



REVIEW

Bioactive Phytochemicals in Plant-Derived Extracellular Vesicles as Novel Therapeutic Agents in Inflammatory Bowel Disease

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ABSTRACT: Inflammatory bowel disease represents a growing global health burden, resulting from a complex interaction among genetic susceptibility, environmental exposome, microbiota dysbiosis, and dysregulation of mucosal immunity. Despite major therapeutic advances achieved over the past decades, current treatments do not always maintain sustainable remission; they primarily target the suppression of the inflammatory cascade rather than the restoration of tissue homeostasis and barrier function. In this context, plant-derived extracellular vesicles (PDEVs) are drawing growing interest. These nanostructures, bound by a lipid bilayer, play an essential role in intercellular and cross-kingdom communication by transporting a multitude of bioactive molecules. The richness of their bioactive phytochemical properties and the botanical diversity of their plant sources including edible fruits, vegetables, and medicinal herbs confer considerable therapeutic potential to PDEVs, along with distinct properties compared to those of mammalian-derived vesicles. They are involved in maintaining the integrity of the intestinal epithelial barrier, in the excretion of cellular metabolites, and in the regulation of very complex interactions between genetic susceptibility, environmental factors, gut microbiota dysbiosis, and dysregulation of the mucosal immune response. Today, PDEVs stand as a natural, highly biocompatible, and promising nanotherapeutic platform; whether used as intrinsic therapeutic agents or as vehicles for the targeted administration of active ingredients, they bring vast clinical perspectives to the management of IBD. This review synthesizes current data regarding the therapeutic potential of PDEVs (in both their natural and artificial forms) and provides an in-depth analysis of their biogenesis, diverse botanical origins, isolation methodologies, and unique biochemical properties, as well as their multi-target mechanisms of action in restoring intestinal homeostasis.

KEYWORDS: Intercellular communication; cargo molecules; bioactive compounds; biogenesis; bioactive phytochemicals; botanical diversity; pathogenesis

1 Introduction

IBD, encompassing Crohn's disease (CD) and ulcerative colitis (UC), is a chronic relapsing inflammatory disorder of the gastrointestinal tract that affects millions of individuals worldwide [1]. The disease arises from a complex interplay among genetic susceptibility, environmental exposures, intestinal microbiota,

epithelial barrier dysfunction, and dysregulated immune responses [2]. Although significant advances have been made in understanding the mechanisms underlying inflammatory bowel disease (IBD), its precise etiology remains incompletely elucidated, and the global burden of the disease continues to increase historically affecting vast regions such as North America, Western Europe, and particularly newly industrialized countries such as China, India, and Brazil [3]. Over the past two decades, the preclinical therapeutic landscape of IBD has evolved considerably, driven by the identification of novel molecular targets. However, the introduction of biologic agents and small-molecule therapies into clinical practice continues to face major challenges, as the number of validated clinical studies remains limited compared to the dynamism of fundamental research. Despite these advances, a considerable proportion of patients fail to achieve sustained remission, lose responsiveness over time, or experience adverse effects associated with long-term treatment. Furthermore, many currently available therapies primarily focus on suppressing inflammation rather than restoring the underlying intestinal homeostasis. These limitations have stimulated growing interest in the development of novel therapeutic approaches capable of simultaneously regulating immune responses, repairing the intestinal barrier, and promoting tissue regeneration [4].

In this context, extracellular vesicles (EVs) have emerged as promising biological mediators and therapeutic tools. EVs are nanosized membrane-bound particles naturally released by virtually all cell types and are increasingly recognized as important mediators of intercellular communication. They can help to regulate numerous physiological and pathological processes, including immune modulation, tissue repair, and inflammatory signaling, among others by carrying proteins, lipids, nucleic acids, and other bioactive molecules [5,6]. Their intrinsic biocompatibility, low immunogenicity, and capacity to carry bioactive cargo have made them highly attractive for use in a wide range of diseases as a therapeutic agent, including IBD [7]. Among the different sources of extracellular vesicles, plant-derived extracellular vesicles (PDEVs) have recently attracted increasing attention as a new class of natural nanotherapeutics. The richness of their bioactive phytochemical compounds, combined with the wide botanical diversity of their sources, including edible fruits, vegetables, medicinal plants and other plant tissues, confers PDEVs unique biological properties and considerable therapeutic potential compared to mammalian-derived extracellular vesicles.

PDEVs represent a heterogeneous group of vesicular structures whose molecular composition depends on their biological origin, the underlying biogenesis mechanisms, and the isolation strategies employed. These vesicles may contain a complex array of bioactive molecules, including membrane lipids such as phosphatidylcholine, phosphatidylethanolamine, sterols, and sphingolipids, which contribute to membrane stability and vesicular functional properties [8,9]. Membrane-associated and intravesicular proteins have also been identified in various PDEV preparations, including transport proteins, receptors, and proteins involved in vesicular trafficking, such as TET8, SNAREs, and syntaxins, as well as chaperone proteins such as HSP70 and various enzymes. In addition, several studies have reported the presence of carbohydrate-containing components, including glycoproteins, glycolipids, glycosylphosphatidylinositols, oligosaccharides, and soluble sugars, associated with plant-derived vesicle preparations [9,10]. Furthermore, nucleic acids such as microRNAs (miRNAs), small interfering RNAs (siRNAs), and messenger RNAs (mRNAs) have been detected in certain plant-derived vesicle preparations, suggesting their potential involvement in intercellular communication and cross-kingdom regulatory mechanisms [11]. However, it remains necessary to determine to what extent these molecules are selectively sorted and actively incorporated into vesicles through regulated biogenesis pathways, or whether some of them may be co-isolated during purification processes. Beyond macromolecular components, PDEVs have also been associated with various plant secondary metabolites, including phenolic compounds, flavonoids, stilbenes, terpenoids, and alkaloids [9]. However, the precise localization of these compounds, whether they are encapsulated within vesicles, associated with the vesicular

membrane, or simply co-purified from the plant matrix remains an important area of investigation. This distinction is essential for accurately interpreting their contribution to the biological activities attributed to PDEVs. Despite these uncertainties, the diversity of molecular cargos associated with PDEVs may contribute to their biological properties, including their ability to modulate mammalian immune responses, regulate intestinal barrier integrity, and interact with the gut microbiota [12,13]. Moreover, their natural origin and availability from plant sources, including edible plants, make PDEVs particularly attractive for the development of innovative therapeutic strategies, especially for gastrointestinal applications.

A remarkable feature of PDEVs is their ability to participate in cross-kingdom communication. Growing evidence suggests that bioactive molecules contained within plant-derived vesicles can be taken up by mammalian cells, where they influence cellular signaling pathways, immune regulation, and interactions with the gut microbiota. This discovery has challenged traditional views of dietary bioactivity and has opened new perspectives on how plant-derived nanoparticles may contribute to intestinal health and disease modulation [14]. In recent years, numerous experimental studies have demonstrated the therapeutic potential of PDEVs in models of intestinal inflammation. Vesicles isolated from various plant sources have been shown to attenuate colitis by suppressing pro-inflammatory signaling pathways, enhancing epithelial barrier integrity, modulating gut microbial composition, and promoting mucosal healing [15–17]. These findings suggest that PDEVs may not only serve as natural therapeutic agents but also function as efficient delivery vehicles for targeted drug administration. Moreover, advances in nanotechnology and bioengineering have expanded the potential applications of PDEVs beyond their native biological functions. Engineered PDEVs can be loaded with therapeutic molecules, modified for enhanced targeting, and optimized to improve therapeutic efficacy, thereby combining the advantages of natural bioactive compounds with modern drug delivery strategies [18–20]. Given the growing body of evidence supporting their biological activity and therapeutic potential, PDEVs have emerged as promising candidates for the management of IBD. This review aims to provide a comprehensive overview of the current knowledge regarding PDEVs, including their biogenesis, isolation and characterization methods, molecular composition, mechanisms of action, therapeutic applications in IBD, safety considerations, and future perspectives.

2 Materials and Methods

2.1 Literature Search Strategy

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) 2020 Statement [21].

A comprehensive literature search was performed independently in PubMed, Web of Science, and Scopus to identify original studies investigating the therapeutic potential of PDEVs in IBD. A master Boolean search strategy was developed and adapted to the syntax of each database using the following combination of terms: (“plant-derived extracellular vesicles” OR “plant extracellular vesicles” OR “plant-derived exosomes” OR “plant-derived nanovesicles” OR “plant nanovesicles” OR “plant-derived vesicle-like nanoparticles” OR “plant exosome-like nanoparticles”) AND (“inflammatory bowel disease” OR IBD OR colitis OR “ulcerative colitis” OR “Crohn’s disease” OR “Crohn disease” OR “experimental colitis” OR DSS OR TNBS OR DNBS OR oxazolone). The search identified 258 records, including 31 from PubMed, 187 from Web of Science, and 40 from Scopus. Following the removal of 61 duplicate records, 197 unique studies remained for title and abstract screening.

2.2 Study Selection

Study selection was performed according to the PRISMA 2020 guidelines. Titles and abstracts of the 197 unique records were screened for relevance based on predefined eligibility criteria. Studies were considered eligible if they were original research articles investigating PDEVs or vesicle-like nanoparticles in relation to in IBD, colitis, or experimental colitis models. Following title and abstract screening, 34 studies met the eligibility criteria for full-text assessment, while 163 studies were excluded. The reasons for exclusion were as follows: 103 review articles, 26 studies not involving plant-derived extracellular vesicles, 27 studies not related to inflammatory bowel disease or colitis, and 7 records for which the abstract was inaccessible, preventing eligibility assessment. The full texts of the remaining 34 articles were subsequently evaluated for inclusion. Of these, 10 studies were excluded because the full text was unavailable, while 24 original studies fulfilled all eligibility criteria and were included in the qualitative synthesis (Fig. 1).

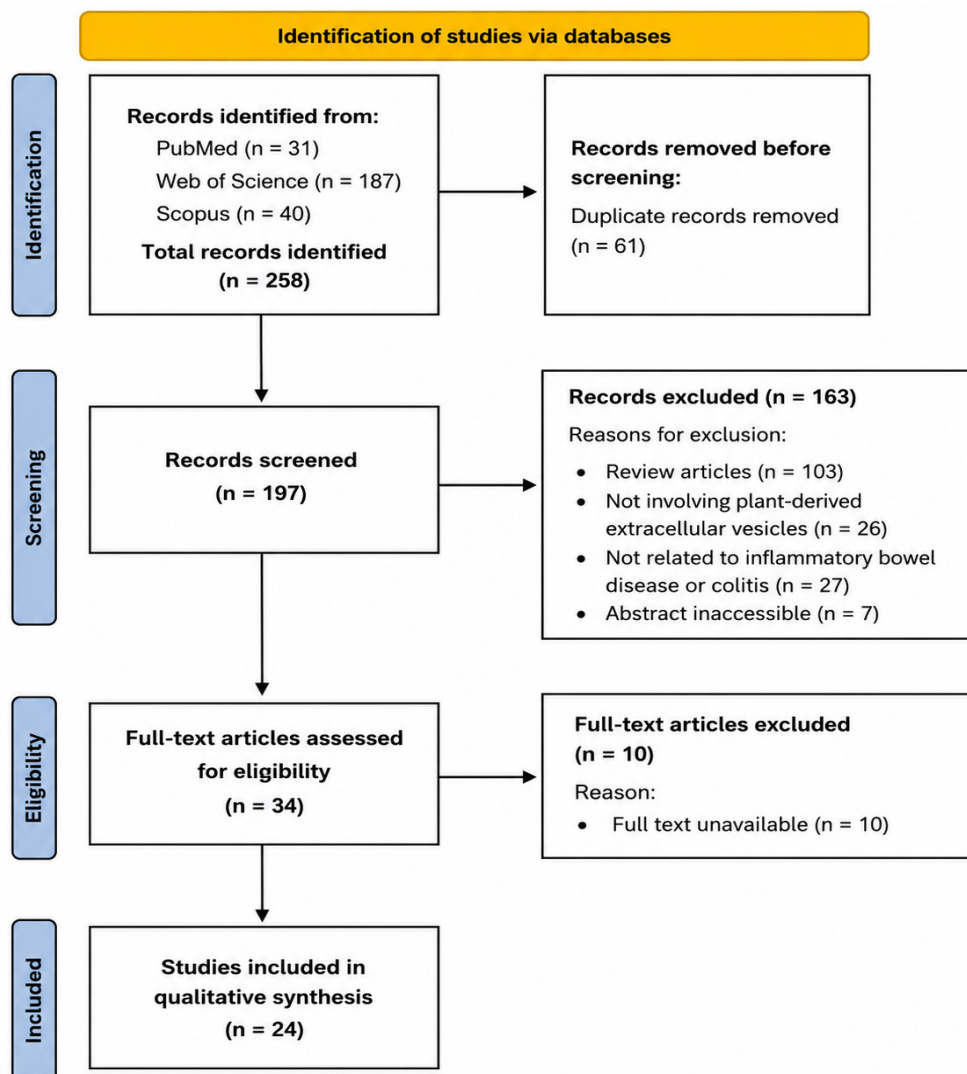


Figure 1: Literature search results according PRISMA guidelines.

3 Plant-Derived Extracellular Vesicles (PDEVs)

3.1 Historical Background and Evolution of the Concept

The concept of plant-derived extracellular vesicles (PDEVs) originated from mid-20th-century discoveries of membrane-bound vesicles in animal cells, initially regarded as cellular debris but later recognized as mediators of intercellular communication [22]. Research expanded rapidly, demonstrating that plant EVs participate in plant–pathogen interactions and cross-kingdom communication by transporting small RNAs, proteins, and metabolites [23]. Based on the general concept that all eukaryotic cells are capable of secreting EVs, research initially developed in parallel in animal and plant systems, with more recent attention focusing on the niche of edible PDEVs [24]. Although the field of PDEVs is relatively young, early ultrastructural investigations in cotton and carrot tissues date back to the 1960s [12].

Early observations reported multi vesicular bodies (MVB)-like structures, Golgi-derived vesicles, and ER-associated vesicular domains in plant cells, revealing a highly active endomembrane trafficking system. Although direct extracellular release was not demonstrated at that time, these findings anticipated the later concept of plant EV [14]. Shortly after, ultrastructural studies showed that multivesicular bodies derived from dictyosomes (Golgi apparatus) fuse with the plasma membrane and release internal vesicles into the apoplast, providing some of the first structural evidence of an exosome-like secretion pathway in plants and laying the foundation for the modern understanding of PDEVs [12]. This development shifted the field from a primarily botanical focus to a broader interdisciplinary framework integrating plant biology, microbiology, nanotechnology, and therapeutic research [25]. Currently, PDEVs are regarded not only as important elements of plant physiology but also as promising natural nanocarriers with applications in both agriculture and medicine [26].

3.2 Classification and Biogenesis of PDEVs

Plant-derived extracellular vesicles (PDEVs) are membrane-bound nanoparticles naturally released by plant cells into the extracellular environment. Unlike animal extracellular vesicles, the classification of plant vesicles remains challenging because specific molecular markers and universally accepted criteria for defining vesicle subtypes are still lacking. Therefore, the terminology “exosomes”, “microvesicles”, and “apoptotic bodies”, which is mainly based on mammalian EV classification, should be used cautiously in plants [14]. Particle size alone is insufficient to assign a vesicle population to a specific biogenetic category, and additional evidence regarding cellular origin and formation mechanisms is required. Based on current knowledge, naturally secreted PDEVs may originate from several biogenetic pathways, including the multivesicular body (MVB) pathway, the exocyst-positive organelle (EXPO) pathway, and the vacuolar pathway [13,27]. In the MVB pathway, endosomal membranes undergo inward budding to generate intraluminal vesicles within multivesicular bodies. Following fusion of MVBs with the plasma membrane, these vesicles are released into the extracellular space as secreted extracellular vesicles. This pathway has been described in several plant species, including carrot cells [28]. The EXPO pathway represents another unconventional secretion mechanism in plants [13]. In this process, double-membrane organelles known as exocyst-positive organelles fuse with the plasma membrane, resulting in the extracellular release of their internal vesicular content. Similarly, the vacuolar pathway involves the fusion of vacuoles with the plasma membrane, allowing the secretion of vesicular structures and soluble components into the extracellular environment [29]. Importantly, many studies investigating edible plant-derived vesicles rely on isolation procedures involving tissue disruption, homogenization, differential centrifugation, ultracentrifugation, filtration, or size-exclusion chromatography. The resulting particles are frequently referred to as plant-

derived nanovesicles (PDNVs), exosome-like nanoparticles, or plant-derived nanoparticles. However, these preparations may represent a heterogeneous population containing naturally secreted vesicles, membrane fragments, and reassembled lipid structures. Consequently, they should not automatically be considered equivalent to naturally secreted PDEVs, as differences may exist in their biogenesis, molecular composition, and biological functions. Therefore, throughout this review, the term PDEVs refers specifically to naturally secreted plant extracellular vesicles when evidence of cellular origin is available, whereas PDNVs or exosome-like nanoparticles are used for isolated plant-derived nanoscale particles whose biogenesis has not been experimentally confirmed.

3.3 Bioactive Phytochemical Compounds in PDEVs

Plant-derived extracellular vesicles (PDEVs) contain a complex and heterogeneous molecular cargo influenced by plant species, tissue origin, physiological status, and isolation procedures. In addition to structural components such as lipids, proteins, and nucleic acids, PDEVs have been reported to contain various low-molecular-weight metabolites and secondary bioactive compounds that may contribute to their biological and therapeutic effects. However, the presence and functional relevance of phytochemicals within PDEVs remain an area of ongoing investigation and require careful interpretation. Unlike lipids, proteins, and nucleic acids, whose association with plant extracellular vesicles is well documented, the incorporation of secondary metabolites into PDEVs remains controversial. The detection of a phytochemical compound in a vesicle preparation does not necessarily confirm that it represents authentic intravesicular cargo, as these molecules may also be associated with the vesicle membrane, adsorbed onto the vesicle surface, or co-isolated from the plant matrix during extraction and purification procedures [9,11]. Lipids represent the most extensively characterized components of plant-derived vesicles and play essential roles in membrane formation, stability, biogenesis, and interactions with recipient cells. Plant vesicle membranes are mainly composed of phospholipids and glycolipids, with phosphatidic acid contributing to membrane remodeling, vesicle formation, and fusion processes [30]. Lipidomic analyses have revealed species-specific lipid profiles among plant-derived vesicles. For example, phosphatidic acid has been identified in nanovesicles isolated from *Uvae-ursi folium* and *Zingiberis rhizoma*, whereas phosphatidylcholine and phosphatidylethanolamine were abundant in *Craterostigma plantagineum*-derived nanovesicles. Phosphatidylinositol was also detected in vesicles derived from *Curcuma zanthorrhiza* and ginger [9]. These membrane-associated lipids may influence vesicle stability, cellular uptake, and interactions with intestinal epithelial cells.

Protein cargo constitutes another important component of PDEVs. Proteomic analyses have identified membrane-associated proteins, trafficking-related proteins, and stress-response proteins involved in vesicle secretion and plant defense mechanisms. Tetraspanin-like proteins, plant-specific syntaxins such as PEN1 (SYP121), GTPases, chitinases, peroxidases, and β -glucosidases have been detected in plant vesicle preparations [10,31,32]. However, protein composition varies considerably depending on plant species and isolation methods, suggesting that some differences may result from technical variations rather than conserved vesicular components. PDEVs are also rich in nucleic acids, including messenger RNAs (mRNAs), microRNAs (miRNAs), and other small non-coding RNAs. Although dietary plant-derived microRNAs have been proposed to enter the mammalian circulation and modulate host gene expression, subsequent studies have yielded inconsistent results, raising questions regarding their physiological abundance, bioavailability, and functional relevance in mammalian systems [33,34]. The discrepancies reported across studies may be attributed to methodological differences, including vesicle isolation and purification procedures, sequencing technologies, contamination control, and bioinformatic analyses. Recent reviews have emphasized that, although plant EV-associated miRNAs may theoretically participate in cross-kingdom communication

under specific experimental conditions, the evidence supporting their absorption, stability, bioavailability, and biological activity in mammals following dietary consumption remains limited and, in some cases, contradictory [35]. Another study using *Arabidopsis thaliana* demonstrated the selective enrichment of specific miRNAs in extracellular vesicles, suggesting that RNA loading into vesicles is a regulated process rather than a passive phenomenon. Therefore, further studies employing standardized experimental protocols, more rigorous purification strategies, and robust functional validation are required to determine whether plant-derived miRNAs transported by extracellular vesicles exert biologically relevant cross-kingdom regulatory functions in mammalian systems.

In addition to macromolecules, several carbohydrates, amino acids, and small metabolites have been reported in plant-derived vesicle preparations. Glucose, fructose, sucrose, alanine, asparagine, leucine, threonine, and isoleucine have been identified in different plant-derived nanovesicle preparations, although their abundance strongly depends on the plant source and purification strategy [12]. However, their precise localization within vesicles (inside the lumen, associated with the membrane, or attached to the external surface) remains poorly characterized. Secondary metabolites, including flavonoids, phenolic acids, alkaloids, terpenoids, and antioxidant molecules represent another important category of plant bioactive compounds associated with PDEVs [9]. However, evidence supporting their true encapsulation within purified vesicles remains limited. For example, analyses of vesicles isolated from *Aconitum napellus*, *Arctostaphylos uva-ursi*, *Nicotiana tabacum*, and *Craterostigma plantagineum* showed that several characteristic alkaloids and phenolic compounds were predominantly detected in soluble fractions rather than in purified vesicle fractions [9]. These findings suggest that some phytochemicals previously attributed to PDEVs may actually correspond to co-isolated soluble compounds or contaminants originating from plant tissues. Nevertheless, certain hydrophobic metabolites, including chlorophyll derivatives and phenylpropanoid compounds, appear to preferentially associate with vesicle membranes due to their lipophilic properties. Rather than being actively encapsulated inside the vesicle lumen, these molecules may interact with the lipid bilayer or accumulate at the vesicle surface. Therefore, future studies should combine rigorous purification strategies with advanced analytical approaches, including LC-MS/MS analysis of highly purified vesicle fractions, density-gradient separation, enzymatic surface digestion, and membrane disruption experiments. These approaches are necessary to distinguish true intravesicular cargo from membrane-associated compounds and co-isolated contaminants. Overall, while lipids, proteins, and nucleic acids represent well-supported components of PDEVs, the contribution of phytochemicals remains promising but requires further experimental validation. A careful distinction between authentic vesicle-associated metabolites and co-purified compounds is essential for accurately understanding the mechanisms underlying the therapeutic potential of PDEVs in inflammatory bowel diseases. Although numerous studies have reported the presence of secondary metabolites in PDEV preparations, the exact origin of these molecules remains controversial [14]. Unlike mammalian extracellular vesicles, for which biogenesis mechanisms, molecular sorting, and cargo-loading processes are relatively well characterized, the mechanisms responsible for the incorporation of secondary metabolites into plant vesicles remain largely unknown [36,37].

Currently, three main hypotheses have been proposed. First, an active and regulated cellular loading mechanism may occur during vesicle biogenesis, although the underlying molecular pathways remain to be elucidated. Second, secondary metabolites may be incorporated passively from compounds present in the cytoplasm or intracellular compartments during vesicle formation. Finally, a widely discussed hypothesis highlights the potential presence of extraction artifacts: homogenization of plant tissues may lead to the release of organelle fragments, lipids, and soluble metabolites that co-isolate with nanovesicles, thereby mimicking an intrinsic intravesicular content [9,36,37]. This issue is particularly relevant when

nanoparticles are isolated from homogenized plant tissues, as these preparations correspond more closely to PDNVs rather than naturally secreted plant extracellular vesicles (PDEVs). Indeed, PDNVs represent a heterogeneous mixture that may contain authentic extracellular vesicles, intracellular vesicles, membrane fragments, subcellular organelles, and various co-isolated metabolites [37]. Consequently, the biological effects reported in many studies may result not only from the vesicles themselves but also from free phytochemicals or contaminants present in the preparations. This methodological limitation must be considered when interpreting the anti-inflammatory, antioxidant, or immunomodulatory properties attributed to PDNVs. To address these uncertainties, several authors have emphasized the need to develop more specific isolation protocols, improve the standardization of purification methods, and combine proteomic, lipidomic, metabolomic, and transcriptomic approaches to identify the biomolecules truly encapsulated within plant-derived vesicles. The diverse molecular cargo carried by PDEVs is likely to underlie their beneficial effects in IBD. Their complex composition including stress-responsive proteins, membrane lipids, regulatory RNAs, carbohydrates, and antioxidant metabolites may act in concert to regulate intestinal inflammation, reduce oxidative stress, preserve epithelial barrier integrity, and influence host microbiota interactions. While the molecular mechanisms responsible for these effects have not yet been fully elucidated, the multifunctional nature of plant-derived extracellular vesicles underscores their promise as natural nanotherapeutic platforms for the prevention and treatment of IBD.

3.4 Isolation Methods of Plant-Derived Extracellular Vesicles (PDEVs)

While animal extracellular vesicles (EVs) are grouped based on their biogenesis mechanism, size and specific protein markers, PDEVs remain undefined [38].

A conceptual classification of PDEVs can be established according to their source and engineering strategy, distinguishing between natural and artificial (bioengineered) vesicles. Following the application of isolation methods, natural PDEVs may be further subdivided into two major categories: (1) physiological PDEVs, also referred to as native EVs [39], which are separated from apoplastic fluids [10] or from other extracellular environment such as water containing root-exudate [40] or conditioned culture medium [41] and (2) edible plant vesicle-like nanoparticles, also described as plant exosome-like nanovesicles, isolated from homogenate [14,42] or from callus-homogenate [41]. The second category was more accurately standardized by Pinedo et al. as “plant-derived nanovesicles” (PDNVs) [39]. The latter term refers to nanoparticles generated through cellular disruption, followed by the reassembly and fusion of membrane fragments and intracellular vesicular components [39]. Furthermore, according to their origin and processing conditions, PDNVs may be divided into two emerging subclasses: those derived from fresh plant juices or homogenates [43] and those obtained from dried plant material [44].

Within intact plants, three principal approaches for obtaining naturally produced EVs have been outlined. These vesicles may be generated under: (1) normal physiological conditions [10], (2) as a part of plant defense mechanisms and intercellular communication processes [38], or (3) under genetically modified conditions manipulating genes involved in EV biogenesis [45]. Stress-related stimuli capable of influencing EV production include pathogen infection, drought, soil salinity, ultraviolet (UV) exposure, and mechanical injury. Under such conditions, the doubled number of EVs may have enriched cargo with defense-related proteins [10], pathogenesis-related proteins, specific small RNAs, and antimicrobial compounds [38]. Nevertheless, stress conditions are not essential for EV biogenesis itself; rather, they modulate vesicular cargo composition, enriching EVs with bioactive molecules [46], a feature that may be particularly relevant for inflammatory bowel disease (IBD) therapy.

As a third group of plant-derived extracellular vesicles may be considered the artificial or bioengineered PDEV systems, also referred to as plant-lipid vesicles or artificial plant-derived nanovectors (APNVs). APNVs are assembled from extracted plant lipids using engineering strategies designed to reconstruct vesicular membranes from plant lipid extracts. These systems often mimic the structural characteristics of natural EVs while providing enhanced functionality or delivery efficiency. To facilitate the effective reconstruction of bilayer membranes, Zhao et al. developed an enzyme-assisted extraction approach from complex plant tissues [47]. Moreover, experimental studies have demonstrated the therapeutic potential of plant-derived nanovesicles; for example, Zhuang et al. reported that orally administered ginger-derived nanoparticles protected mice against alcohol-induced liver injury, supporting their potential as naturally derived nanotherapeutic systems [48].

Collectively, biologically native plant vesicles and engineered vesicular platforms represent a synergistic source of natural bioactive compounds and advanced delivery systems [18]. Consequently, the bioengineering of lipid membranes derived from biological sources may inspire cross-disciplinary methodologies, as membrane manipulation and cargo-loading strategies developed in other systems are increasingly being adapted to plant-based vesicular platforms, an emerging field of considerable scientific interest. Further optimization of membrane composition, cargo loading, and vesicle physicochemical properties may expand their biomedical applications. Fig. 2 presents the main isolation methods of natural and artificial PDEVs, as well as the different steps involved in their engineering and modification.

3.4.1 Naturally Secreted Plant Extracellular Vesicles (EVs)

Naturally secreted plant EVs can be isolated from extracellular fluids, including apoplastic fluid [10,49] and root exudates-water of hydroponically grown tomato plants collected after 2 days [40], thereby providing a source of vesicles released by living plant cells under relatively physiological conditions. Plant cell cultures provide another source of naturally released EVs because vesicles secreted by living cells accumulate in the conditioned culture medium (similarly as water-root exudate), which can subsequently be collected and processed for EV isolation [41].

3.4.2 Plant-Derived Nanovesicles (PDNVs) Obtained from Disrupted Plant Tissues

PDNVs can be obtained from mechanically disrupted plant tissues, including fruits, vegetables, roots, and edible plant organs. In contrast to naturally secreted EVs, tissue disruption releases intracellular and membrane-derived nanovesicles together with soluble and particulate plant material, making the biological origin of the final preparation more heterogeneous. Juices represent a particularly accessible source of PDNVs because mechanical processing disrupts plant tissues and releases membrane-bound nanovesicles into the liquid fraction. These preparations have been obtained from edible fruits and vegetables and investigated for their physicochemical properties, biological activity, and potential use as delivery systems [14]. Moreover, plant callus cultures represent a potentially scalable and controllable source of plant-derived nanovesicles. When callus biomass is mechanically or enzymatically disrupted before vesicle isolation, the resulting preparations should be classified as tissue-derived PDNVs rather than naturally secreted EVs, because vesicle recovery is associated with disruption of the cellular material [41].

Particularly, enzymatic cell-wall digestion represents a less mechanically disruptive approach than grinding and may reduce the extensive physical shearing and cellular disruption associated with mechanical homogenization. In a comparative study, enzymatic degradation of the plant cell wall yielded a higher number of smaller and more uniformly sized vesicular particles than grinding-based extraction, suggesting that this approach may facilitate the recovery of vesicles that are less physically disrupted

during isolation [47]. However, the enzymatically released particles should not automatically be considered equivalent to naturally secreted EVs isolated directly from apoplastic fluid or conditioned culture medium. Although enzymatic digestion may release pre-existing vesicles located within or associated with the plant cell-wall/apoplastic compartment, the resulting preparation may still contain heterogeneous vesicular and non-vesicular components, depending on the plant tissue, enzymatic treatment, and downstream purification procedures. Therefore, until their composition and relationship to naturally secreted plant EVs are more comprehensively established, these preparations should be regarded as a distinct category of enzyme-released, EV-enriched plant vesicle preparations rather than being automatically classified as either naturally secreted plant EVs or PDNVs. This could be a provisional descriptive category, not as an established biological vesicle class. The “enzyme-released, EV-enriched plant vesicle preparations” describes the method of production and the likely enrichment in vesicular structures; it does not, by itself, prove a specific biogenetic origin.

3.4.3 Artificial Plant-Derived Nanovesicles (APNVs)

Artificial plant-derived nanovesicles (APNVs) are engineered vesicular systems derived from plant materials or plant-derived membrane components that are deliberately modified to incorporate selected therapeutic cargo, surface ligands, or specific membrane properties [50]. Unlike naturally secreted plant extracellular vesicles (native plant EVs), whose composition is determined by endogenous cellular biogenesis pathways, APNVs are generated through engineering approaches that allow greater control over vesicle composition, surface characteristics, and cargo incorporation. These approaches enable the optimization of physicochemical properties and provide opportunities for enhanced cargo loading through strategies such as passive loading, electroporation, sonication, or extrusion. Compared with naturally secreted plant EVs, APNVs may offer advantages in terms of loading efficiency, production reproducibility, and scalability, as their membrane composition and assembly parameters can be more precisely controlled. Furthermore, surface modification strategies may improve their targeting capacity and facilitate tissue-specific delivery. Plant-derived vesicle platforms have demonstrated the ability to carry endogenous plant miRNAs, protect RNA molecules from RNase degradation, and incorporate exogenous synthetic miRNAs, highlighting their potential as customizable delivery systems [51]. However, several challenges remain before APNVs can be translated into biomedical applications. Unlike native plant EVs, APNVs may lack naturally organized molecular components responsible for intrinsic biological activity, and the reconstruction of functional membranes may not fully reproduce the complexity of naturally secreted vesicles. In addition, large-scale manufacturing requires further optimization to ensure batch-to-batch consistency, stability, purity, and reproducible biological performance. From a regulatory perspective, APNVs will require rigorous characterization and safety evaluation, including assessment of composition, residual contaminants, biodistribution, and potential immunogenicity. Therefore, APNVs should be considered complementary engineered platforms rather than direct replacements for naturally secreted plant EVs. Their ability to combine plant-derived biological components with nanotechnology-based engineering approaches provides promising opportunities for targeted therapeutic delivery while requiring further investigation to overcome current production and regulatory challenges. Accordingly, the following terminology is used throughout this review: PDEVs as a general term for plant-derived extracellular vesicle comprising any of them, previously described. For clarity, the terminology used throughout this review is summarized as follows in Table 1. A comparative overview of the main isolation and purification methods for plant-derived extracellular vesicles (PDEVs), based on their vesicle category, purity, yield, scalability, major contaminants, advantages, limitations, and differences from mammalian extracellular vesicle isolation methods, is presented in Table 2.

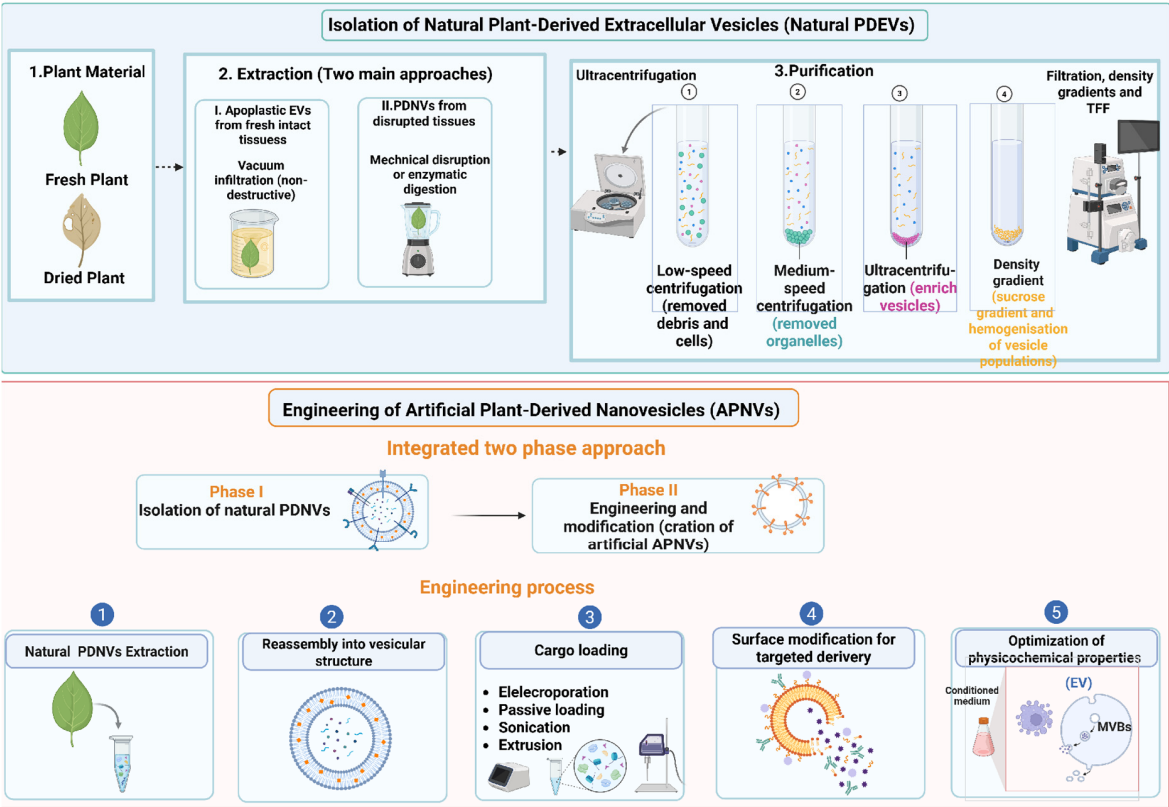


Figure 2: Isolation methods for Natural PDEVs and Artificial PDEVs. Phase I consists of isolating natural EVs or PDEVs, and Phase II corresponds to their engineering and modification. This process comprises five steps: (1) extraction of natural PDEVs, (2) their reassembly into vesicular structures, (3) cargo loading of a content/active ingredient (via electroporation, passive loading, sonication, or extrusion), (4) surface modification for precise targeted delivery, and (5) optimization of the physicochemical properties of the vesicles.

Table 1: The definitions of main abbreviations used throughout this work.

Category	Definition	Typical Source	Ref.
(Native/plant) EVs	Naturally secreted membrane-bound extracellular vesicles released by intact plant cells with no disruption of plasma membrane.	Apoplastic fluid or extracellular space or exudate or conditioned culture media	[10,40,41,49]
PDEVs	Heterogeneous nanoscale membrane-associated particle preparations obtained from disrupted plasma membrane of plant cells	Homogenates, juices, mechanically or enzymatically disrupted tissues or callus homogenate.	[14,41,47]
APNVs	Engineered vesicles reconstructed from plant-derived lipids, membranes, or related components	Deliberate laboratory engineering	[48]

Table 2: Comparison of isolation and purification methods for plant-derived vesicles (PDEVs) according to the category of vesicles obtained.

Isolation Method	Vesicle Category Obtained	Principle	Purity	Yield	Scalability	Main Contaminants	Advantages	Limitations	Differences from mammalian EVs	Ref.
Vacuum infiltration	Native/plant EVs	Extraction of apoplastic fluid containing naturally secreted EVs from plant cells using negative pressure	Very high	Low	Low	Apoplastic proteins, extracellular enzymes	Enables the isolation of native EVs without cellular disruption and under physiological conditions	Limited yield and highly dependent on plant tissue type	Plant-specific approach with no direct equivalent in mammalian EV systems	[10]
Root exudate collection	Native/plant EVs	Recovery of EVs naturally released into the extracellular environment of roots grown under hydroponic conditions	High	Low to medium	Low	Root metabolites, soluble proteins	Preserves the natural biological origin of EVs	Limited production requiring controlled culture conditions	Comparable to mammalian biological fluids but with a different plant-specific matrix	[40]
Conditioned culture medium isolation	Native/plant EVs	Collection of EVs secreted by living plant cells accumulating in conditioned culture medium	High	Medium	Medium	Medium-derived proteins, extracellular metabolites	Allows controlled production of native plant EVs	Requires optimized plant cell culture systems	Similar to EV secretion by mammalian cultured cells	[41]
Differential ultracentrifugation (UC)	Native/plant EVs + PDNVs	Separation based on particle size and density through sequential high-speed centrifugation steps	Low to medium	High	Medium	Cellular debris, soluble proteins, membrane fragments, plant organelles	Widely used enrichment method for vesicles	Low purity, possible aggregation and vesicle damage	Plant matrices contain more structural contaminants than mammalian biological fluids	[14,38]
Density gradient ultracentrifugation (sucrose/iodixanol)	Native/plant EVs + PDNVs	Separation according to vesicle buoyant density	High	Low to medium	Low	Associated proteins, non-vesicular membranes	Improves vesicle purity and enables better characterization of vesicle populations	Time-consuming and difficult to scale up	Similar to approaches used for mammalian exosome purification	[10,38]
Size-exclusion chromatography (SEC)	Native/plant EVs + PDNVs	Separation of particles according to size through a porous chromatographic matrix	High	Medium	Good	Soluble proteins, small metabolites	Gentle method preserving vesicle integrity and biological activity	Sample dilution and limited column capacity	Also widely used for mammalian EV purification	[39,41]
Tangential flow filtration (TFF)	Native/plant EVs + PDNVs	Concentration and purification using membrane filtration under tangential flow conditions	Medium to high	Very high	Excellent	Polysaccharides, soluble proteins, small molecules	Suitable for scale-up production and industrial applications	Requires optimization depending on plant matrix	More suitable for large-scale production compared with conventional mammalian EV isolation methods	[52]
Mechanical homogenization (plant juice, tissue grinding)	PDNVs	Mechanical disruption of plant tissues releasing membrane-derived and intracellular vesicular components	Low to medium	Very high	Excellent	Cellular debris, DNA, proteins, pigments, secondary metabolites	Simple method allowing high production of PDNVs	Heterogeneous preparations that do not always represent native EVs	Unlike mammalian EVs, PDNVs may result from membrane reassembly after cellular disruption	[14,42]

Table 2: Cont.

Isolation Method	Vesicle Category Obtained	Principle	Purity	Yield	Scalability	Main Contaminants	Advantages	Limitations	Differences from mammalian EVs	Ref.
Enzymatic digestion (cellulase, pectinase, macerozyme)	PDNVs	Enzymatic degradation of the plant cell wall to facilitate the release of vesicular components	Medium to high	High	Medium	Enzyme residues, cell wall fragments, polysaccharides	Improves recovery from resistant plant tissues	Risk of membrane alteration and need for optimization	Plant-specific approach due to the presence of a rigid cell wall	[41,47]
Isolation from plant callus homogenates	PDNVs	Mechanical or enzymatic disruption of callus biomass followed by nanovesicle recovery	Medium	High	Good	Cellular debris and intracellular components	Potentially scalable and controllable source of PDNVs	Does not represent natural vesicle secretion	No direct equivalent in mammalian EV systems	[41]
Plant lipid extraction and membrane reassembly	APNVs	Extraction of plant-derived lipids followed by artificial reconstruction of vesicular membranes	Very high	Variable	Good	Residual lipids or extraction reagents	Enables controlled cargo loading and therapeutic engineering	Complex process requiring advanced engineering strategies	More comparable to artificial liposomes than native mammalian EVs	[47,53]

3.5 Characterization of Plant-Derived Extracellular Vesicles

To evaluate the physicochemical properties of plant-derived extracellular vesicles, several characterization techniques are commonly employed. These methods allow the determination of various parameters, including particle size, concentration, morphology, zeta potential, and size distribution. Among the most widely used approaches are nanoparticle tracking analysis (NTA) [54], dynamic light scattering (DLS), transmission electron microscopy (TEM) [55], scanning electron microscopy (SEM) [56], atomic force microscopy (AFM) [55], and tunable resistive pulse sensing (TRPS) [57,58].

Each technique presents specific advantages but also important limitations. NTA enables the individual measurement of nanoparticle size and concentration in suspension, whereas DLS provides a rapid estimation of particle size distribution based on light scattering properties. Electron microscopy techniques (TEM and SEM) allow direct visualization of particle morphology, while AFM provides three-dimensional information regarding particle surface topography and mechanical properties. TRPS, on the other hand, enables accurate measurement of particle size and concentration based on the passage of individual particles through a nanopore.

To date, no single method can definitively confirm the identity and biogenetic origin of extracellular vesicles. Therefore, recent recommendations from the International Society for Extracellular Vesicles (ISEV) advocate for a multiparametric approach combining physical, morphological, biochemical, and functional analyses [22]. Furthermore, when nanoparticles are obtained from homogenized plant tissues, the term plant-derived nanovesicles (PDNVs) is preferred over plant extracellular vesicles (PEVs), as it better reflects the heterogeneous nature of these preparations and the lack of direct evidence confirming their biogenesis.

3.6 Physiological and Pathological Roles of Plant-Derived Nanovesicle Vesicles in IBD

Plant-derived extracellular vesicles have emerged as potential modulators of intestinal homeostasis and promising therapeutic candidates for inflammatory bowel disease (IBD), owing to their reported effects on immune responses, epithelial-barrier integrity [59], inflammatory signalling, and the gut microbiota [17,60]. More broadly, edible plant-derived nanovesicles have been proposed to mediate interspecies communication [61] through both direct interactions with mammalian cells, as suggested by in relation to grape-derived nanoparticles and plant miRNA-mediated regulation of mammalian targets [14], and indirect effects mediated through the gut microbiota. Particularly, PDNVs from garlic, “altered the gut microbiota profile of colitis mice by recovering the relative abundance of Lachnospiraceae and reducing the relative abundance of *Helicobacter*” [62]. Additionally, the bioinformatic sequence similarity reported by Mu et al. between vvi-miR169 and human miRNAs could be interpreted as potential evidence of functional cross-kingdom regulation [14]. Therefore, lamina propria macrophages continuously sample the intestinal environment. Subsequently, by phagocytosis and endocytosis [63] and following internalization and degradation of PDNVs, plant RNAs, particularly microRNAs, can interact with the host gene regulatory machinery and modulate the expression of genes involved in metabolism and immune responses: for instance, miR-159, miR-166, ref. [51,64], miR-146 [59], miR-8126 [65], miR-2911 [66], the miR-482 family [67].

PDNVs possess characteristic lipid and protein compositions that may influence their interactions with intestinal epithelial cells [59], stem cells [68], macrophages [60], and the gut microbiota [60,68]. Tetraspanins organize specialized membrane microdomains involved in vesicle biogenesis and cargo sorting [69,70]. In plants, TET8 has been implicated in EV biogenesis and in the sorting and trafficking of glycosyl-inositol-phosphoceramides (GIPCs), supporting a role for TET8–lipid interactions in plant EV production [71]. Functionally, plant TET8 belongs to the broader tetraspanin family being a homolog to mammalian EV-associated proteins such as CD9, CD63, and CD81 [69]. Following uptake through mechanisms including

endocytosis, macropinocytosis, phagocytosis, or potentially membrane fusion, plant-derived nanovesicles may deliver molecular cargo to recipient cells [72]. Experimental studies have reported the internalization of plant-derived vesicle preparations by mammalian cells [41] and biological effects of miRNA-containing plant vesicles on mammalian tumour-cell proliferation and apoptosis [64,65]. Moreover, recent investigations have shown that specific edible plant miRNAs can modulate metabolic and inflammatory responses in human-cell models, including lipid accumulation in hepatocytes and pro-inflammatory gene expression in THP-1 macrophages [65,67,73]. Together with reports proposing plant miRNA–mammalian gene interactions [14], plant-derived vesicle–microbiota–host communication composition [62,65], and the use of plant EVs as potential carriers for exogenous miRNAs [51], these findings support the potential of plant-derived nanovesicles and their molecular cargo to participate in cross-kingdom communication. Nevertheless, the extent to which plant miRNAs remain intact [67], are delivered to specific mammalian cells, and exert physiologically relevant effects following oral administration remains under investigation. A summary of PDEV cargo molecules and their biological functions in IBD is presented in Table 3.

Table 3: Overview of plant-derived EV cargo molecules and their biological functions in IBD.

Cargo Category	Examples Identified in PDEVs	Biological Function in IBD
Proteins	Enzymes, signaling proteins, immune regulators	Regulation of inflammation and cellular communication
Lipids	Phospholipids, fatty acids, membrane components	Vesicle stability, cell targeting, immune modulation
mRNA	IL-22 mRNA, Foxa2 mRNA	Regulation of epithelial regeneration and microbial adaptation
miRNA	miR-4057 miR-159, miR-166, miR-146, miR-8126, the miR-482 family.	Post-transcriptional regulation of inflammatory genes
Secondary metabolites	Curcuminoids, phenolic compounds	Antioxidant and anti-inflammatory effects
RNA-lipid complexes	Ginger-derived lipid nanoparticles	Drug delivery and targeted therapeutic applications

4 Etiology and Pathogenesis of IBD

4.1 Genetic Factors

Inflammatory bowel disease (IBD) can have either a monogenic or a polygenic genetic basis [74]. Monogenic forms, which are more frequently seen in cases of early-onset disease, are caused by mutations in genes that play a role in immune regulation and intestinal homeostasis. Examples of such genes include IL10RA/IL10RB, XIAP, CYBB, LRBA and TTC7A [75]. These forms are often linked to severe clinical symptoms and resistance to conventional treatments [76]. Conversely, the majority of IBD cases have a polygenic background involving multiple genes related to innate immunity, autophagy and cytokine signaling pathways. The most well-characterised susceptibility genes include NOD2 [70] and autophagy-related genes such as ATG16L1 and IRGM [77]. Variants in genes involved in interleukin signaling pathways, including STAT3, TYK2 and JAK2, together with PTPN2, have also been implicated in disease predisposition [78]. Other genes that contribute to IBD pathogenesis include OCTN1/Slc22a4 [79], which may influence the transport of bacterial molecules in the gut; CARD9, which is a key mediator of innate immune activation through the MAPK and NF- κ B pathways [80] and TNFSF15, which encodes the cytokine TL1A and interacts with its receptor DR3 (TNFRSF25) to regulate intestinal inflammatory responses [81].

4.2 Environmental Factors

Environmental exposures are increasingly recognized as important modulators of inflammatory bowel disease (IBD) risk. The exposome is the concept that encompasses the cumulative environmental influences encountered throughout life that may affect disease susceptibility and progression [3]. Early-life exposures appear to be particularly relevant. For example, factors such as maternal smoking [82], diet [83], infections [84], not breastfeeding and reduced microbial exposure [85] may alter immune system maturation and influence the development of the gut microbiota, thereby increasing the risk of intestinal inflammation. Throughout life, cumulative environmental exposures may further influence microbial diversity and immune responses. These include the use of antibiotics such as metronidazole and fluoroquinolones as well as other medications like oral contraceptive pills. In addition, various infections (bacterial, viral, and parasitic), psychiatric conditions such as mood disorders, and urban living have been associated with alterations in immune regulation and gut microbiota composition. Certain surgical procedures, including appendectomy and tonsillectomy, have also been investigated for their potential role in modifying the risk of developing IBD. In adulthood, additional environmental factors such as air pollution [86], smoking [87], sleep disturbances [88], and water contamination with different pollutants such as, antagonist of steroid receptor [89], or metal elements [90], have been linked to an increased risk of IBD. Also, epigenetic modifications in intestinal epithelial cells have been linked to dietary patterns characteristic of Western diets, which are marked by low consumption of fibre, fruits, and vegetables, limited micronutrient intake, and high fat content. These alterations include the reduced expression of certain microRNAs (miRNAs), such as miR-143/145a, miR-148a and miR-152, in colonocytes, which may exacerbate colonic inflammation [91].

4.3 Gut Microbiota Factor

The intestinal microbiome plays a fundamental role in maintaining gut homeostasis and regulating immune responses, and its disruption represents a key mechanism in the pathogenesis of IBD. In healthy individuals, the gut ecosystem is dominated by diverse bacterial communities primarily belonging to the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria that contribute to metabolic functions, epithelial barrier maintenance, and immune modulation [92]. These microorganisms produce metabolites such as short-chain fatty acids (SCFAs), particularly butyrate, which support epithelial integrity and exert anti-inflammatory effects. In IBD, however, this balanced ecosystem becomes disrupted, leading to microbial dysbiosis, characterized by reduced microbial diversity, depletion of beneficial commensal bacteria, and expansion of potentially pathogenic taxa. Beneficial bacteria such as *Faecalibacterium prausnitzii*, *Bifidobacterium*, *Lactobacillus*, and other SCFA-producing Firmicutes are typically reduced, while pro-inflammatory microorganisms including adherent-invasive *Escherichia coli* [93], *Klebsiella* [94], and sulfate-reducing bacteria such as *Desulfovibrio* become much abundant [95]. These microbial alterations contribute to intestinal inflammation by promoting immune activation, increasing epithelial permeability, and disrupting the mucosal barrier. Beyond bacterial communities, changes also occur in other components of the intestinal ecosystem, including the virome and mycobiome. Increased abundance of bacteriophages belonging to the Caudovirales order [96] and shifts in fungal populations particularly increased *Candida* [97] and *Malassezia* [98] species have been associated with enhanced mucosal immune activation and Th17-mediated inflammatory responses. These alterations further destabilize microbial communities and amplify immune dysregulation. Collectively, these changes in microbial composition and function interact with host genetic susceptibility, environmental exposures, and immune responses, ultimately disrupting intestinal homeostasis and contributing to the initiation and perpetuation of chronic inflammation characteristic of IBD [99].

4.4 Immunological Factors

Inflammatory bowel disease (IBD) result from an abnormal immune response to normal luminal stimuli in genetically predisposed individuals, characterized by dysregulation of mucosal immunity, impairment of the intestinal epithelial barrier, and excessive activation of immune responses, as demonstrated by clinical studies and animal models [100]. At the cellular level, the lamina propria predominantly contains CD4⁺ and CD8⁺ T lymphocytes, with an increased population of CD45RO⁺ memory T cells in the intestinal mucosa of patients with IBD [101]. These T cells express activation markers (IL-2R, CD98, HLA-DR) and produce numerous cytokines, thereby contributing to the regulation of immune responses [102]. CD has historically been associated with Th1-dominated responses, although it is now understood to also involve Th17/IL-23-driven inflammation), whereas UC primarily involves Th2-type responses (IL-4, IL-10), reflecting distinct immune profiles depending on the type of IBD [100]. The Th3 subpopulation, which produces TGF- β , plays a key role in oral tolerance and in the regulation of Th1 responses [103]. Finally, IBD is characterized by a marked immunoregulatory imbalance, with excessive production of pro-inflammatory cytokines (TNF- α , IL-1, IL-6, IFN- γ) and infiltration of lymphocytes, macrophages, and polymorphonuclear neutrophils, which are responsible for tissue damage and clinical manifestations [104]. More recently, the IL-23/IL-17 axis has been identified as a key driver of intestinal inflammation, with IL-23 acting upstream to sustain pathogenic Th17 responses and innate immune activation, leading to persistent cytokine production and epithelial barrier dysfunction [105]. Persistent activation of the immune system requires continuous antigenic stimulation derived from pathogenic bacteria, the normal intestinal flora, or dietary antigens, and this interacts with the host's genetic susceptibility to trigger disease onset [94]. According to the model proposed by Sartor, the pathogenesis of IBD progresses through sequential stages: initiating events, epithelial barrier dysfunction, antigen exposure, immunoregulatory abnormalities, tissue injury, and the development of clinical symptoms [100,101].

4.5 Oxidative Stress Factors

Oxidative stress is a key factor in the pathogenesis of ulcerative colitis (U.C.). Under physiological conditions, organisms continuously generate reactive oxygen (ROS) and nitrogen species (RNS) through oxidative metabolism and mitochondrial activity. However, excessive production of these reactive species can cause significant oxidative damage [106]. Moreover, disruption of gut microbiota balance characterized by reduced bacterial diversity and overgrowth of Gram-negative bacteria contributes to high lipopolysaccharide (LPS) production, which in turn promotes increased ROS and RNS generation [107,108]. The accumulation of these reactive species impairs the integrity of colonic epithelial and immune cells, compromises the intestinal barrier, and amplifies the inflammatory response [109,110].

4.6 Intestinal Barrier Disruption Factors

The intestinal mechanical barrier primarily depends on tight junction (TJ) proteins, such as Claudins, Occludin, and ZO-1, which are crucial for maintaining intestinal homeostasis. These proteins regulate the passage of water, electrolytes, and nutrients across epithelial cells [111]. When this barrier is disrupted, intestinal bacteria and antigens can translocate into the mucosal lamina propria, exacerbating tissue damage. This translocation activates local immune cells, triggering elevated production of cytokines and other inflammatory mediators [112]. A schematic representation of the multifactorial etiopathogenesis of IBD is presented in Fig. 3, highlighting the interactions between genetic, immunological, microbiota-related, environmental, oxidative stress, and intestinal barrier disruption factors.

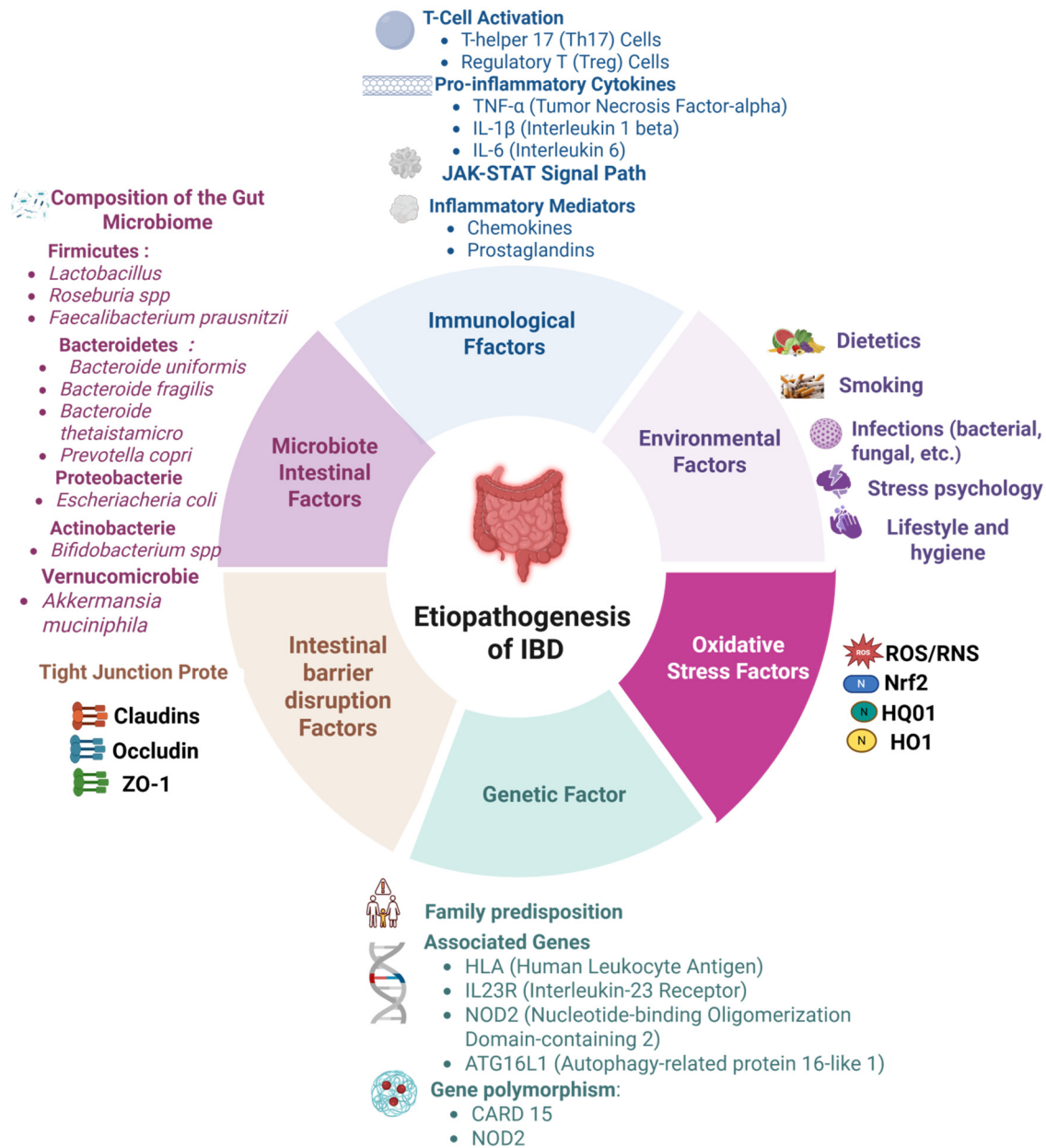


Figure 3: Multifactorial etiopathogenesis of IBD, interplay between genetic, immunological, microbiota, environmental, oxidative stress, and intestinal barrier disruption factors.

5 Functional and Therapeutic Roles of PDEVs in IBD Pathogenesis

Extracellular vesicles (EVs) transport a diverse cargo of bioactive molecules, including proteins, nucleic acids, lipids, and metabolites, which are closely involved in IBD pathogenesis [5]. These components contribute to the maintenance of intestinal homeostasis by modulating immune responses, shaping the gut microbiota, and preserving the integrity of the intestinal barrier. Recent studies indicate that plant derived extracellular vesicles (PDEVs) make a key contribution to IBD [16,112]. They are especially noteworthy because the human gut is continuously exposed to dietary nanoparticles from edible and medicinal plants. In a healthy intestine, the microbiota and the immune system act together to preserve balance by carefully controlling responses to foreign antigens [113]. In this context, we review the main mechanisms and

functions of PDEVs, focusing on their roles in regulating immune activity, repairing the intestinal epithelial barrier, and remodeling the gut microbiota (Fig. 3, Tables 4–7).

5.1 EVs in the Regulation of the Intestinal Barrier

Disruption of the epithelial barrier is a central feature of inflammatory bowel disease (IBD) and contributes to increased permeability, microbial translocation, and sustained inflammation [114–116]. Plant-derived extracellular vesicles (PDEVs) have been shown to counteract these alterations by promoting epithelial repair, supporting junctional stability, and regulating signalling pathways involved in tissue regeneration. Several experimental studies have demonstrated their therapeutic potential. Grape-derived exosome-like nanoparticles can cross the intestinal mucosa in DSS-induced colitis models and activate the Wnt/ β -catenin signaling pathway, promoting Lgr5⁺ intestinal stem cell proliferation and accelerating epithelial regeneration. Similarly, lipid-rich grape-derived extracellular vesicles specifically target intestinal stem cells, enhance organoid formation, and regulate tissue renewal and remodeling while protecting against DSS-induced colitis [68]. Grapefruit-derived EVs have also been shown to attenuate DSS-induced colitis by maintaining intestinal macrophage homeostasis and reducing inflammatory responses, highlighting their immunomodulatory properties [117]. Likewise, vesicles isolated from *Cannabis sativa* L. protect against DSS-induced intestinal hyperpermeability by enhancing barrier-related protein expression, inhibiting NF- κ B activation, and reducing oxidative stress markers [116]. Aloe vera nanovesicles decrease acute colonic inflammation and promote the restoration of junctional proteins, resulting in improved epithelial integrity [118]. Other plant-derived vesicles regulate genes associated with inflammation and epithelial function. Sweet orange (*Citrus sinensis*)-derived vesicles restore barrier-associated pathways by modulating pro-inflammatory genes, including ICAM-1 and HMOX-1, as well as junction-related genes such as OCLN, CLDN-1, and MLCK [119]. Turmeric (*Curcuma longa*)-derived vesicles exert anti-inflammatory effects through MMP-9 inhibition and increased expression of TJP1 and MUC2, thereby supporting mucosal recovery [120]. The RNA cargo of PDEVs may also contribute to epithelial restoration. Ginger (*Zingiber officinale*)-derived vesicles containing mRNA enhance Foxa2 expression in intestinal epithelial cells, promoting functional recovery and cellular protection [15]. Finally, apple derived vesicles enriched in microRNAs regulate intestinal transporter expression, including the reduction of OATP2B1 expression in Caco-2 cells [121].

5.2 EVs in the Immune Response

Immune dysregulation is a central feature of inflammatory bowel disease (IBD), characterized by excessive activation of inflammatory pathways, increased production of pro-inflammatory cytokines, and impaired mechanisms responsible for maintaining immune tolerance. In this context, macrophages, dendritic cells, and T lymphocytes represent major cellular targets involved in the initiation and amplification of intestinal inflammation. Therefore, restoring immune balance represents an important therapeutic objective in IBD management [122]. Plant-derived extracellular vesicles (PDEVs) have emerged as potential immunomodulatory nanoplatforms capable of regulating inflammatory responses through their bioactive cargo, including proteins, lipids, and regulatory RNAs. Several studies have demonstrated their ability to modulate key inflammatory pathways and immune cell functions. Blueberry derived vesicles contain proteins, lipids, and bioactive mRNAs and/or miRNAs that protect intestinal cells against TNF- α -induced oxidative stress and reverse inflammation-associated gene expression changes, including the regulation of IL-6, IL1RL1, MAPK1, ICAM1, TLR8, and TNF expression [123]. Similarly, Tea leaf (*Camellia sinensis*)-derived vesicles contain bioactive lipids and proteins that contribute to the attenuation of intestinal inflammation

by reducing pro-inflammatory cytokine production and promoting IL-10 secretion by macrophages [124]. Ginseng derived exosomes (GEN) exert anti-inflammatory effects through inhibition of the NF- κ B pathway and promotion of macrophage polarization toward the M2 phenotype, thereby favoring immune resolution and tissue recovery [125]. Ginger-derived exosomes (GELN) are efficiently internalized by intestinal cells and reduce LPS-induced inflammatory responses by inhibiting NF- κ B activation and decreasing the expression of inflammatory mediators, including IL-6, IL-8, and TNF- α [126]. Moreover, extracellular vesicles derived from ginger and shiitake (*Lentinula edodes*) inhibit NLRP3 inflammasome activation, reduce IL-1 β and IL-18 secretion, and limit macrophage pyroptosis. Vesicles derived from grapefruit, carrot, and ginger also influence nuclear factor signaling pathways in macrophages, further supporting their role as modulators of immune responses in intestinal inflammation [127].

5.3 EVs in the Regulation of the Gut Microbiota

Gut microbiota imbalance is a major feature of (inflammatory bowel disease) IBD and contributes to disease progression through alterations in microbial composition, reduced beneficial bacterial populations, and increased abundance of potentially harmful microorganisms [128]. Therefore, restoring microbial equilibrium represents an important therapeutic target, and plant derived extracellular vesicles (PDEVs) have recently emerged as potential modulators of microbiota composition and function. Several studies suggest that PDEVs can influence microbial survival, diversity, and metabolite production through their bioactive cargo. Lemon (*Citrus limon*)-derived vesicles containing interleukin-22 (IL-22) mRNA enhance bacterial adaptation under stress conditions by promoting specific tRNA degradation through RNase P activity. Their mRNA and tRNA cargo also improve bile resistance and intestinal persistence of beneficial bacteria, including *Lactobacillus rhamnosus* GG and *Streptococcus thermophilus* ST-21. In addition, these vesicles stimulate IL-22 production through activation of aryl hydrocarbon receptor (AhR) ligands, thereby supporting epithelial protection and regeneration [129]. Similarly, tea leaf-derived vesicles containing miR-4057 promote microbial diversity and beneficial changes in bacterial composition, including an increased Bacteroidetes/Firmicutes ratio and higher total bacterial abundance in fecal samples [124]. Vesicles derived from coconut (*Cocos nucifera*) and Tartary buckwheat (*Fagopyrum tataricum* L. Gaertn) also enhance microbial diversity, increase the abundance of beneficial bacterial species such as *Escherichia coli* and *Lactobacillus rhamnosus*, and promote short-chain fatty acid production, which contributes to intestinal homeostasis [130]. Furthermore, lemon-derived exosome-like nanoparticles (LELN) improve probiotic viability, stimulate IL-22 production, and enhance epithelial repair mechanisms. They also increase lactic acid production while reducing pathogenic bacteria, including *Clostridioides difficile*, suggesting a potential role in restoring microbial balance during intestinal inflammation [131]. Beyond their direct effects on microbiota composition, PDEVs are increasingly investigated as natural delivery platforms capable of transporting therapeutic molecules and bioactive compounds to the intestine. For example, ginger-derived exosome-like nanoparticles (GELN) and their lipids, when reassembled and loaded with doxorubicin (Dox), achieve significantly higher targeted delivery efficiency compared to free Dox. Moreover, this system provides a more favorable pH-dependent drug release profile compared to commercially available doxorubicin [132]. A schematic overview of the functional roles of plant-derived extracellular vesicles (PDEVs) in regulating the gut microbiota, modulating immune responses, and preserving intestinal barrier integrity is presented in Fig. 4. A comprehensive overview of the roles of plant-derived extracellular vesicles (PDEVs) in IBD, including intestinal barrier modulation, immunomodulatory effects, gut microbiota regulation, and therapeutic mechanisms, is presented in Tables 4–7.

Table 4: Plant-derived extracellular vesicles involved in intestinal barrier modulation.

Plant Source	Vesicle Type	Main Bioactive Cargo	Disease Model/Experimental System	Molecular Targets and Signaling Pathways	Main Therapeutic Effects	Ref.
Grape	Grape-derived exosome-like nanoparticles (GELNs)	Lipids, proteins, RNAs	DSS-induced colitis mouse model; intestinal organoids	Activation of Wnt/ β -catenin pathway; stimulation of Lgr5 ⁺ intestinal stem cells	Promotes epithelial regeneration, enhances organoid formation, accelerates mucosal repair	[68]
Grapefruit	Grapefruit-derived EVs	Lipids, proteins, RNAs	DSS-induced colitis mice	Regulation of macrophage homeostasis; inhibition of inflammatory signaling	Maintains intestinal macrophage balance and reduces inflammation	[117]
<i>Cannabis sativa</i> L.	Cannabis-derived nanovesicles	Bioactive lipids and proteins	DSS-induced intestinal permeability model	Decrease NF- κ B activation; decrease oxidative stress markers; Increase tight junction proteins	Restoration of epithelial barrier integrity and reduced permeability	[116]
<i>Aloe vera</i>	Aloe-derived nanovesicles	Lipids, proteins, small RNAs	DSS-induced acute colitis mice	Regulation of tight junction and adherens junction proteins	Reduces inflammation and restores epithelial barrier function	[118]
<i>Citrus sinensis</i> (Sweet orange)	Orange-derived EVs	RNAs, lipids, proteins	Intestinal epithelial cell models	Regulation of ICAM-1, HMOX-1, OCLN, CLDN-1 and MLCK expression	Restoration of epithelial barrier function	[133]
Turmeric	Turmeric-derived vesicles	Curcuminoid-associated cargo, RNAs	Intestinal inflammation models	Decrease MMP-9; Increase TJP1 and MUC2 expression	Enhances mucosal integrity and reduces inflammation	[120]
<i>Malus domestica</i> (Apple)	Apple-derived vesicles	miRNAs	Caco-2 cell model	Regulation of intestinal transporter expression (OATP2B1)	Modulates epithelial transport functions	[121]

Table 5: Immunomodulatory effects of plant-derived extracellular vesicles in inflammatory bowel disease.

Plant Source	Vesicle Designation	Cargo Molecules	Experimental Model	Immune Targets	Signaling Pathways Affected	Biological Effects	Ref.
Blueberry	Blueberry-derived EVs	Proteins, lipids, mRNA/miRNA	TNF- α stimulated intestinal cells	IL-6, IL1RL1, MAPK1, ICAM1, TLR8, TNF genes	Regulation of inflammatory gene expression	Protects cells against oxidative stress and inflammatory activation	[123]
<i>Camellia sinensis</i> (Tea)	Tea-derived EVs	Lipids and proteins	Colitis and colitis-associated cancer models	Macrophages, cytokines	Decrease Pro-inflammatory cytokines; Increased IL-10	Suppresses inflammation and promotes immune tolerance	[134]
Ginseng	Ginseng-derived exosomes (GEN)	Proteins, RNAs	Inflammatory models	Macrophage polarization	Decrease NF- κ B activation; M1 $\rightarrow$ M2 polarization	Restores macrophage homeostasis	[125]

Table 5: Cont.

Plant Source	Vesicle Designation	Cargo Molecules	Experimental Model	Immune Targets	Signaling Pathways Affected	Biological Effects	Ref.
Ginger	Ginger-derived exosome-like nanoparticles (GELNs)	Lipids, RNAs	LPS-induced inflammatory macrophages	NF- κ B, IL-6, IL-8, TNF- α	Inhibition of inflammatory response	Reduces cytokine production	[126]
Ginger + Shiitake (<i>Lentinula edodes</i>)	Plant-derived EVs	RNAs, proteins	Macrophage models	NLRP3 inflammasome	Decrease IL-1 β , IL-18 secretion; decrease pyroptosis	Limits inflammatory macrophage activation	[127]

Table 6: Modulation of intestinal microbiota by plant-derived extracellular vesicles.

Plant Source	Vesicle Cargo	Microbiota Model/Disease Context	Molecular Mechanism	Microbial Changes	Therapeutic Outcome	Ref.
<i>Citrus × limon</i> (Lemon)	IL-22 mRNA, RNAs	Gut microbiota models	Activation of AhR pathway; RNase P-mediated tRNA degradation	Increased survival of <i>Lactobacillus rhamnosus</i> GG and <i>Streptococcus thermophilus</i> ST-21	Improved bacterial adaptation and intestinal protection	[129]
<i>Camellia sinensis</i> (Tea)	miR-4057	Mouse microbiota model	Regulation of microbial composition	Increased microbial diversity; Increased Bacteroidetes/Firmicutes ratio	Improved microbiota balance	[124]
Coconut	Lipids, RNAs	Gut microbiota models	Microbial community regulation	Increased beneficial bacteria and short-chain fatty acid (SCFA) production	Improved intestinal homeostasis	[56]
Tartary buckwheat	Bioactive RNAs and lipids	Gut microbiota models	Microbiota remodeling	Increased microbial diversity and SCFA levels	Supports intestinal health	[56,130]
<i>Citrus × limon</i> (Lemon)	Lemon-derived exosome-like nanoparticles (LELN)	Microbial infection models	AhR activation; probiotic support	Increased <i>Lactobacillus</i> ; Decrease <i>Clostridioides difficile</i>	Enhanced epithelial repair and probiotic survival	[131]

Table 7: Therapeutic mechanisms of PDEVs in IBD pathogenesis.

Biological Process Affected	Main Mechanisms	Representative PDEVs
Intestinal barrier restoration	Increased Claudins, Occludin, ZO-1, MUC2; epithelial regeneration	Grape, Aloe vera, Cannabis, Turmeric
Immune regulation	decreased NF-κB, MAPK; macrophage M1/M2 reprogramming	Ginger, Ginseng, Tea, Onion
Oxidative stress control	Reduction of ROS and inflammatory mediators	Blueberry, Cannabis
Microbiota remodeling	Increased beneficial bacteria, Increased short-chain fatty acid (SCFA) production	Lemon, Tea, Coconut
Drug delivery applications	Natural nanocarriers for therapeutic molecules	Ginger-derived EVs loaded with doxorubicin

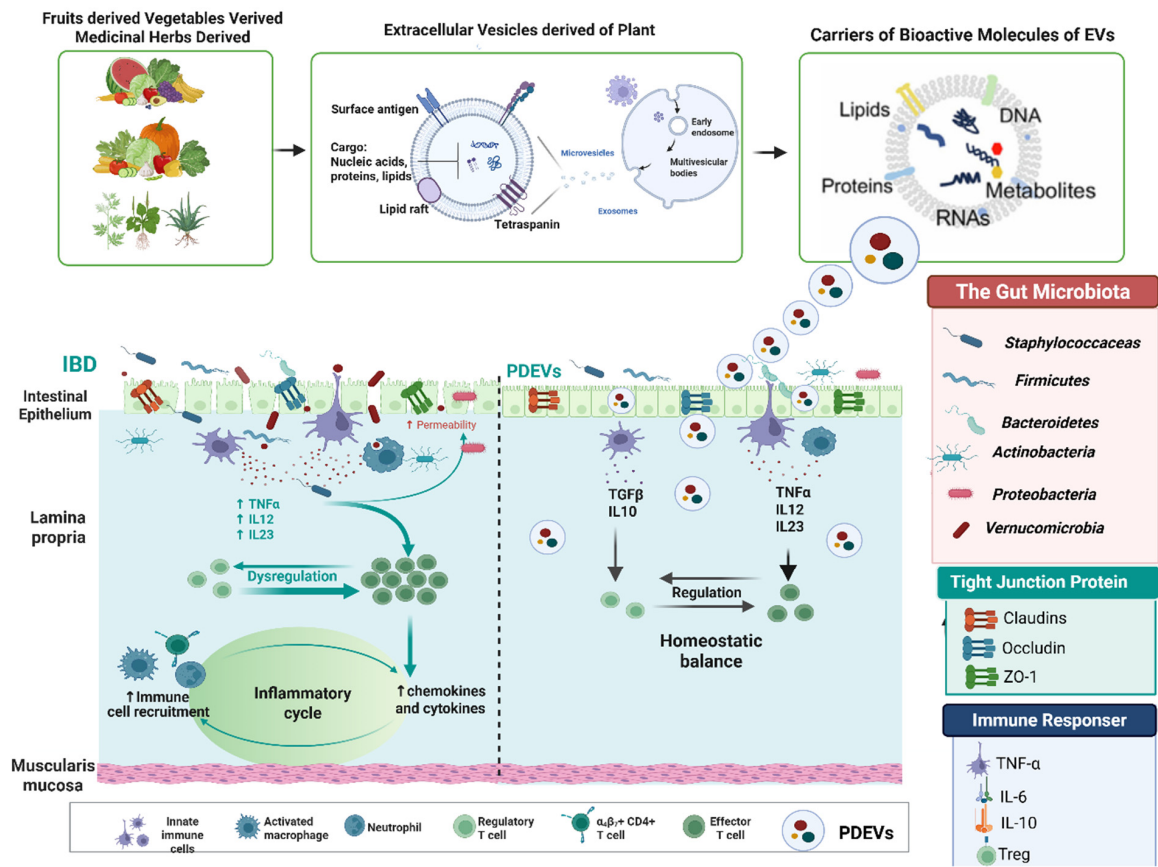


Figure 4: Functional of PDEVs in regulating gut microbiota, immune response, and intestinal barrier integrity. PDEVs, composed of bioactive biomolecules such as lipids, proteins, RNAs, and metabolites, play an essential role in maintaining intestinal homeostasis. They have the potential to restore intestinal imbalance by reshaping microbial communities, modulating the production of pro- and anti-inflammatory cytokines, and strengthening tight junction proteins within the intestinal epithelium, thereby helping to preserve barrier integrity and promote immune regulation and intestinal dysbiosis.

6 Preclinical Evaluation of the Therapeutic Potential of Plant Derived Extracellular Vesicles (PDEV) on the Colorectal Inflammatory Process

In recent years, many experiments have been conducted using plant derived extracellular vesicles (PDEV)s on colitis and colo-rectal cancer models, both from edible and medicinal plants [30]. Their therapeutic potential arises not only from their natural cargo load, but also from the ability to be loaded with certain therapeutic agents to enable more targeted delivery [7].

6.1 Acute Inflammation Models

Although the acute inflammatory process is an immediate defense response of the organism, its runaway escalation in the colon requires strict therapeutic regulation; in this context, plant derived extracellular vesicles (PDEVs) emerge as an innovative solution thanks to their properties. By far most of the studies have been focused on effects of EVs in models of acute inflammation. *Portulaca oleracea*-derived exosome-like nanoparticles were found to alleviate DSS-induced colitis in C57BL/6 mice by limiting the expression of pro-inflammatory cytokines (IL-6, IL-12, IL-1 β , TNF- α) and of myeloperoxidase (MPO), along with increasing levels of anti-inflammatory cytokines (IL-10). A remodelling of the gut microbiota was also observed [61]. In another model of acute murine colitis, *Prunus mume*-derived vesicles were shown to inhibit the activation of NLRP3 inflammasome activation in macrophages, thus reducing the intensity of inflammation [135]. *Centella asiatica*-derived exosomes were also shown to reduce the negative effects of acute DSS exposure in mice, the main roles of regulating the immune response and of modulating the gut microbiota being attributed to specific miRNAs [136]. Turmeric-derived nanoparticles (TDNPs) containing curcumin were proven to minimise inflammation by decreasing concentrations of pro-inflammatory cytokines (TNF- α , IL-6, IL-1 β) and increased expression of the antioxidant gene HO-1 [137]. TDNPs prevented LPS-induced inflammation, while also specifically targeting the inflamed colon [138]. Ginger-derived nanoparticles were shown to prevent acute colitis, confirmed histologically and corroborated with colonic levels of MPO and an increased expression of E-cadherin [134].

They also were found to promote macrophage polarization towards the M2 phenotype, along with reducing expression of pro-inflammatory cytokines (IL-6, IL-1 β) and increasing levels of TGF- β and IL-10. In a murine DSS-induced colitis model, pretreatment with grapefruit-derived nanovesicles reduced disease severity by promoting the anti-inflammatory phenotype of intestinal macrophages, as evidenced by increased expression of IL-10 and HO-1 [117]. Grape exosome-like nanoparticles are able to promote proliferation of Lgr5hi intestinal stem cells through the Wnt/ β -catenin signaling pathway, an essential feature in protecting mice from DSS-induced colitis [68]. Broccoli-derived nanoparticles were also found to have a beneficial effect by modulating pro-inflammatory mediators, by lowering the rate of monocyte recruitment, and by inducing tolerogenic intestinal dendritic cells (DCs) via the AMPK pathway [16]. Bitter melon (*Momordica charantia*)-derived EVs have shown potential in reducing colitis by regulating the macrophage response in inflammation, scavenging ROS and protecting cells and mitochondria from oxidative damage, thus reducing mitochondria-dependent apoptosis [139]. Red cabbage (*Brassica oleracea* var. *capitata* f. *rubra*)-derived EVs (Rabex) were shown to inhibit the inflammatory action of macrophages and stimulate the proliferation of epithelial cells in a murine DSS colitis model. Engineering Rabex with hyaluronic acid proved more effective in targeting intestinal epithelial cells [140].

In the realm of medicinal plants, *Taxus chinensis*-derived nanovesicles have been shown to reduce inflammation in an acute colitis model. Gong et al. have found that nanovesicles extracted from the leaves reduced lesions by inhibiting the TLR4/MyD88/NF- κ B pathway activation, and consequently inhibiting the expression of pro-inflammatory cytokines [139]. In another study, *Sophora flavescens* exosome-like

nanoparticles were found to inhibit the *in vitro* proliferation of macrophages, as well as polarizing them towards the M2 phenotype, thus significantly decreasing pro-inflammatory factors (TNF- α , IL-6) [141].

6.2 Chronic Inflammation Models

Unlike acute inflammation, colorectal chronic inflammation models remain largely underexplored in the scientific literature due to the high logistical cost and biological complexity of long-term models, which involve adaptive immune mechanisms that are much more difficult to standardize. The long-term evaluation of plant derived extracellular vesicles (PDEVs) thus relies on specific models, notably genetically modified mice, to understand their impact against persistent pathology, to assess their capacity to induce sustainable immune regulation, and to potentially prevent colonic tumorigenesis [139,141,142]. Administration of *Portulaca oleracea*-derived exosome-like nanoparticles prevented the development of acute DSS-induced colitis in IL-10^{-/-} mice. The therapeutic effect was accompanied by the reprogramming of CD4⁺ T cells into CD4⁺CD8⁺ double-positive T cells, leading to a marked reduction in intestinal inflammation [61]. Another model that used IL-10 knockout mice showed that ginger-derived nanoparticles could prevent chronic colitis, the treatment group exhibiting reduced histologic evidence of inflammation and lower expression of pro-inflammatory cytokines (TNF- α , IL-1 β). The same study suggests that ginger-derived nanoparticles have a potential of preventing colitis-associated cancer (CAC) by regulating a series of proteins thought to be involved in the genesis of this type of neoplasm [132].

6.3 Effects on Tumor Models

Beyond their anti-inflammatory properties, PDEVs are garnering growing interest in preclinical oncology, particularly in gastrointestinal cancers. Raimondo et al. found that *Citrus x limon*-derived nanovesicles could slow down the growth of human colorectal adenocarcinoma cells *in vitro*, as well as that of tumor xenografts *in vivo*, via TRAIL-mediated apoptosis and by inhibiting the secretion of cytokines involved in neoangiogenesis. These events seem to be the result of the downregulation of Acetyl-CoA Carboxylase 1 (ACACA), which inhibits lipid metabolism in neoplastic cells [143]. Lemon-derived nanovesicles also showed an effect on gastric cancer cells and a mouse tumor model, by generation of ROS and consequent S-phase arrest of the cell cycle followed by apoptosis [144]. Corn (*Zea mays*)-derived nanoparticles were observed to reduce the growth of colon26 cell line *in vitro*, as well as suppressing tumor growth in mice *in vivo*. The antitumoral effect was noted to be a direct one via G2-phase cell cycle arrest, and an indirect one by activating infiltrating leukocytes (macrophages especially), and thus by production of TNF- α [145]. Apart from the direct antitumoral use of PDEVs, some extracts show promise as an adjuvant treatment alongside chemotherapeutic drugs. In a study, *Catharantus roseus* exosome-like nanovesicles were shown to reduce cyclophosphamide-induced immunosuppression via the TNF- α /NF- κ B/PU.1 axis, by modulating hematopoietic stem cell differentiation and decreasing the known negative effects of cell cycle arrest by the chemotherapeutic drug used [145].

7 PDEVs as Nanocarriers for Drug Delivery in IBD

Over the past decade, nanoparticle-based drug delivery systems have emerged as promising alternatives to conventional therapies by improving drug stability, reducing toxicity, and enabling targeted delivery. Among them, PDEVs have gained increasing attention due to their biocompatibility, low toxicity, and natural targeting properties. However, their clinical translation remains limited by challenges such as heterogeneity, lack of standardization, unclear biodistribution and *in vivo* behavior, as well as difficulties in large-scale production, drug loading efficiency, dose control, and regulatory constraints [146].

7.1 Oral Administration of PDEVs for Drug Delivery

The choice of administration route for PDEVs plays a key role in their therapeutic efficacy. Among the available options, the oral route is often preferred because it is simple, non-invasive, and well accepted by patients. However, the gastrointestinal tract is not a benign environment: It is characterized by pH variations and the presence of digestive enzymes. These conditions can degrade active compounds, reduce intestinal absorption and bioavailability, and increase the risk of side effects [147]. This is where PDEVs emerge as promising carriers for oral drug delivery. Thanks to their lipid bilayer structure, they are highly resistant to gastrointestinal conditions and effectively protect the molecules they encapsulate. In addition, their natural origin confers excellent biocompatibility and a favorable safety profile. However, the mechanisms by which they cross intestinal barriers and target specific cells remain poorly understood and require further investigation [148]. Previous studies suggest that the oral route influences their distribution, stability, and biological activity, which may explain their resistance in the digestive environment. For example, silymarin (*Silybum marianum*)-derived vesicles have shown anti-inflammatory and antioxidant effects by reducing the expression of TNF- α , IL-1 β , NF- κ B, and MPO, as well as lipid peroxidation and protein carbonylation in a TNBS-induced colitis model. Zhang M. and colleagues reported that orally administered ginger-derived vesicles remain highly stable in simulated gastrointestinal fluids and are efficiently taken up by intestinal epithelial cells and macrophages [15]. Moreover, ginger-derived nanovesicles loaded with siRNA successfully delivered siRNA-CD98 to the intestine, resulting in a significant downregulation of CD98 gene expression in colonic cells [149]. Similarly, grapefruit-derived vesicles maintained high stability in acidic conditions and simulated gastrointestinal fluids, demonstrating strong resistance to enzymatic degradation [150].

7.2 Drug Loading Methods

PDEVs are highly promising drug delivery vectors due to their lipid bilayer structure, which enables the encapsulation of both hydrophobic and hydrophilic compounds while protecting their cargo from gastrointestinal degradation. Several loading strategies have been developed for these vesicles, including simple incubation, sonication, electroporation, freeze–thaw cycles, and co-extrusion. These methods facilitate the efficient incorporation of therapeutic agents, including small-molecule drugs, biological agents, and nucleic acids such as siRNA and miRNA. Simple incubation is the most straightforward and cost-effective method. It requires minimal equipment and technical expertise while preserving the structural integrity and biological functions of EVs. However, its main limitation is a low loading efficiency, particularly for hydrophilic molecules. In addition, this method is restricted to compounds capable of passive diffusion into EVs, and residual transfection reagents may affect encapsulation efficiency and alter the behavior of modified EVs [151,152].

Sonication is suitable for both hydrophobic and hydrophilic molecules by enhancing cargo entry through temporary permeabilization of EV membranes. This method uses ultrasonic waves to induce cavitation in the liquid medium, briefly disrupting vesicular membranes and facilitating drug uptake. However, it requires careful optimization of operational parameters, as it may lead to EV damage, aggregation, and partial loss of functionality [153,154]. Electroporation is widely used for loading nucleic acids and macromolecules. It offers high loading efficiency, particularly for RNA, proteins, and certain small molecules. Nevertheless, this technique may induce structural alterations, vesicle aggregation, and morphological changes. Moreover, its efficiency varies depending on the type of cargo, requiring careful optimization to preserve EV integrity [155,156]. Freeze–thaw cycles represent a simple and widely used loading method, with satisfactory efficiency for certain compounds and relatively good vesicle stability. This technique

involves freezing a mixture of EVs and drug, followed by thawing. This process creates temporary pores in vesicular membranes, facilitating drug entry. However, repeated cycles may compromise EV integrity, leading to increased particle size, reduced surface charge, lipid degradation, and greater heterogeneity [157]. Co-extrusion is an efficient loading method that ensures uniform size distribution and high encapsulation efficiency, particularly for hydrophobic molecules. It involves simultaneously extruding the drug and EVs through calibrated membrane pores. However, this technique requires specialized equipment and may result in vesicle loss during the process [158,159].

7.3 Drug-Loaded PDEVs in IBD Applications

PDEVs are increasingly being investigated as versatile delivery platforms for therapeutic molecules in IBD. Their intrinsic biological properties and ability to interact with intestinal cells make them attractive candidates for enhancing the local delivery and efficacy of therapeutic agents.

Among them, acerola (*Malpighia emarginata*)- and ginger-derived vesicles have been particularly well studied as drug delivery systems. Acerola vesicles, for instance, have shown relatively good efficiency in transporting microRNAs such as hsa-miR-340, thereby reducing the expression of specific target genes in the intestine. Their loading efficiency remains moderate, at around 60% [160]. Ginger-derived vesicles demonstrate superior loading capacity and greater therapeutic versatility. They efficiently encapsulate bioactive compounds such as 6-shogaol, improving its sustained release and uptake by macrophages, thereby enhancing its anti-inflammatory effects in models of ulcerative colitis [132]. Moreover, they achieve an impressive loading efficiency for chemotherapeutic agents such as doxorubicin, reaching nearly 96%, highlighting their potential for colorectal cancer therapy [150]. Grapefruit-derived vesicles are particularly valuable for intestinal disorders due to their strong targeting ability. They have been used to deliver methotrexate directly to intestinal macrophages, thereby modulating the inflammatory response in a targeted manner [117]. They have also been employed to transport doxorubicin and curcumin directly to colonic tissue, showing promising results in inflammatory colitis model. Finally, orange-derived vesicles have been used to deliver messenger RNA into T cells, opening new avenues for immunomodulatory strategies in inflammatory diseases [161].

8 Toxicity of PDEVs

PDEVs originate from edible plants, herbs, or plant tissues, therefore, they are generally considered to exhibit low toxicity according to current literature [162]. However, this assumption should be interpreted cautiously, as the toxicological profile of these vesicles may vary depending on several factors, including the route of administration, dosage, and the plant source from which they are isolated [163]. For example, intravenous administration of tea flower-derived PDVLNs resulted in significantly increased blood levels of ALT (Alanine Aminotransferase) and AST (Aspartate Aminotransferase) compared with both orally treated and control groups, suggesting potential hepatotoxicity associated with intravenous delivery. In contrast, repeated oral administration of the same vesicles appeared to be relatively safe [163]. To evaluate their potential toxic effects, studies have assessed the cytotoxicity of plant-derived extracellular vesicle extracts using both *in vitro* and *in vivo* experimental models. Among the different fractions of PDEVs, plant-derived exosome-like nanoparticles appear to represent a more defined subpopulation and have consistently demonstrated relatively low cytotoxicity in both experimental settings [164]. In the context of IBD therapy, PDEVs follow a similar trend reported in the broader literature, exhibiting comparatively weak cytotoxic effects when evaluated alongside other therapeutic approaches [163]. In contrast, the toxicological profile of engineered nanoparticles is often associated with mechanisms such as oxidative

stress induction and DNA damage [26,163]. Moreover, synthetic nanomaterials may provoke undesirable immune responses. For example, animal studies have shown that mice treated with liposomes exhibit increased production of pro-inflammatory cytokines [12], a process that may aggravate the progression of IBD [163]. Unlike engineered nanoparticles, which are frequently associated with higher cytotoxicity, PDVLNs demonstrate improved tissue-targeting capabilities and generally lower toxicity [165]. Their biological activity is partly attributed to the presence of bioactive molecules derived from plants, including microRNAs with immunomodulatory properties [20].

Beyond their biological activity, plant-derived nanoparticles exhibit enhanced stability and broader potential applications compared with many conventional plant-based products. Evidence from *in vivo* experiments further supports their favorable safety profile. Oral administration of grapefruit-derived PDVLNs did not produce detectable local or systemic adverse effects, supporting their favorable safety profile [166]. Likewise, cabbage-derived PDVLNs (cabex) exhibited no significant cytotoxicity or other adverse effects in either mouse or human cells, further highlighting the low toxicity of plant-derived vesicles. Earlier research also indicated that orally administered PDVLNs did not alter serum levels of liver enzymes such as AST and ALT, nor did they trigger immunogenic or toxic responses [163]. Due to their high biocompatibility [167], low toxicity, and favorable immune tolerance, PDVLNs therefore present several advantages over synthetic drug delivery systems and are increasingly considered promising candidates for biomedical applications. In addition to their low cytotoxicity, PDEVs may also exert cytoprotective effects by protecting cells from damage induced by harmful molecule [168]. Growing evidence supports the cytoprotective potential of PDEVs and plant bioactive compounds in biomedical applications. For example, ginger has demonstrated hepatoprotective effects against liver damage induced by ethanol, acetaminophen, and carbon tetrachloride [48,169,170]. In the same manner, carrot-derived EVs (Carex) significantly reduced ROS production and apoptosis in cardiomyoblast and neuroblastoma cell models, while showing minimal cytotoxicity in H9C2 and SH-SY5Y cells. These vesicles also prevented the downregulation of antioxidant-related molecules such as Nrf-2, HO-1, and NQO-1 [168,171]. Similarly, grapefruit-derived nanovectors have shown high biocompatibility and low toxicity *in vitro*, inducing lower apoptosis levels in macrophages and colon-26 cells compared with conventional DC-Chol/DOPE liposomes [20]. Further studies demonstrated their ability to promote mucosal tissue regeneration in mouse models of colitis [127]. Additionally, plant-derived EVs from carrots, grapes, grapefruit, and ginger have been shown to regulate intestinal intercellular communication and contribute to intestinal homeostasis [14]. The safety of plant-derived nanovesicles has been extensively evaluated in studies investigating their application as drug delivery platforms. Wang et al. demonstrated that tumor-targeted grapefruit-derived nanovectors (GNVs) exhibited favorable biodistribution, with reduced accumulation in the liver and spleen, potentially minimizing systemic toxicity while improving therapeutic delivery. Histopathological examination of the heart, liver, spleen, lungs, and kidneys revealed no significant tissue alterations compared with control animals, supporting the use of plant-derived nanovesicles as safe and biocompatible nanocarriers capable of enhancing therapeutic efficacy while reducing adverse effects [166]. Current evidence suggests that PDEVs and PDVLNs exhibit favorable biocompatibility, low cytotoxicity, and promising therapeutic potential compared with synthetic nanomaterials. Their ability to deliver bioactive molecules, modulate cellular communication, and exert antioxidant and cytoprotective effects highlights their relevance for biomedical applications, particularly in inflammatory conditions such as IBD. Nevertheless, factors such as dosage, plant origin, and route of administration remain important determinants of their safety profile. Therefore, further well-designed *in vitro* and *in vivo* studies are necessary to fully elucidate their mechanisms of action and to ensure their safe and effective application as drug delivery systems and therapeutic agents. Fig. 5

present cytotoxic potential of plant-derived extracellular vesicles (PDEVs). Evaluation of the effects of PDEVs on cell viability and their safety profile in different cellular models.

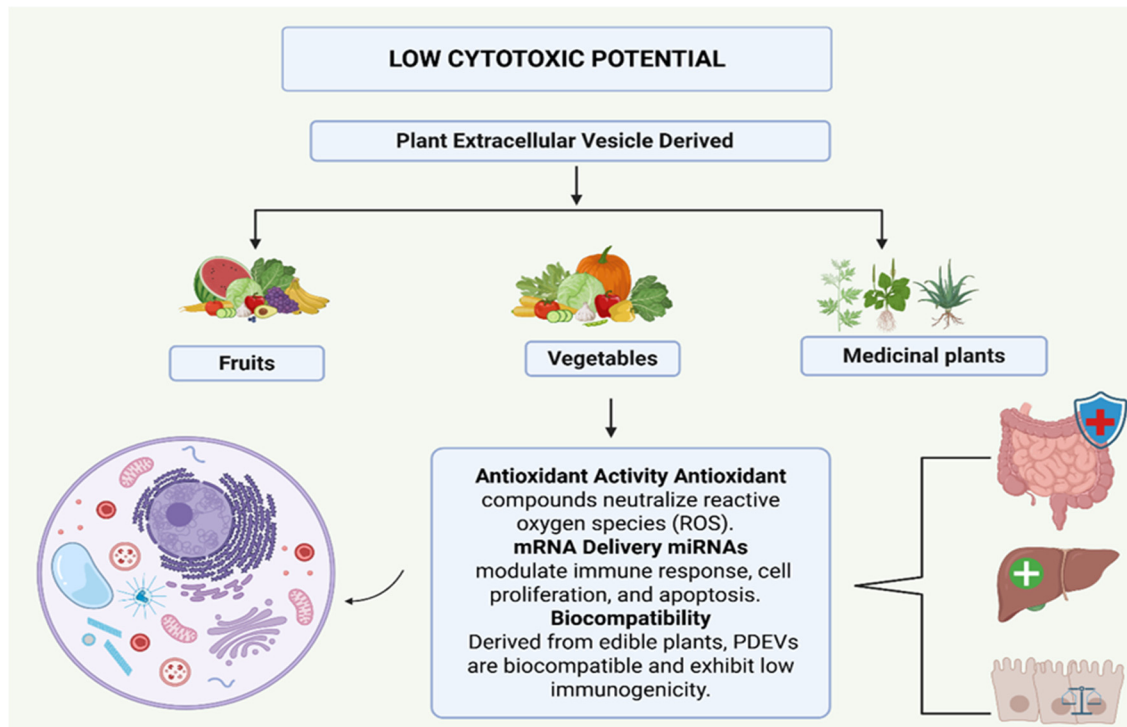


Figure 5: Cytotoxic potential of PDEVs.

9 Challenges, Limitations and Future Perspectives

Despite the growing interest in PDEVs as therapeutic agents for IBD, several important challenges and limitations remain. One of the major challenges is the lack of standardized terminology, as well as standardized isolation, purification, and characterization protocols. As discussed throughout this review, PDEVs comprise a heterogeneous group of vesicular systems, including native plant extracellular vesicles (EVs), plant-derived nanovesicles (PDNVs), and artificial plant-derived nanovesicles (APNVs), which differ in their origin, biogenesis, molecular composition, and production methods. The inconsistent use of these terms has contributed to confusion in the literature and complicates comparisons among published studies. Different extraction methods, including ultracentrifugation, density gradients, filtration, tangential flow filtration, vacuum infiltration, and enzymatic digestion, may produce vesicle populations with variable purity, yield, size distribution, and molecular composition, making direct comparisons between studies difficult [44]. Another important challenge concerns the heterogeneity of PDEVs. Vesicle composition is strongly influenced by plant species, tissue origin, physiological state, environmental conditions, and processing methods, including the use of fresh versus dried plant material. These variations may significantly affect vesicle stability, cargo composition, targeting properties, and biological activity [167]. From a botanical perspective, the real challenge is to understand how the specific physiology of each plant and its natural defense systems influence the chemical composition of these small vesicles, depending on whether they originate from food plants or medicinal plants. Furthermore, the isolation strategy itself may influence the molecular composition of vesicle preparations. In particular, PDNVs obtained from homogenized plant tissues may contain co-isolated membrane fragments, polysaccharides, proteins, and

phytochemicals released during tissue disruption, resulting in heterogeneous nanoparticle populations. Consequently, some of the reported biological activities may not originate exclusively from authentic vesicles but could also be influenced by co-purified plant components. In addition, many studies remain limited to *in vitro* systems or experimental animal models, while clinical validation in humans is still scarce [172]. The precise mechanisms underlying cross-kingdom communication between PDEVs and mammalian cells also remain incompletely understood. Although several studies suggest that plant-derived RNAs, lipids, proteins, and metabolites can modulate immune responses, intestinal barrier integrity, and gut microbiota composition, the efficiency of vesicle uptake, intracellular trafficking, cargo release, and long-term biological effects require further investigation [148]. Similarly, although plant-derived miRNAs have been proposed as mediators of cross-kingdom communication, their uptake efficiency, physiological abundance, reproducibility, and functional relevance in mammalian systems remain subjects of ongoing debate. Furthermore, the stability and bioavailability of PDEVs following oral administration under gastrointestinal conditions remain insufficiently characterized [173]. Current characterization techniques also present important limitations. Conventional methods such as nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), and transmission electron microscopy (TEM) provide valuable information regarding particle size, morphology, and concentration but cannot reliably distinguish authentic plant-derived vesicles from membrane fragments or other nanoparticles generated during tissue disruption. Therefore, the identification of robust plant-specific vesicular markers and complementary characterization approaches remains essential to improve vesicle purity, reproducibility, and standardization. Additional limitations involve large-scale production, storage stability, reproducibility, and regulatory aspects [25]. While artificial plant-derived nanovesicles offer improved control over composition and functionality, challenges regarding manufacturing standardization, scalability, and quality control still need to be addressed before clinical translation becomes feasible [174]. In addition, although APNVs provide advantages in terms of cargo loading efficiency, surface engineering, and targeting capability, naturally secreted plant EVs and engineered APNVs should be considered complementary rather than interchangeable therapeutic platforms. Future research should focus on establishing standardized methodologies for the nomenclature, PDEV isolation, purification, characterization, and quantification [29]. More studies are also needed to clarify the molecular mechanisms involved in host–microbiota–PDEV interactions, particularly the role of vesicular RNAs, lipids, and metabolites in immune modulation and intestinal homeostasis [13]. Furthermore, in order to truly understand how PDEVs derived from different plant families function, it will be necessary to map their unique biochemical and structural properties. This is a key step toward establishing a standardized reference framework of phytoactive compounds, which could then be tailored according to the desired therapeutic effects. In addition, comparative investigations between native plant EVs, PDNVs, and engineered APNVs may help optimize their therapeutic efficacy and targeting capabilities [27]. Finally, well-designed *in vivo* studies and clinical trials are required to evaluate the safety, biodistribution, pharmacokinetics, long-term effects, and therapeutic efficacy of PDEVs in IBD patients. The integration of nanotechnology, molecular biology, microbiome research, and precision medicine approaches may further support the development of PDEVs as innovative therapeutic platforms for IBD [175].

10 Conclusion

PDEVs have emerged as promising therapeutic candidates for IBD. Experimental studies have demonstrated their ability to modulate inflammatory and immune responses, promote intestinal barrier repair and mucosal regeneration, restore gut microbial homeostasis, and serve as efficient carriers for targeted drug delivery. Their favorable biocompatibility, low toxicity, and potential antitumoral effects further support their

therapeutic relevance. Crucially, these benefits are deeply rooted in the botanical diversity of their sources, as the unique plant physiology and defense mechanisms endow these vesicles with distinct biochemical and structural properties. Despite these encouraging findings, several challenges remain. Current evidence is largely derived from preclinical studies, and discrepancies in experimental outcomes may arise from variations in PDEV source, composition, dosage, administration route, and methodological approaches. In addition, the lack of standardized protocols for isolation, production, characterization, and storage limits the reproducibility and comparability of existing studies. The mechanisms governing PDEV interaction with mammalian cells and their long-term safety profile also require further clarification. Future research should focus on overcoming these limitations in order to facilitate clinical translation. The development of standardized methodologies, well-designed *in vivo* and clinical studies, and a deeper understanding of the molecular mechanisms underlying PDEV activity will be essential for validating their therapeutic safety and efficacy. In this pursuit, a comprehensive characterization of the bioactive phytochemicals naturally packaged within diverse plant families will open new paradigms for plant-to-mammal translation. Furthermore, advances in bioengineering may expand the application of PDEVs as next-generation platforms for targeted drug delivery and personalized treatment strategies in IBD. Therefore, further mechanistic, preclinical, and clinical studies are needed to establish standardized methodologies and comprehensively evaluate the efficacy and safety of PDEVs as novel therapeutic agents for IBD.

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Abbreviations

The following abbreviations are used in this manuscript:

IBD	Inflammatory bowel disease
CD	Crohn's disease
UC	ulcerative colitis
EVs	extracellular vesicles
PDEVs	Plant derived extracellular vesicles

MVB	Multi vesicular bodies
EXPO	exocyst-positive organelle
APNVs	artificial plant-derived nanovesicles
PDNVs	plant-derived nanovesicles
PDVLNs	plant-derived vesicle-like nanoparticles
TFF	tangential flow filtration
TEMs	transmission electron microscopy
GIPCs	glycosyl-inositol-phosphoceramides
PA	phosphatidic acid
miRNAs	microRNAs
SCFAs	short-chain fatty acids
ROS	reactive oxygen species
RNS	reactive nitrogen species
LPS	lipopolysaccharide
PRRs	pattern recognition receptors
TLRs	Toll-like receptors
PAMPs	pathogen-associated molecular patterns
GEN	ginseng-derived exosomes
Onex	Onion-derived EVS
IL-22	interleukin-22
AhR	aryl hydrocarbon receptor
LELNs	lemon-derived exosome-like nanoparticles
GELNs	ginger exosome-like nanoparticles
Dox	doxorubicin
MPO	myeloperoxidase
TDNPs	Turmeric-derived nanoparticle
DCs	intestinal dendritic cells
Rabex	Red cabbage-derived EVS
CAC	colitis-associated cancer
ACACA	Acetyl-CoA Carboxylase 1
PDVLNs	Plant-Derived Vesicle-Like Nanoparticles
XenomiRs	microRNAs
cabex	cabbage-derived PDVLNs
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
Carex	carrot-derived EVs
GNVs	grapefruit-derived nanovectors
MVBs	Multivesicular bodies
Th17	T helper 17 cells
Treg	Regulatory T cells
TNF- α	Tumor Necrosis Factor-alpha
IL-1 β	Interleukin-1 beta
IL-6	Interleukin-6
JAK	Janus Kinase
STAT	Signal Transducer and Activator of Transcription
HLA	Human Leukocyte Antigen
DNA	Deoxyribonucleic Acid
RNA	Ribonucleic Acid
miRNA	MicroRNA
mRNA	Messenger RNA
IL-10	Interleukin-10
IL-12	Interleukin-12
IL-23	Interleukin-23
TGF- β	Transforming Growth Factor beta
Treg	Regulatory T cells

CD4 ⁺	Cluster of Differentiation 4-positive T cells
ZO-1	Zonula Occludens-1
DNA	Deoxyribonucleic Acid

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