified into M1 and M2 subtypes. M1 macrophages mainly exert anti-tumor effects, and they can secrete a large amount of pro-inflammatory cytokines, such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and interleukin-6 (IL-6), which promote inflammatory responses. Additionally, they release reactive oxygen species (ROS) and reactive nitrogen species (RNS), which can effectively kill tumor cells. Many TCMs have been reported to promote the transformation of M2 type macrophages to M1 type to enhance antitumor immunity, such as Astragaloside IV extracted from astragalus<sup>46</sup> and soyasapogenols extracted from soybean.<sup>47</sup> Kaneko et al also found that Xiao-chai-hu-tang augmented the activity of natural killer (NK) cells in a dose-dependent manner in mice.<sup>48</sup> Lupeol promotes the proliferation of NK cells and increases their killing effect on gastric cancer cells.<sup>49</sup> As for adaptive immunity, compound

Chinese formula 1 (CFF-1) is a classic TCM-derived formula used in prostate cancer and has been proven to be therapeutic. Previous studies have found that CFF-1 induces apoptosis in prostate cancer by inhibiting the EGFR/PI3K/AKT signaling pathway. Interestingly, Zhang et al found that CFF-1 could also reduce PD-L1 expression in prostate cancer cells, increase the population of CD4<sup>+</sup> T lymphocyte subsets, and simultaneously downregulate the population of regulatory T cells (Treg cells) at the same time, thereby dismantling the immunosuppressive environment in prostate cancer and enhancing anticancer immunity.<sup>50</sup> Some TCMs can also exert antitumor effects by promoting T cell differentiation into Th1 cells, which can release several cytokines and chemokines to recruit CD8<sup>+</sup> T cells and enhance their antitumor abilities.<sup>45</sup>

## Challenges of Using Plant-Based Anticancer Therapy

Plant-based anticancer therapy has many advantages compared to other anticancer therapies; for example, they are mainly administered orally or by injection; therefore, they are more convenient and less painful for patients and have proven effective in many cancers with relatively few side effects.<sup>51</sup> However, the challenges and drawbacks of plant-based anticancer therapies need to be addressed. It must be recognized that many compounds with anticancer activity in plants cannot be synthesized artificially, so the consumption is mainly dependent on natural resources.<sup>51</sup> Take paclitaxel for example, paclitaxel is mainly found in the bark of the Pacific yew tree with low concentration of only 0.01%-0.05%, and the amount of paclitaxel extracted from 10 tons of bark is sufficient to treat 500 patients.<sup>30</sup> Besides, the Pacific yew tree is a slow-growing tree, and stripping the bark usually leads to the death of the tree; therefore, over-harvesting once brought it to the brink of extinction.<sup>52</sup> Although scientists have suggested the production of paclitaxel using plant cell cultures or genetically modified plants to reduce the consumption of natural sources, the purification efficiency is as low as 0.004%<sup>52</sup> and many bioactive compounds are lost during the purification process. In addition, some plant-derived compounds can exhibit drug synergism and may be less effective after purification and administration as single molecules.<sup>51</sup> The specific mechanisms underlying the anticancer effects of some plants remain unknown, so it is difficult to extract and purify their bioactive compounds. Moreover, there still exists some concerns about the bioavailability of plant-derived compounds. The absorption of many plant-derived compounds has not been well investigated; therefore, some of them may not be soluble properly in the systemic fluid and cannot be well absorbed and stably transferred to the tumor area to achieve a therapeutic concentration.<sup>51</sup> Many methodologies have been adopted to improve bioavailability and absorption, such as encapsulation of drugs in liposomes or micelles, which can be modified to increase the targeting ability of drug delivery. However, synthetic materials also raise concerns regarding their safety; therefore, PDVLNs have unique advantages in this regard. They are less toxic or immunogenic because of their natural sources. In addition, many bioactive compounds can be retained in PDVLNs during the isolation process to exert synergistic anticancer effects, thereby significantly reducing losses during the purification process. Therefore, PDVLNs are playing increasingly crucial roles in plant based anticancer therapies.<sup>53</sup>

## Characterization of the PDVLNs

As early as the 1960s, scientists first observed the release of exosome-like vesicles by the fusion of multivesicular bodies and plasma membranes from cultured carrot cells.<sup>54</sup> In 2009, Regente et al successfully isolated exosome-like vesicles from the apoplast washing fluids of sunflower seeds,<sup>13</sup> which led to decades of research on PDVLNs. To date, PDVLNs have been isolated from various plant species and shown to exhibit therapeutic effects in many human diseases.<sup>55,56</sup> Similar to mammalian EVs, PDVLNs are a group of heterogeneous nano-sized lipid bilayer capsules that carry lipids, proteins, nucleic acids, and some metabolites.<sup>18</sup>

## Size and Potential

Two commonly used methods for measuring the size of PDVLNs are dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA). DLS serves as a non-single-particle characterization method, yet it suffers from the drawback that larger particulates can overshadow the signals emanating from smaller particles.<sup>57</sup> Therefore, when dealing with samples that have a broad particle size distribution, the particle sizes measured by DLS may deviate from the actual values. NTA records the Brownian motion trajectories of individual particles, and quantitatively calculates particle size and concentration. However, it requires sufficient trace length and an optimal concentration range.<sup>58</sup> The size distribution

of PDVLNs varies among source plants, and the reported size ranges from 50 nm to 1000 nm.<sup>59</sup> Notably, PDVLNs derived from the same plant source may exhibit different size distribution peaks. For example, orange PDVLNs exhibit a primary peak and a secondary smaller peak in their size distribution.<sup>60</sup> The zeta potential of PDVLNs is mostly negative, and the zeta potential varies significantly among different PDVLNs. The reported zeta potential distribution ranges from  $-1.5$  to  $-49$ .<sup>61</sup>

## Protein Markers

Proteomics methods have been widely used to identify proteins in various PDVLNs, but our understanding of the functional roles these proteins play within PDVLNs remains limited. In contrast to the well-established marker proteins such as CD9, TSG101, and CD63 in mammalian EVs,<sup>62</sup> there is currently a lack of consensus on specific marker proteins within PDVLNs. Proteins associated with membrane stability or PDVLN secretion may emerge as candidate marker proteins. Interestingly, in the latest research, Ma et al found that TSG101, CD63, and CD9 are highly expressed in nanoparticles derived from ginseng.<sup>63</sup> A family of proteins that has been widely detected in PDVLNs is the heat shock proteins (HSPs). For example, HSP70 has been found to be highly expressed in nanoparticles derived from grapes and olive pollen grains.<sup>64,65</sup> HSP90 has been reported to be present in PDVLNs from tea plants.<sup>66</sup> PEN1 has been found to be highly expressed in PDVLNs derived from *Arabidopsis* and is associated with membrane surface components.<sup>67</sup> However, to be recognized as a widely accepted vesicle surface marker protein, it still needs to be confirmed in nanoparticles from many different plant sources.

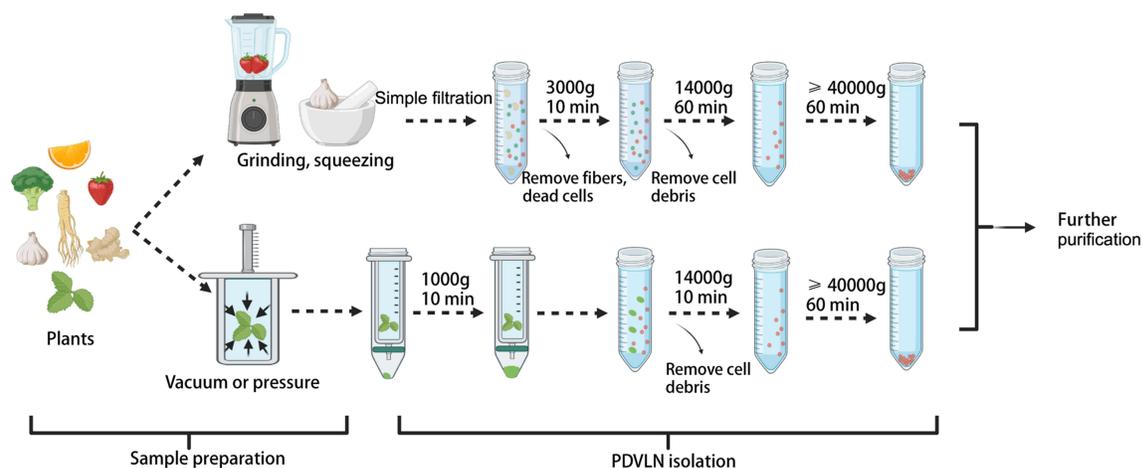
## Morphology

Researchers have verified the successful isolation of PDVLNs by observing their bilayer lipid membrane structure through transmission electron microscopy. PDVLNs have been reported to have oval, spherical, or cup-shaped morphology.<sup>68</sup> These morphological differences may originate from variations in plant species or electron microscopy imaging techniques. For instance, Mu et al used the same method to extract vesicles from grapes and grapefruits, but morphological differences could be observed.<sup>59</sup> The transmission electron microscope (TEM), which is commonly used to observe the morphology of EVs, requires repeated dehydration during sample preparation, which may cause the EVs to take on a cup-like shape.<sup>69</sup> Atomic force microscopy (AFM), a type of high-resolution microscope, has also been widely used for PDVLNs imaging.<sup>70</sup>

## Isolation and Purification

### Sample Preparation

PDVLNs are secreted into the apoplast space; therefore, before the formal isolation of PDVLNs, the juice containing PDVLNs was collected first. Figure 3 shows the general process of the PDVLNs isolation. There are two main methods to acquire PDVLNs juice: tissue disruption and tissue infiltration. For tissue disrupt method, physical methods (including grinding, blending, and squeezing) are used to disrupt plant tissues and obtain plant juice.<sup>71–73</sup> For some plants with low juice content, phosphate buffer saline (PBS) can be added to plants and ground with plants to load PDVLNs.<sup>66,74</sup> Following this, several low-speed centrifugation steps (usually two or three) are conducted to remove the fibers and cell debris. It is worth noting that during the duration of disruption, the extracellular matrix may contaminate the extracted juice, and subjected methods should be used to eliminate the influence of the matrix. A typical example is citrus, in which traditional differential centrifugation is difficult to separate from pectin. Tris-HCl can be added to citrus plant-derived juices to remove pectin.<sup>75</sup> Moreover, the tissue-disrupt method inevitably destroys the cellular structure, and some organelles and cell fragments may be purified together with PDVLNs.<sup>76</sup> As for the tissue-infiltration method, simply put, this method involves infiltrating the isolation buffer to apoplast to load the PDVLNs, and then extracting it out by centrifugation. Rutter et al successfully extracted PDVLNs from *Arabidopsis* using this method.<sup>67</sup> The infiltration was driven by the pressure difference (using vacuum equipment), and centrifugation was usually performed at a low speed (700–5000 g).



**Figure 3** General process of the isolation of PDVLNs. The whole process can be divided into two parts: sample preparation and PDVLNs isolation. Tissue-disrupt method acquire plant juice rich in PDVLNs by grinding or squeezing, and tissue-infiltration method infiltrate buffer to load PDVLNs by pressure or vacuum.

The tissue infiltration method causes less damage than tissue disruption and can thus obtain purer PDVLNs.<sup>53</sup> However, the yield of PDVLNs extracted by the tissue infiltration method was lower than that extracted by the tissue disruption method. This is because the isolation buffer may dilute fluid from the apoplast. Therefore, it is important to select the appropriate method for this purpose.

## Isolation and Purification of PDVLNs

As the interest of researchers in mammalian EVs has increased in the last decade, isolation methods for EVs have developed greatly, and numerous methods have been explored to isolate EVs. Similar to mammal-derived EVs, PDVLNs have been isolated based on their density, size, charge, and immunoaffinity. The most commonly reported method is ultracentrifugation (UC).<sup>61</sup> After pre-processing the plant tissue, the juice is centrifuged one to three times at low speed (300–10,000 g) to remove impurities such as fibers and debris. Then, an ultracentrifugation step (> 40,000 g) is taken to finally collect the PDVLNs.<sup>77</sup> The selection of centrifugation speed and time largely depends on the plant source and experience of the researchers. The advantage of UC is that researchers can simultaneously centrifuge hundreds of milliliters of juice to obtain good yields. Some organelle fragments and non-vesicular substances may have a density similar to that of PDVLNs, and may mix with PDVLNs in the last UC steps. In this context, some recent studies have combined UC and density gradient centrifugation (DGC) to obtain PDVLNs with higher purity.<sup>78</sup> Two widely used gradient buffer are sucrose and iodixanol.<sup>79,80</sup> However, considering the losses during the process and the potential interaction between the PDVLNs and the gradient medium, the combination of UC and DGC inevitably reduces the yield of PDVLNs.<sup>61</sup> Notably, owing to the diversity of plant sources, specific isolation processes should be carefully evaluated and adjusted according to specific plant species. We look forward to more collaborations between researchers and botanists in the future to further improve the extraction process. The ultrafiltration method uses a membrane to separate PDVLNs from other contaminants based on their size. Lee et al isolated EVs from plant leaves using a 100kDa centrifugal filter.<sup>81</sup> Although ultrafiltration does not require specialized equipment such as ultracentrifugation, it is usually applied in combination with other isolation methods owing to problems such as low purity and possible membrane blockage. Size exclusion chromatography (SEC) relies on the relationship between the hydrodynamic radius of the particles and pore size of the resin beads. By utilizing SEC, the PDVLNs can be separated based on their size. Kim et al combined ultrafiltration and SEC to isolate carrot derived PDVLNs and achieved relatively higher yields.<sup>82</sup> Polymer-based precipitation uses polymers such as polyethylene glycol (PEG) to change the solubility of PDVLNs and increase their sedimentation. For example, Kalarikkal used PEG6000 to purify ginger PDVLNs and achieved a yield of 60% to 90% compared to UC.<sup>83</sup> This method has a simple protocol and relatively high yield, but may lead to the aggregation of EVs and mixing with other proteins.<sup>84</sup> Other methods, such as immunoaffinity, field-flow fractionation, and electrophoresis-coupled dialysis, have also been developed for PDVLN isolation; their principles and characteristics are shown in Table 1.

**Table 1** Preparation Methods of PDVLNs

Preparation Methods	Theories	Strengths	Weaknesses	Refs.
Differential ultracentrifugation (DUC)	Different sizes and sedimentation rates between PDVLNs and other ingredients	Widely used, low cost, high recovery, large sample capacity	Time-consuming, compromise structural integrity, pellets aggregation, requires expensive equipment	[85,86]
Density gradient centrifugation (DGC)	Different buoyant densities between PDVLNs and other ingredients across a density gradients	Widely used, low cost, high purity	Time-consuming, contamination of density gradient medium, requires expensive equipment, difficulty in expanded production	[60,86,87]
Ultrafiltration (UF)	Using a membrane filter to trap large particles while allowing smaller particles to flow through	Time-saving, allows for processing different samples simultaneously, preserve structural integrity	Large particles may cause membrane blockage, particle loss, low purity	[74,81,88]
Size exclusive chromatography (SEC)	Different hydrodynamic radius between PDVLNs and other ingredients	High purity, preserve structural integrity	Time-consuming, High cost, difficulty in expanded production	[82,89]
Immunoaffinity capture	Using specific antibodies to bind specific proteins on the surface of PDVLNs	High specificity	Lack of commercial antibodies and universal PDVLNs markers, low recovery	[90]
Asymmetric-flow field-flow fractionation (AF4)	Different sizes between PDVLNs and other ingredients	High automatic, various mobile eluents, combine with light scattering detection	Under developing, time-consuming, requires specialized equipment	[91]
Polymer-based precipitation	Using polymers to reduce the solubility of PDVLNs and increase their sedimentation	Time-saving, convenient operation, low cost, large sample capacity	Low purity	[92]
Electrophoresis coupled dialysis (ELD)	Using electric current to draw other ingredients out of dialysis bags while remain PDVLNs in it	Time-saving, convenient operation	Low recovery, pellets aggregation	[93,94]
Aqueous Two-Phase System (ATPS)	Different partition coefficient between PDVLNs and other ingredients between immiscible solvents	Time-saving	The existence of dextran may affect cell viability and increase the viscosity of samples	[95,96]

## Anticancer Effects of PDVLNs

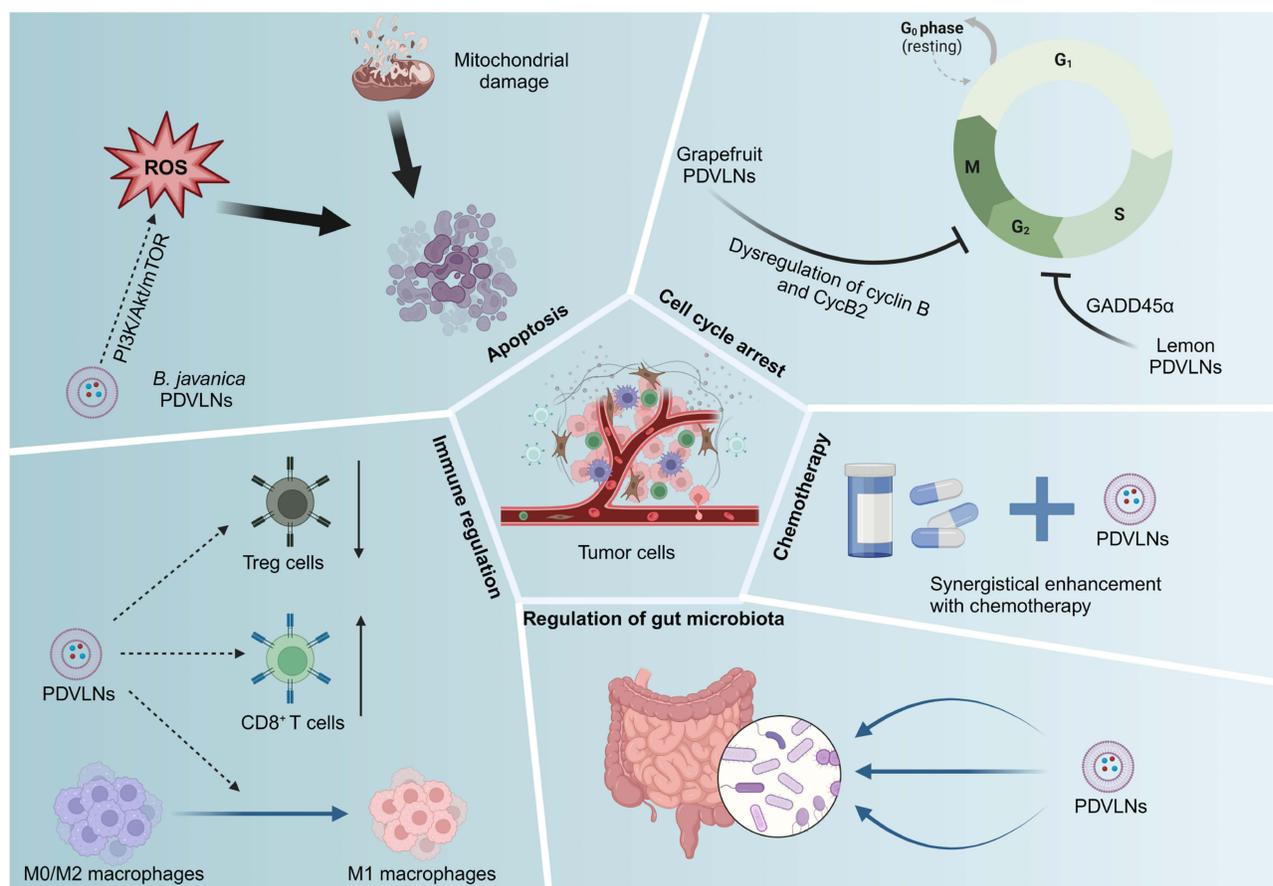
In mammals, EVs play a vital role in intercellular communication and intercellular substance transport to regulate biological and pathological processes. Similarly, PDVLNs transport various bioactive substances among cells in plants. It has been proven that bioactive molecules encapsulated in PDVLNs play a role in interspecies communication,<sup>59</sup> indicating the potential of PDVLNs for the treatment of human diseases. As mentioned earlier, there are many anticancer substances in plants, including proteins, miRNAs, and metabolites. Therefore, PDVLNs carrying anticancer substances have emerged as promising anticancer agents. [Table 2](#) lists the current use of PDVLNs for cancer treatment, and [Figure 4](#) shows the mechanisms underlying their anticancer activities.

## Inducing the Apoptosis of Cancer Cells

Although many PDVLNs from various plants have been reported to have anticancer activity,<sup>95,107,108</sup> the underlying mechanisms have not been well demonstrated, and further research is needed. Briefly, the currently reported anticancer mechanisms of PDVLNs mainly involve three aspects: apoptosis, cell cycle arrest, and immune regulation. Most studies have reported the ability of PDVLNs to induce apoptosis in cancer cells, although the specific signaling pathways vary across studies. Bitter melon derived PDVLNs stimulate the generation of intracellular reactive oxygen species (ROS) in breast cancer cells, thus inducing apoptosis.<sup>97</sup> In this study, the fluorescence intensity of DCFH-DA (20,70–dichlorofluorescein diacetate)

**Table 2** The Current Use of Plant Derived Vesicle Like Nanoparticles for Tumor Treatment

Plant Source	Type of Tumor	Type of Study	Isolation Method	Remarks	Ref.
Bitter melon	Breast cancer	In vitro and in vivo	UC	Bitter melon derived PDVLNs induces apoptosis of 4T-1 (mouse breast cancer cells) and MCF-7 (human breast cancer cells) cells by generation of ROS and the decrease in mitochondrial membrane potential.	Feng et al <sup>97</sup>
	Oral squamous cell carcinoma	In vitro and in vivo	Electrophoresis- and dialysis-based methods.	Bitter melon derived PDVLNs induces apoptosis of WSU-HN6 and CAL 27 cells (human tongue squamous cell carcinoma cells) by generation of Erlotinib ROS. Enhancing the cytotoxic effect of 5-FU in vivo.	Yang et al <sup>93</sup>
Ginseng	Melanoma	In vitro and in vivo	UC and DGC	Ginseng derived PDVLNs alter M2 macrophage polarization in a TLR4-MyD88-dependent manner.	Cao et al <sup>98</sup>
	Glioma	In vitro and in vivo	UC and DGC	Ginseng derived PDVLNs suppress M2 macrophage polarization. Fast penetration of the blood–brain barrier. PDVLNs mediate the gene silencing effect of ptc-miR396f on c-MYC.	Kim et al <sup>99</sup>
Lemon	A549, SW480 and LAMA84 cells	In vitro	UC and DGC	Lemon derived PDVLNs TRAIL-mediated cell death of A549 (human non-small cell lung cancer cells), SW480 (human colon cancer cells) and LAMA84 (human chronic myeloid leukemia) cells.	Raimondo et al <sup>100</sup>
	Gastric cancer	In vitro and in vivo	Electrophoretic technique with 300 kDa cut-off dialysis bag	Lemon derived PDVLNs induce S-phase arrest and cell apoptosis of AGS, BGC-823 and SGC-7901 cells (human gastric adenocarcinoma cells). Upregulating the expression of GADD45a and induce the generation of ROS.	Yang et al <sup>94</sup>
Grapefruit	Melanoma	In vitro	Phase separation method	Grapefruit derived PDVLNs induce G2/M checkpoint arrest of A375 cells.	Stanly et al <sup>73</sup>
Cannabis with high CBD content	Hepatocellular carcinoma	In vitro	UC and DGC	Cannabis derived PDVLNs induce G0/G1 arrest of HepG2 and Huh-7 cells (human hepatoma cells).	You et al <sup>101</sup>
<i>Moringa oleifera</i>	Hela and Jurkat cells	In vitro	UC	<i>Moringa oleifera</i> derived PDVLNs exhibit proapoptotic effects of Hela (human cervical cancer cells) and Jurkat (human T lymphocyte leukemia cells) cells.	Potestà <sup>102</sup>
Garlic	A549 and A498 cells	In vitro	Aqueous two-phase systems	Garlic derived PDVLNs induce apoptosis of A549 and A498 (human renal cell carcinoma cells) cells	Özkan et al <sup>95</sup>
	Pan-cancers	In vitro and in vivo	UC	PDVLNs promote gamma-delta T cells activation and migration from the gut to the tumor. PDVLNs enhancing immunity by synergizing with anti-PD-L1. PDVLNs reduce the expression of Ki67 and PCNA. PEGylated PDVLNs enhanced the therapeutic effects in vivo.	Xu et al <sup>103</sup>
<i>Asparagus cochinchinensis</i>	Hepatocellular carcinoma	In vitro and in vivo	UC and DGC	PDVLNs reduce the expression of Ki67 and PCNA. PEGylated PDVLNs enhanced the therapeutic effects in vivo.	Zhang et al <sup>104</sup>
Tea flower	Breast cancer	In vitro and in vivo	UC and DGC	Tea flowers derived PDVLNs induce the generation of ROS and cell cycle arrest of MCF-7 and 4T1. PDVLNs inhibit the lung metastasis of breast cancer in vivo. Taking PDVLNs orally maintain the abundance of the typical beneficial symbiotic bacteria and decrease the abundance of the typical harmful bacteria.	Chen et al <sup>66</sup>
<i>Dendropanax morbifera</i> , <i>Pinus densiflora</i> , <i>Thuja occidentalis</i> , and <i>Chamaecyparis obtusa</i>	MCF-7 and A431 cells	In vitro	UC	<i>D. morbifera</i> and <i>P. densiflora</i> derived PDVLNs display cytotoxic effects on MCF-7 and A431 (human epidermal carcinoma cells) cells. These two PDVLNs synergistically kill MDA-MB-231 (human breast cancer cells) and MCF-7 cells.	Kim et al <sup>105</sup>
Corn <i>Brucea javanica</i>	Colon26 cells	In vitro and in vivo	UC	RAW264.7 cells treated with corn derived PDVLNs reduce the number of colon26 cells.	Sasaki et al <sup>106</sup>
	TNBC	In vitro and in vivo	UC	PDVLNs induce the ROS/caspase-mediated apoptosis of breast cancer cells through PI3K/Akt/mTOR signaling pathway.	Yan et al <sup>15</sup>



**Figure 4** Various mechanisms of the anticancer effect of PDVLNs. There are five main aspects: induction of apoptosis, induction of cell cycle arrest, regulation of immune system, regulation of gut microbiota and synergistical enhancement with chemotherapy.

was used to detect the level of ROS, which is a commonly used method in PDVLNs research. ROS-induced apoptosis is completed within 60 min in a caspase-dependent manner.<sup>109</sup> Indeed, radiotherapy and chemotherapy induce the accumulation of ROS in cancer cells, resulting in apoptosis of cancer cells,<sup>110</sup> and many types of anticancer agents can generate oxidative stress. For example, Erlotinib and Imatinib induce ROS-dependent apoptosis in non-small-cell lung cancer cells and melanoma, respectively.<sup>111,112</sup> Furthermore, 5-fluorouracil (5-FU) induces the accumulation of ROS in a p53-dependent manner, leading to apoptosis in colorectal cancer cells.<sup>113</sup> Interestingly, Yang et al found that bitter melon derived PDVLNs induced the apoptosis of oral squamous cell carcinoma by ROS and synergistically enhanced the cytotoxic effects of 5-FU.<sup>93</sup> Although they pointed out that the synergistic effect was generated by the PDVLNs mediated downregulation of NLRP3, PDVLNs induced ROS may also play a role in this effect. Therefore, PDVLNs from various plants may act as promising adjuvants for enhancing the therapeutic effects of chemotherapy. Apart from ROS, Chen et al found that the levels of intracellular superoxide anions and reactive nitrogen species also increased in tea flower derived PDVLNs treated breast cancer cells.<sup>66</sup> In the meantime, Chen et al attempted to identify the substances responsible for the induction of ROS. Using liquid chromatography coupled with tandem mass spectrometry, they found several flavonoids and polyphenols, such as epicatechin gallate and epicatechin, in tea flower derived PDVLNs, which have been reported to induce increased oxidative stress in cancer cells.<sup>114,115</sup> Another proapoptotic effect of PDVLNs is mitochondrial damage. Although ROS can also induce mitochondrial damage,<sup>116</sup> little is known about whether this damage is caused by ROS or PDVLNs. JC-1 (5,5',6,6'-tetrachloro-1,10,3,3'-tetraethyl-imidacarbocyanine iodide) assay was used to detect the membrane potential of mitochondrial to evaluate the degree of mitochondrial damage.<sup>66,97</sup> Specifically, lemon derived PDVLNs can induce cancer cell apoptosis via the tumor necrosis factor related apoptosis-inducing ligand (TRAIL)/Dr5 pathway.<sup>100</sup> A latest study indicated that *Brucea*

*javanica* (a medicinal plant) derived PDVLNs could induce the ROS/caspase-mediated apoptosis of breast cancer cells through the PI3K/Akt/mTOR signaling pathway.<sup>15</sup>

## Induction of Cell Cycle Arrest

The second anticancer effect of PDVLNs is cell cycle arrest. Several plants derived PDVLNs have exhibited the ability to induce cell cycle arrest of cancer cells. However, the specific arrest phases are different. Lemon derived PDVLNs can induce the cell cycle in gastric cancer cells at S phase.<sup>94</sup> Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis revealed that this cell cycle arrest effect might be caused by the GADD45 $\alpha$  protein, and after treatment with lemon derived PDVLNs, the expression level of GADD45 $\alpha$  increased. It is reported that GADD45 $\alpha$  could inhibit the G2/M checkpoint and arrest the cell cycle at G2.<sup>117</sup> Moreover, GADD45 $\alpha$  can induce S-phase cell cycle arrest and further inhibit the proliferation of human hepatoma G2 cells.<sup>118</sup> Another study on grapefruit derived PDVLNs indicated that PDVLNs induce the G2/M arrest of A375 cells (human melanoma cells).<sup>73</sup> This effect is attributed to the dysregulation of cyclin B and CycB2. Cyclin B and CycB2 are two essential proteins that participate in the G2/M cell cycle progression, and it is reported that the downregulation of CycB2 inhibits the proliferation and metastasis of cancer cells.<sup>119,120</sup> In general, PDVLNs derived from citrus plants appear to have a favorable ability to induce cell cycle arrest of cancer cells, and further research is needed in the future. Similarly, tea flower derived PDVLNs also induce G2/M arrest of cancer cells. Tajik et al reported that cannabis derived PDVLNs induced G0/G1 arrest of hepatocellular carcinoma cells, which might be attributed to the alteration of other cell cycle regulatory proteins.<sup>101</sup>

## Regulating the Immune Microenvironment of Tumor Tissue

The immune regulatory effects of PDVLNs mainly involve two immune cells currently, namely, macrophages and T cells. Most studies have reported on the regulation of macrophages. Two studies reported the alteration of M2 macrophages by ginseng derived PDVLNs. Cao et al conducted a comprehensive study on the polarization of macrophages induced by ginseng derived PDVLNs. They found that PDVLNs altered the polarization of M2-like macrophages and inhibited melanoma growth. Using flow cytometry, they verified the polarization and function of the M1-like macrophages *in vivo*.<sup>98</sup> Moreover, they found that when lacking the surface proteins (treated with proteinase K), PDVLNs did not induce alterations in M2 macrophages. Another study reported that ginseng derived PDVLNs could suppress M2 macrophage polarization, thus suppressing glioma growth.<sup>99</sup> Meanwhile, the authors reported an alteration of T cells in tumor microenvironment, and after treatment with ginseng derived PDVLNs, the number of CD8<sup>+</sup> T cells significantly increased, while the abundance of Treg cells (FoxP3<sup>+</sup> and CD25<sup>+</sup>) decreased. Sasaki et al reported that corn derived PDVLNs altered the polarization of macrophages by using RAW264.7 cells (mouse monocyte macrophage leukemia cells).<sup>106</sup> Xu et al have described the status of intestinal immune cells following oral administration of garlic PDVLNs through single-cell sequencing.<sup>103</sup> They found that oral administration of PDVLNs could promote proliferation and activation of intestine gamma-delta T cells, thereby increasing the secretion of IFN- $\gamma$ . At the same time, activated gamma-delta T increased the expression of CXCR3, which prompted them to migrate from the intestine to the tumor tissue.

Other potential anticancer effects include specific bioactive molecules (such as non-coding RNA) and regulation of the gut microbiota. Accumulating evidence has demonstrated that plant-derived miRNAs exist in the human body and play a role in human health.<sup>121,122</sup> It has been reported that plants derived miRNAs are administered orally and are observed in the intestine.<sup>123</sup> Due to the characteristics of interspecies communication of PDVLNs, the manner in which these miRNAs enter human bodies may also be mediated by PDVLNs. PDVLNs contain several RNAs (such as miRNAs),<sup>124,125</sup> and these miRNAs vary among different source plants or parts of plants. Many plant-derived miRNAs have been shown to exert anticancer effects in humans.<sup>126,127</sup> Therefore, exploring potential plants that contain anticancer miRNAs may be a promising method for identifying new PDVLNs for tumor therapy.

Another potential anticancer effect is the regulation of the gut microbiota. The oral intake of PDVLNs is considered a safe method for absorbing PDVLNs and rarely causes adverse effects. Several studies have used oral administration of PDVLNs to treat intestinal diseases, such as colitis.<sup>128</sup> Orally administered tea flower-derived PDVLNs have been

reported to remodel the gut microbiota and may play a role in anticancer effects.<sup>66</sup> Teng et al also showed that PDVLNs could regulate gut microbiota and alleviate specific diseases.<sup>129</sup>

In general, PDVLNs are promising agents or tools for tumor therapy. Compared with mammal derived EVs, PDVLNs themselves have anticancer effects. However, unlike mammal derived EVs (cell line derived EVs), PDVLNs are extracted directly from plants rather than from cultured cells. This approach allows for the extraction of large quantities of PDVLNs in each extraction, but it also makes it difficult to genetically engineer PDVLNs.<sup>130</sup> For example, engineered mammal derived EVs can express specific targeted proteins on their surfaces to achieve targeted delivery of drugs or fluorescent molecules to tumors.<sup>131</sup> Notably, some PDVLNs show great biosafety because they significantly inhibit the proliferation of cancer cells while having no effect on normal cells,<sup>66,100</sup> which compensates for the shortcomings of insufficient targeting to some extent. The engineering of PDVLNs is in its early stages, and there is still a long way to go in the future. In the next section, we review the current articles on the engineering of PDVLNs and provide insights into their future development.

## Methods for PDVLNs Engineering

Although some PDVLNs were discovered to be tumor-suppressive on their own, researchers have proposed various methods to modify PDVLNs to improve their targeting ability and anticancer effects.

### Loading Cargoes into PDVLNs

A crucial aspect in evaluating the feasibility of PDVLNs as delivery platforms is their ability to carry therapeutic drugs. Hydrophilic drugs can be encapsulated into PDVLNs' cavity, whereas hydrophobic drugs can be loaded onto the lipid bilayer membrane. The drug loading strategies employed by PDVLNs are similar to those used by mammal-derived EVs, such as co-incubation, ultrasound, electroporation, freeze-thaw cycles, and co-extrusion.<sup>132,133</sup>

Co-incubation utilizes diffusion and lipophilic interactions between therapeutic drugs and the lipid bilayer of the PDVLNs. Xiao et al co-incubated doxorubicin (DOX) with lemon-derived PDVLNs and proved that encapsulation did not impair the anticancer efficiency of DOX while reducing its side effects.<sup>134</sup> Umezu et al also attempted to package miRNA into cherry derived PDVLNs through co-incubation and observed downregulation of the miRNA target gene after oral administration.<sup>135</sup> Although co-incubation is a widely used and simple method for drug loading, some limitations must be considered. First, co-incubation is a passive process that lacks additional forces to promote diffusion, which usually results in a relatively low encapsulation efficiency. In addition, the encapsulation efficiency may also be influenced by the characteristics of the loaded drugs because lipophilic and oppositely charged molecules make it easier to approach PDVLNs.<sup>136,137</sup>

Ultrasound, freeze-thaw cycles, and electroporation have similar mechanisms for drug loading. Ultrasound can temporarily damage the lipid bilayer of PDVLNs using mechanical energy and increase the chances for drugs to penetrate the PDVLNs.<sup>93</sup> Similarly, freeze-thaw cycles and electroporation can form pores in the lipid bilayer, allowing drugs to enter.<sup>138</sup> Yang et al used ultrasound to load 5-fluorouracil (5-FU) into bitter melon derived PDVLNs to enhance the therapeutic effects of 5-FU on oral squamous cell carcinoma.<sup>93</sup> Kilasoniya et al loaded heat shock protein 70 (HSP70) into grapefruit derived PDVLNs using an ultrasonic bath and demonstrated its anti-tumor effect on glioma cells.<sup>139</sup> These methods show a higher encapsulation efficiency than co-incubation.<sup>93,138,140</sup> However, the damage can distort the integrity of the lipid bilayer membrane. Freeze-thaw cycles have been reported to cause less damage to vesicles and have simpler operation methods than ultrasound, but frequent temperature changes may lead to protein inactivation and an increase in vesicle size.<sup>141</sup> Electroporation is usually applied for loading small molecules, such as siRNA, and the loading efficiency can be adjusted by adjusting the EVs, drug ratio, and voltage supplied.<sup>8</sup>

Co-extrusion refers to the extrusion of PDVLNs and drugs through a polycarbonate membrane filter, and the diameter of the obtained nanovesicles can be regulated by controlling the pore size of the filter.<sup>142</sup> This method can produce uniform-sized vesicles with a satisfactory loading efficiency. Zhang et al encapsulated siRNA-CD98 into ginger derived PDVLNs by co-extrusion and successfully reduced the expression of CD98 in colon tissues.<sup>143</sup> Co-extrusion can also be used to modify PDVLNs by fusing their membranes with those of other vesicles. However, this method also has several limitations. Repeated co-extrusion process may impair the biological activity of PDVLNs, which has been proved by

Fuhrmann's study.<sup>136</sup> Loss of lipid bilayers on the filter membrane is also a vital issue that cannot be ignored.<sup>144</sup> In addition, the microfluidic method has also been recently reported as a method for drug loading. Shoko et al used microfluidic method to load siRNA into grapefruit derived PDVLNs, achieving a loading efficiency of 11% and successfully reducing the expression of target gene in HaCaT cells.<sup>145</sup> The advantage of microfluidic methods is that they can control the diameter of nanoparticles to obtain stable nanoparticles.

In general, these are the main strategies for loading anti-tumor molecules into PDVLNs. Co-incubation can protect the integrity of the membrane, but has a lower loading efficiency. In contrast, ultrasound, freeze-thaw cycles, and electroporation have better loading efficiency, but damage the membrane of nanoparticles. Co-extrusion can produce uniform-sized vesicles but may diminish the biological activity of PDVLNs. The characteristics of anti-tumor molecules can also affect the loading process. Researchers should take these aspects into consideration before deciding on a proper encapsulation method.

## Modifying PDVLNs

Unlike mammal-derived EVs isolated from cell culture medium, PDVLNs are directly isolated from plants, indicating the absence of a cell culture process. This characteristic makes PDVLNs easier to isolate and less costly but blocks the possibility of engineering PDVLNs from their biogenesis, such as using lentivirus to transduce genes into cells and express targeting peptides on their membrane. The culture of plant cells is a complex and expensive process. Thus, it is rarely considered in PDVLN production. Most studies that engineered PDVLNs have focused on modifying their lipid bilayer membranes. Table 3 compares the advantages and disadvantages of the three types of PDVLNs in terms of preparation and cost.

To enhance the targeting capability of PDVLNs, a common strategy involves immobilizing molecules such as cyclic arginine-glycine-aspartic acid (cRGD) onto their surface. cRGD can recognize and competitively bind to  $\alpha v\beta 3$  integrins that are highly expressed on the surface of many solid tumors,<sup>146</sup> which can also block the adhesion of tumor cells to the extracellular matrix or directly induce tumor cell apoptosis. Taking advantage of these features, Chen et al modified lemon derived PDVLNs with cRGD using the EDC/NHS chemistry coupling method and identified an increased accumulation at the tumor site.<sup>147</sup> Some studies have also applied heparin as a bridge between cRGD and PDVLNs since it can increase the stability and biocompatibility of EVs in vivo owing to its good anti-complement activation ability.<sup>134</sup> Niu et al first linked pH-sensitive adipic acid dihydrazide (ADH) to DOX to promote its release in an acidic tumor microenvironment. They then conjugated ADH-DOX and cRGD to the carboxyl group of heparin. The remaining carboxyl groups of heparin were then bound to the active amino groups on the membrane of grapefruit derived PDVLNs to establish a novel delivery platform that could not only target gliomas but also control drug release.<sup>148</sup> Folic acid (FA) is another frequently used targeting ligand, since folate receptors are reported to be highly expressed in many cancer cells but have limited distribution in normal organs.<sup>149</sup> Zhang et al mixed total lipids extracted from ginger derived nanovesicles (GDNV) with DOX and FA in dimethyl sulfoxide (DMSO) in chloroform, and then dried the mixture under nitrogen to obtain a thin lipid film. A standard method based on lipid film hydration was applied to obtain DOX-FA-GDNVs, which exhibited an improved targeting ability towards colon tumors.<sup>137</sup>

Another strategy for modifying PDVLNs involves the infusion of different types of lipid membranes. Wang et al coated grapefruit derived nanovector with a leukocyte-derived plasma membrane (IGNV) by extruding the mixture 20

**Table 3** Comparison of Three Kinds of PDVLNs in Property

	PDVLNs	Surface-modified PDVLNs	Drug-carrying PDVLNs
Hardship in production	+	+++	++
Cost	+	+++	++
Targeting ability	++	+++	++
Anti-tumor effect	++	++	+++
Stability	+++	++	++

**Notes:** +, low; ++, medium; +++, high.

**Table 4** Clinical Trials of PDVLNs

Trial Title	Disease or Condition	Intervention or Treatment	Primary Outcome Measures	Current Status	NCT Number
Edible Plant Exosome Ability to Prevent Oral Mucositis Associated With Chemoradiation Treatment of Head and Neck Cancer	Head and Neck Cancer, Oral Mucositis	Two arms: 1. Grape PDVLNs, 2. Lortab, Fentanyl patch, mouthwash	Pain caused by oral mucositis (Extent of pain from oral mucositis will be evaluated weekly during treatment (6 to 7 weeks) and for six months following the completion of treatment which will last approximately 30 days).	Completed	NCT01668849
Plant Exosomes ± Curcumin to Abrogate Symptoms of Inflammatory Bowel Disease	Irritable Bowel Disease	Three arms: 1. Ginger PDVLNs, 2. Curcumin, 3. Ginger PDVLNs plus Curcumin	Change in inflammation on Colonoscopy (Decrease in inflammatory cells in the biopsy after treatment versus before treatment).	Completed	NCT04879810
Study Investigating the Ability of Plant Exosomes to Deliver Curcumin to Normal and Colon Cancer Tissue	Colon Cancer	Three arms: 1. Curcumin alone, 2. Curcumin with PDVLNs, 3. No treatment	Concentration of curcumin in normal and cancerous tissue	Recruiting	NCT01294072
Plant Exosomes and Patients Diagnosed With Polycystic Ovary Syndrome (PCOS) 17	Polycystic Ovary Syndrome	Four arms: 1. Ginger PDVLNs, 2. Aloe PDVLNs, 3. Ginger and Aloe PDVLNs, 4. Placebo	Change in glucose tolerance as measured by a glucose tolerance test	Withdrawn	NCT03493984

times through a 200 nm polycarbonate membrane. They found that IGVN showed an enhanced ability to home to inflammatory tumor tissues.<sup>150</sup> Chen et al proposed a conceptually novel design of a lemon derived PDVLNs engineered structural droplet drug (ESDD). They mixed cRGD- PDVLNs with DOX in a PBS solution as the aqueous phase, used squalene as the organic phase, and then used an ultrasonic emulsification method to fabricate the ESDD. The structure of the ESDD was similar to that of encapsulating DOX into a squalene droplet and coating it with cRGD-PDVLNs. ESDD could carry more DOX than PDVLNs and had better deformability, which could benefit the transcytosis process and improve its penetration into tumor tissues. ESDD also inherits the advantages of PDVLNs, such as their good bioavailability and targeting capability.<sup>147</sup>

## Clinical Trials of PDVLNs

Currently, research on PDVLNs is primarily preclinical. Given the promising performance of PDVLNs in disease treatment, there is an urgent need to know whether PDVLNs will demonstrate good efficacy and safety when applied in humans. There are currently four registered clinical trials related to PDVLNs, two of which have been completed, one is recruiting participants, and one had to be withdrawn due to lack of patient enrollment. Table 4 summarizes the details of these four clinical trials.

## Conclusion and Prospective

Before the first successful isolation of PDVLNs, plant-based anticancer therapy was mainly focused on the extraction of specific anticancer molecules or adjuvant therapy with other therapies. Although these two methods have yielded valuable achievements, the development of plant-based anticancer therapies has been limited by several factors. Along with the successful isolation of PDVLNs, plant-based anticancer therapy is a new development. Several plants with potential medicinal properties have been used to treat cancer. Currently, PDVLNs are primarily isolated from fruits and vegetables. Given the diversity of medicinal plants used for cancer therapy, medicinal plant-derived PDVLNs can be

isolated in future research to further concentrate on effective substances and reduce their toxicities. Bioactive molecules in PDVLNs are derived from source plants. Therefore, plants that have been proven to have active molecules that work against specific diseases may be the focus of future PDVLNs research.

PDVLNs have emerged as promising agents and tools for cancer therapies. PDVLNs exhibit ant-cancer effects and can act as anticancer agents. Moreover, similar to mammal derived EVs, PDVLNs can be designed as drug delivery platforms to carry drugs to the lesion area. The characteristic of PDVLNs to resist digestive enzymes in the gastrointestinal tract makes them ideal for oral medicine. However, compared to mammal derived EVs, research on PDVLNs is still in its infancy, and many problems need to be further studied. Although it is relatively easy to obtain PDVLNs that can be isolated directly from plant leaves, seeds, and stems of fruit juice, the process of planting plants requires a lot of manpower and resources. In the meantime, different soils, climates, and seasons may have an impact on the production of plants and the abundance of bioactive molecules. It is currently unclear whether these natural factors affect the composition of the PDVLNs. The isolation methods for PDVLNs are highly similar to those used for mammal derived EVs. However, this does not mean that these methods are suitable for extracting PDVLNs, because they originate from plants.

To further improve the quality of PDVLNs research and enhance the translational applications of PDVLNs, efforts can be made in several directions. The first is the development of more suitable isolation methods for PDVLNs and the improvement of the yield and purity of PDVLNs. Exploring the most suitable isolation method for specific plants. Second, qualitative and quantitative analyses of PDVLN components and the identification of effective substances in PDVLNs were performed. Multi-omics analysis can help to explore the differences in components among different PDVLNs and the mechanisms of PDVLNs in the biological effects of PDVLNs. Quantitative analysis of specific bioactive molecules can aid the translational application of PDVLNs. Third, comprehensively evaluating the safety of PDVLNs. Although some studies have reported the selective killing effect of PDVLNs on tumor cells, it is unclear whether PDVLNs are safe for all normal cells. Due to the complexity of PDVLNs components, the potential interactions between PDVLNs and other drugs should be carefully investigated.

## Data Sharing Statement

All relevant data are within the paper.

## Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

## Funding

This work was supported by the China Postdoctoral Science Foundation (2023M741278), Key R & D Program of Hubei Province (2023BCB149), Tongji Hospital Medical Innovation and Transformation Incubation Project (2022ZHFY02), and the National Natural Science Foundation of China (NSFC, 82370770).

## Disclosure

The authors have declared no conflicts of interest.

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