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Review

Sea Cucumber-Derived Frondoside A for Colorectal Cancer Therapy: Production, Functionalization, and Colon-Targeted Delivery Using pH-Responsive Microcapsules

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Abstract

This review examines a marine-biopolymer-enabled strategy for the colon-targeted oral delivery of Frondoside A, a sulfated triterpene glycoside obtained from Cucumaria frondosa, for colorectal cancer (CRC) therapy. Particular attention is given to marine and naturally derived polysaccharides—including chitin, chitosan, alginate, carrageenan, and fucoidan—as functional carriers for protecting Frondoside A during upper gastrointestinal transit and controlling its release in the colon. Current preclinical evidence indicates that Frondoside A induces mitochondrial and caspase-dependent apoptosis, inhibits proliferation, migration, and invasion, modulates innate immune responses (including natural killer cell interferon-γ preservation and macrophage activation), and enhances the activity of 5-fluorouracil and oxaliplatin in human CRC cell lines in vitro, with broader in vivo efficacy demonstrated in non-CRC solid tumour models. However, oral translation remains constrained by acidic glycosidic hydrolysis, inter-individual gastrointestinal pH and transit variability, premature absorption, and potential off-target toxicity. Chitosan–alginate polyelectrolyte complexes and ligand-functionalized polysaccharide microcapsules offer a plausible formulation platform because polymer charge, molecular weight, degree of deacetylation, and crosslinking density can be tuned to regulate gastric protection, mucoadhesion, and colonic release, alongside active receptor-mediated tumour uptake. Because direct in vivo data from Frondoside A-loaded colon-targeted formulations remain limited, this production-to-functionalization-to-application framework defines the key physicochemical, pharmacokinetic, and translational benchmarks required to advance marine-biopolymer microcapsules toward preclinical validation.

Keywords: colorectal cancer; *Cucumaria frondosa*; Frondoside A; marine biopolymers; marine polysaccharides; chitin; chitosan; alginate; pH-responsive microcapsules; colon-targeted drug delivery

Academic Editor: Jessica Amber Jennings

Received: 26 October 2026
Revised: 24 November 2026
Accepted: 25 November 2026
Published: 28 November 2026

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1. Introduction

Marine organisms are important sources of bioactive compounds with pharmaceutical potential. The North Atlantic sea cucumber *Cucumaria frondosa* is notable for its nutritional value and abundance of triterpene glycosides, or saponins [1,2]. These amphiphilic metabolites contribute to chemical defence and display anticancer, anti-inflammatory, antimicrobial, antioxidant, and anticoagulant activities [1–3]. Sea cucumbers also have a long histoProofreadingry as food-medicines in Asian traditions, particularly for wound healing, inflammation, and digestive health [4]. Among C. frondosa saponins, Frondoside A has received particular attention for its immunomodulatory and anticancer properties [5].

Frondoside A is a sulfated triterpene glycoside composed of a lanostane-derived aglycone and a carbohydrate chain, an architecture that underlies its amphiphilic and membrane-interactive behaviour [6–9]. Preclinical studies indicate that it can modulate inflammatory signalling, cytokine production, platelet activation, immune responses, and pathways involved in cancer progression [7,10,11]. These findings identify Frondoside A as a promising marine-derived therapeutic lead, while also emphasizing the need to distinguish evidence obtained directly in colorectal cancer from results generated in other disease models.

In colorectal cancer (CRC) models, Frondoside A has been associated with reduced proliferation, migration, and invasion, as well as activation of mitochondrial and caspase-dependent apoptosis [7,12,13]. It may also enhance the effects of standard agents such as 5-fluorouracil under experimental conditions. However, these observations remain preclinical and do not establish clinical superiority, tumour selectivity, or a safe dose-reduction strategy. Translation therefore requires a delivery approach that preserves the compound, concentrates exposure near colonic lesions, and limits unnecessary systemic distribution.

Oral delivery is attractive for localized CRC therapy, but Frondoside A must remain stable while traversing the stomach and small intestine. Its glycosidic structure may be altered by acidic hydrolysis or enzymatic activity, whereas gastrointestinal pH, mucus, epithelial barriers, microbial activity, and variable transit can cause premature degradation, absorption, or release [14–20]. Systemic chemotherapy avoids some absorption barriers but exposes healthy tissues and can produce substantial toxicity while achieving limited tumour accumulation [21–23] (Table 1). These limitations support the development of colon-targeted carriers capable of protecting Frondoside A during upper gastrointestinal passage and releasing it near the intended site.

Table 1. Current clinical standard-of-care therapies for colorectal cancer (CRC) stratified by disease stage, molecular subtype, and key translational limitations.

Disease Stage/Setting	Standard Clinical Regimen	Therapeutic Class & Mechanism	Key Clinical Limitations
Stage I–II (Localized, High-Risk)	Surgical resection ± adjuvant capecitabine or 5-FU/leucovorin	Antimetabolite (thymidylate synthase inhibition; DNA/RNA disruption)	Systemic mucosal toxicity, hand–foot syndrome, neutropenia; modest benefit in low-risk Stage II
Stage III (Node-Positive Adjuvant)	FOLFOX (5-FU, LV, oxaliplatin) or CAPEOX	Fluoropyrimidine + platinum DNA crosslinker	Cumulative dose-limiting peripheral neuropathy (oxaliplatin), myelosuppression, and chemoresistance

Stage IV/Metastatic (RAS/BRAF Wild-Type)	FOLFOX or FOLFIRI + cetuximab or panitumumab	Anti-EGFR monoclonal antibodies	Acquired KRAS/NRAS/BRAF or EGFR ectodomain resistance, severe acneiform rash, hypomagnesemia
Stage IV/Metastatic (RAS-Mutant/Unselected)	FOLFOX/FOLFIRI/FOLFOXIRI + bevacizumab	Anti-VEGF-A monoclonal antibody (anti-angiogenic)	Hypertension, thromboembolism, GI perforation, and poor penetration due to elevated tumour IFP [24]
Stage IV (BRAF V600E-Mutant, 2nd-Line)	Encorafenib + cetuximab	BRAF V600E kinase inhibitor + anti-EGFR mAb	Rapid pathway reactivation via MAPK feedback loops and cutaneous/gastrointestinal toxicities
Stage IV (dMMR/MSI-H)	Pembrolizumab, nivolumab ± ipilimumab	Immune checkpoint inhibitors (anti-PD-1/anti-CTLA-4)	Restricted to ~5% of metastatic CRCs with dMMR/MSI-H; immune-related adverse events
Refractory Metastatic (≥3rd-Line)	Regorafenib, fruquintinib, or trifluridine/tipiracil ±bevacizumab	Multi-kinase/VEGFR inhibitors or nucleoside analogue	Modest overall survival gains, fatigue, hepatotoxicity, and poor tolerance in frail patients

pH-responsive polymers can delay release by remaining compact or insoluble in the acidic stomach and swelling or dissolving under intestinal conditions. Natural biopolymers are especially relevant to this strategy. Chitosan, produced by deacetylation of chitin, is cationic and mucoadhesive [25,26], whereas alginate is an anionic, ionically crosslinkable polysaccharide. Their electrostatic complexation can create a protective matrix whose permeability changes during gastrointestinal transit [27–29]. A chitosan–alginate formulation could therefore improve gastric protection and localized colonic release of Frondoside A, although compound-specific loading, stability, release, efficacy, and safety must be demonstrated.

Accordingly, this review examines Frondoside A through a production-to-application framework: sea-cucumber sourcing and compound production; the structure and preclinical activity of the payload; the origin, processing, and properties of chitin, chitosan, alginate, and related polysaccharides; and functionalization through crosslinking, coating, and pH- or microbiota-responsive design. It distinguishes direct Frondoside A evidence from findings extrapolated from other compounds, tumour models, or carrier systems and assesses whether marine-biopolymer formulations provide a credible basis for colon-targeted biomedical application.

2. Sea Cucumber Biology, Traditional Uses, and Chemical Defence

2.1. Taxonomy and Morphology of Cucumaria frondosa

Sea cucumbers are marine invertebrates classified within the phylum Echinodermata and class Holothuroidea, comprising a diverse group of benthic organisms widely distributed across marine environments [1]. Members of this class are characterized by elongated, cylindrical, and soft-bodied morphologies with a leathery integument, adaptations that facilitate survival in sediment-associated habitats. Among these, Cucumaria frondosa is one of the most abundant species in the North Atlantic Ocean, particularly along the eastern coast of Canada, where it contributes significantly to regional marine ecosystems and fisheries [2] (Figure 1). This species exhibits defining morphological features, including a flexible body wall, oral tentacles used for feeding, and tube feet that support locomotion and environmental interaction [2]. Its feeding behavior,

which involves the consumption of phytoplankton, zooplankton, and organic particulate matter, reflects its ecological adaptation to benthic environments [1,2].

Beyond its morphological characteristics, Cucumaria frondosa demonstrates a nutritionally and biochemically rich composition that underpins its increasing scientific and industrial relevance. Sea cucumbers are recognized as valuable sources of essential nutrients, including vitamins (A, B-complex), minerals such as calcium, magnesium, iron, and zinc, and a wide range of bioactive compounds [1]. In particular, the body wall of C. frondosa contains high levels of protein, predominantly collagen, which contributes to both structural integrity and functional bioactivity [2]. Additionally, the species contains essential amino acids, lipids, polysaccharides, and bioactive constituents such as saponins, glycosides, carotenoids, and phenolic compounds [9]. Collectively, these compositional attributes highlight the biological significance of C. frondosa and support its emerging applications in nutraceutical, pharmaceutical, and functional food development.

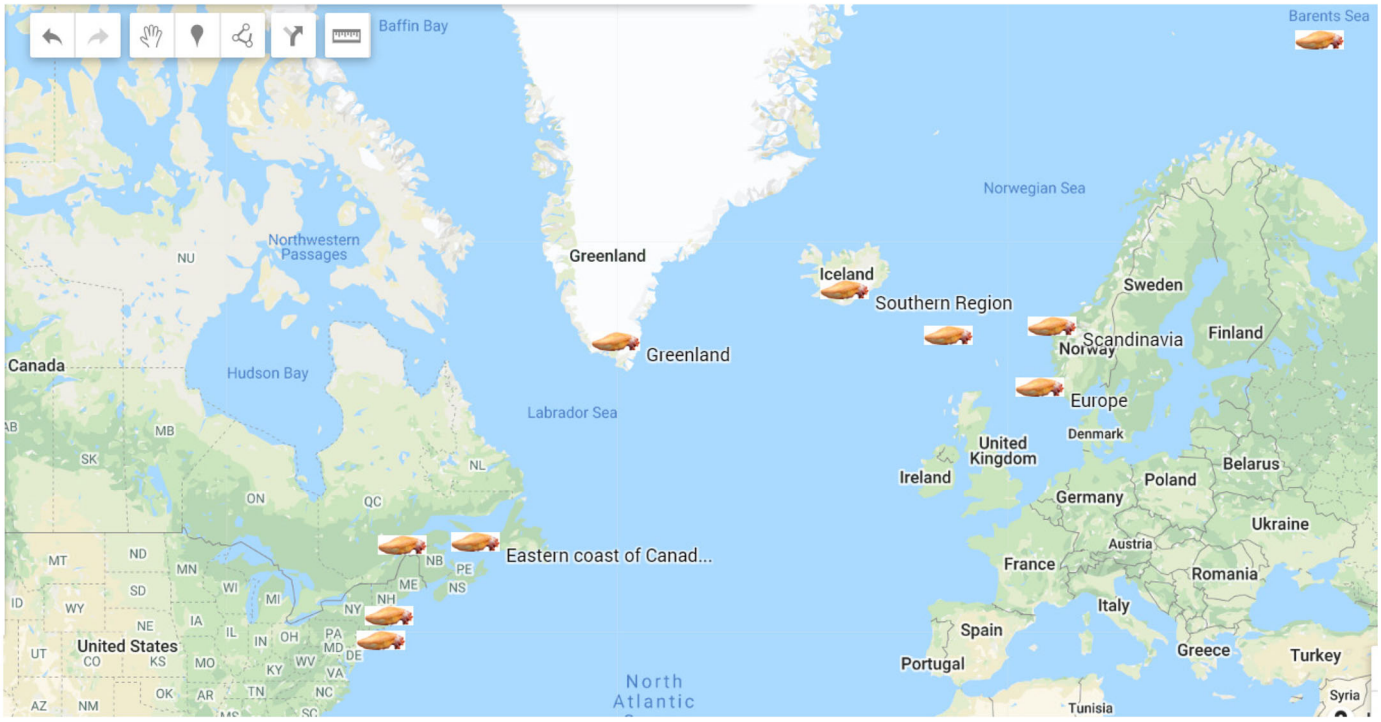

Figure 1. Geographic distribution of Cucumaria frondosa across the North Atlantic Ocean, including eastern Canada and northern European regions [2].

Sea cucumbers, belonging to the phylum Echinodermata and class Holothuroidea, are marine invertebrates recognized for both their ecological importance and unique biochemical composition [1,2]. Among these species, Cucumaria frondosa, commonly referred to as the orange-footed sea cucumber, has received increasing scientific attention because of its high abundance in the North Atlantic Ocean and its rich concentration of bioactive compounds, particularly triterpene glycosides known as saponins. Distributed primarily along the eastern coast of Canada and other northern Atlantic regions, C. frondosa plays an important ecological role in nutrient cycling and organic matter processing within benthic marine ecosystems. At the same time, its biochemical composition has contributed to growing interest in its applications within the nutraceutical, pharmaceutical, and biomedical industries.

Morphologically, sea cucumbers possess elongated, soft-bodied structures with a collagen-rich body wall strengthened by microscopic calcareous ossicles (Figure 2). This structural organization provides both flexibility and protection, enabling adaptation to

benthic environments characterized by fluctuating environmental conditions. In C. frondosa, the body plan includes oral tentacles specialized for suspension feeding and tube feet that facilitate locomotion and substrate attachment [2]. Through these feeding mechanisms, the organism contributes to the recycling of organic particulate matter within marine sediments and surrounding waters. The body wall constitutes the majority of the organism's mass and is consistently identified as the principal reservoir for structural proteins such as collagen, as well as numerous biologically active compounds [2]. This dual structural and biochemical function distinguishes sea cucumbers from many other marine invertebrates and underlies their growing relevance in biomedical research.

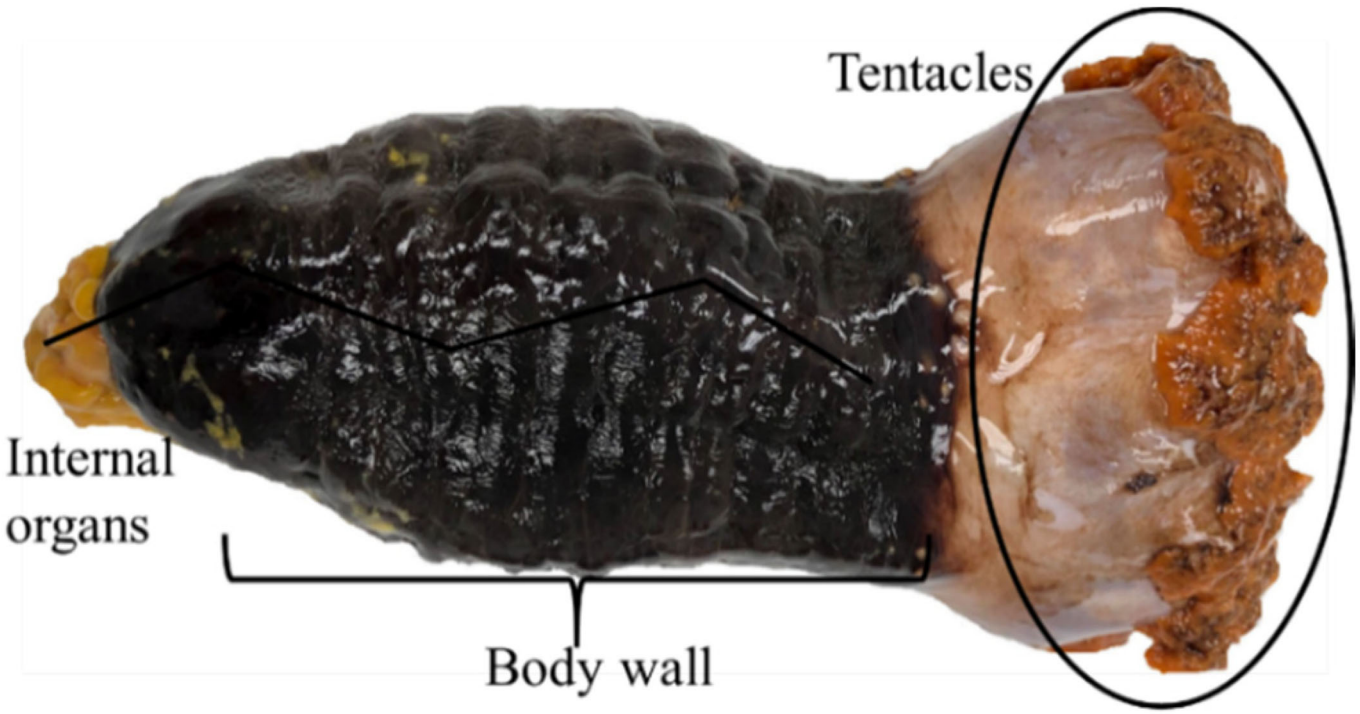

Figure 2. Morphological characteristics of Cucumaria frondosa, showing an elongated body structure, oral feeding tentacles, and tube feet used for suspension feeding and locomotion [2].

In addition to structural proteins, C. frondosa contains a broad range of nutrients and secondary metabolites, including essential amino acids, vitamins, minerals, fatty acids, and polysaccharides [1]. However, studies frequently report variability in the concentration and composition of these compounds. Such inconsistencies are commonly attributed to differences in geographical habitat, seasonal variation, diet, environmental conditions, and analytical methodologies used during biochemical characterization [1,30]. Consequently, the literature highlights an ongoing methodological challenge in establishing standardized compositional analyses for sea cucumber-derived compounds, limiting direct comparisons across studies.

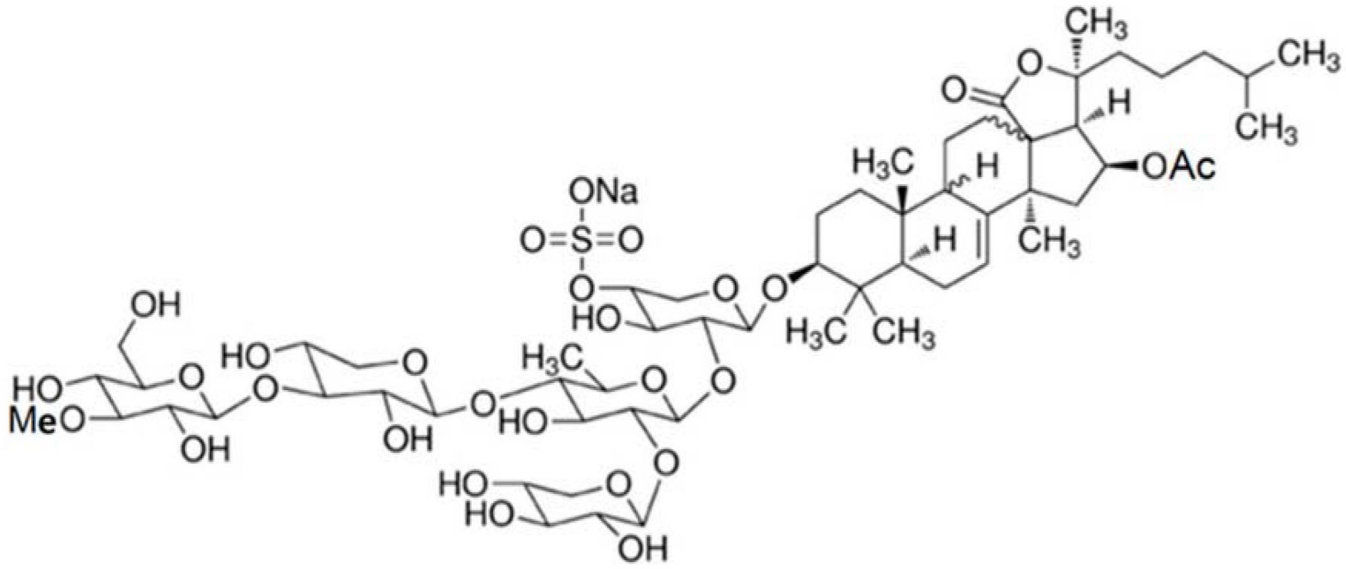

Figure 3. Molecular structure of Frondoside A, a sulfated triterpenoid glycoside isolated from the sea cucumber *Cucumaria frondosa* [7].

Among the various bioactive constituents identified in sea cucumbers, triterpene glycosides, commonly referred to as saponins, have emerged as compounds of particular scientific interest. These secondary metabolites are predominantly localized within the body wall and are widely believed to function as chemical defense agents against predators, parasites, and microbial pathogens [3,31]. Structurally, saponins possess amphipathic properties, consisting of hydrophobic aglycone backbones linked to hydrophilic sugar chains. This molecular arrangement enables interaction with lipid bilayers and cellular membranes, a feature strongly associated with their biological activities, including antifungal, anti-inflammatory, anticancer, and immunomodulatory effects [5]. Their membrane-active properties have also generated interest in pharmaceutical applications, particularly in drug delivery systems where enhanced membrane permeability and targeted transport are desirable. The molecular structure of Frondoside A, a representative sulfated triterpene glycoside of C. frondosa, is shown in Figure 3 [7].

Despite growing interest in sea cucumber saponins, the literature demonstrates substantial variability in the characterization of these compounds and their reported biological activities. Differences in extraction procedures, purification techniques, and structural identification methods have resulted in inconsistencies across studies, making it difficult to establish standardized relationships between saponin structure and biological function. Furthermore, although several investigations suggest that saponins may improve drug permeability and therapeutic targeting, relatively few studies systematically evaluate these compounds within controlled drug delivery models. As a result, an important gap remains in the integration of detailed biochemical characterization with applied pharmaceutical and translational research. Therefore, while Cucumaria frondosa is consistently recognized as a promising source of bioactive saponins, additional research is required to standardize analytical methodologies and better evaluate its potential in drug delivery applications.

2.2. Ethnomedicine and Traditional Uses

Sea cucumbers have a long history of use in ethnomedicine, particularly within Asian traditional medical systems, where they are consumed as both food and therapeutic agents for maintaining health and treating disease [32]. Historically, they have been regarded as tonics within Chinese medicine, often prepared in powdered form, because

their collagen, sulfated polysaccharides, and n-3 polyunsaturated fatty acids contribute to their nutritional and restorative value [33,34]. In Malaysian traditional medicine, sea-cucumber body-wall derivatives such as gamat gel or fluid are used internally to support recovery from trauma and childbirth, while gamat oil is applied externally for wound healing, aches, and skin conditions [35]. Modern research reports antimicrobial, anticoagulant, and antitumour activities associated with sea-cucumber constituents [33]. These properties are attributed to compounds including saponins, cerebrosides, polysaccharides, and peptides, some of which have demonstrated anti-inflammatory and anti-angiogenic effects [33]. Sulfated glycosaminoglycans from species such as *Stichopus hermanni* have also been associated with wound-healing activity [4]. These findings provide a biomedical context for the traditional classification of sea cucumbers as functional food-medicines.

2.3. Chemical Defence Mechanisms

As soft-bodied, slow-moving echinoderms, sea cucumbers produce glycosylated steroids and triterpenes known as saponins, which provide chemical defence against predators. Some sea cucumbers expel sticky threads called Cuvierian tubes that immobilize and entangle predators [3]. The saponins tend to kill the immobilized enemy. Saponins have antifungal activity attributed to their ability to form complexes with sterols such as cholesterol, causing membrane permeabilization and cell death [3]. Sea cucumbers produce different sterols, including lathosterol and 14α-methylcholest-9(11)-en-3b-ol, which protect the creatures from self-poisoning [3]. Sea cucumber sterols are triterpenoids.

In sea cucumbers, saponins are concentrated within the body wall and internal organs, particularly in the specialized sticky structures known as Cuvierian tubules [31,36]. On a molecular level, these compounds are triterpene glycosides featuring an aglycone derived from holostane-3β-ol linked to an oligosaccharide chain [31,36]. This sugar chain, attached at the C3 position, typically contains up to six units, such as glucose, xylose, quinovose, or 3-O-methylglucose—and may occasionally feature sulfation on the xylose residue [31,36].

The amphiphilic nature of these molecules allows them to disrupt cellular membranes, resulting in toxic effects that serve as a potent defense against most organisms [31,36]. Furthermore, sea cucumbers can actively release these saponins into the surrounding water to signal their unpalatability, acting as a chemical warning to potential predators [37].

Saponins represent a class of naturally occurring amphiphilic glycosides biosynthesized by various plants, bacteria, and select marine invertebrates [3,31,36]. For over fifty years, these secondary metabolites have been a focal point in the field of natural product discovery. As research increasingly investigates their bioactive potential, new opportunities are emerging across the pharmaceutical, biotechnological, and ecological sectors [3,31,36]. Echinoderms lack adaptive immunity and rely solely on innate immunity for growth and survival in their environment [3]. In sea cucumbers, innate immunity is composed of specialized metabolites such as glycosylated steroids and triterpenes, also known as saponins, which provide chemical defence against predators by forming a poisonous barrier on their body wall and Cuvierian tubules [3].

When stressed, *Cucumaria frondosa* expels its Cuvierian tubules to immobilize a predator [38]. Frondoside A acts as a defence compound by interacting with cell membranes, increasing membrane permeability and promoting membrane lysis [31] (Figure 4). Beyond this ecological role, Frondoside A has attracted interest for its therapeutic potential. Administration at doses below those required to inhibit cancer-cell growth has been reported to stimulate innate immune responses in mammalian models [39].

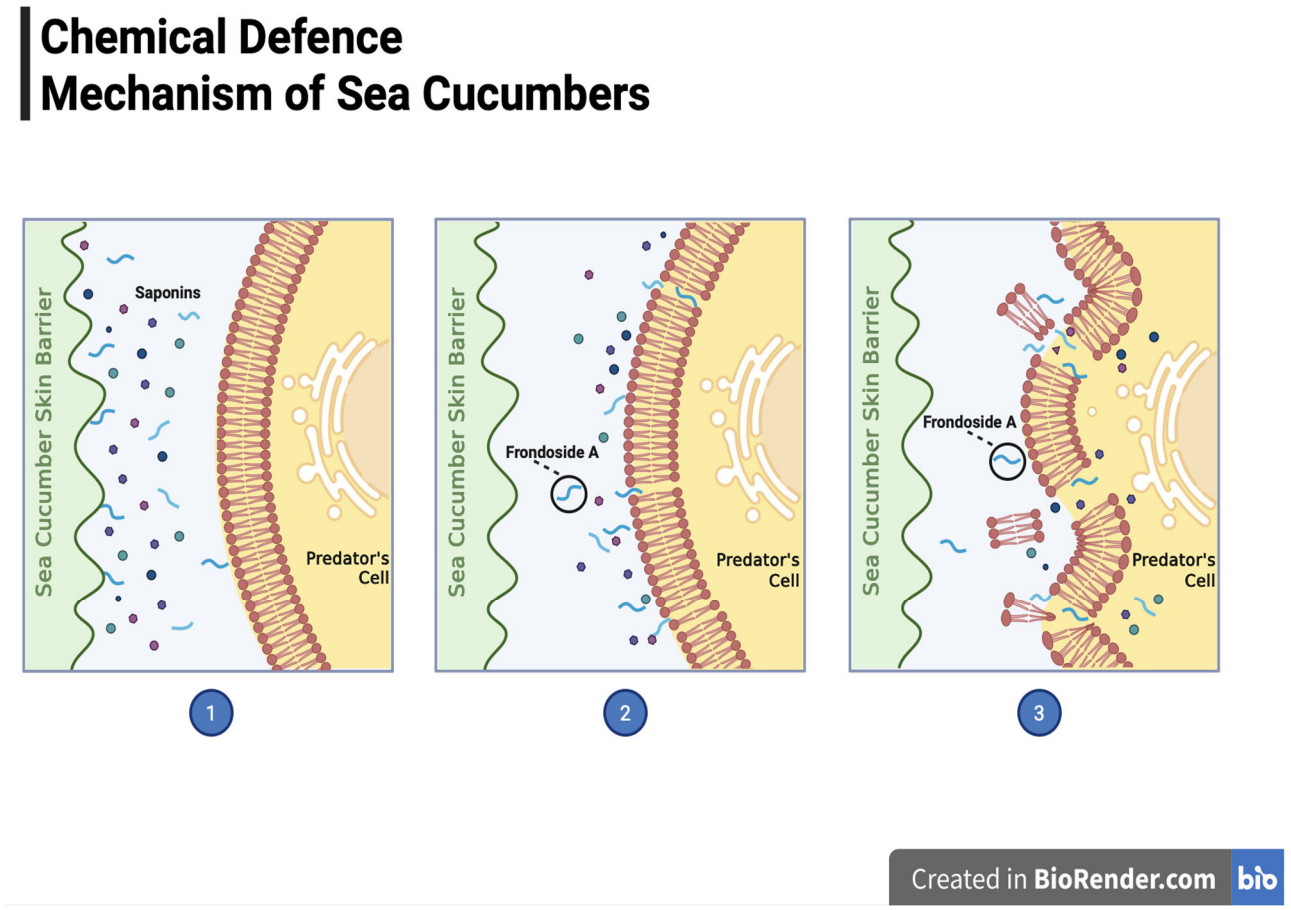

Figure 4. Schematic illustration of the chemical defence mechanism of Cucumaria *frondosa*. (1) C. *frondosa* releases saponins in response to predator attack. (2) Frondoside A interacts with the predator's cell membrane, increasing membrane permeability and disrupting membrane integrity. (3) Membrane disruption leads to cellular lysis and death of predator cells. Created using BioRender.com.

3. Chemical Structure and Biological Activity of Frondoside A

3.1. Molecular Composition

Sea cucumbers produce triterpene glycosides as part of their chemical defence system, with these compounds ultimately derived from the isoprenoid (mevalonate) pathway. In this pathway, multiple isoprene units are assembled into the linear C30 precursor squalene, a largely nonpolar molecule that is then converted into 2,3-oxidosqualene, which acts as a key branching intermediate for triterpene biosynthesis [3,6]. From this point, oxidosqualene cyclases (OSCs) catalyze the folding and cyclization of 2,3-oxidosqualene into a variety of triterpene carbon skeletons. Subsequent enzymatic tailoring introduces functional groups such as hydroxyls, carboxyls, and sugars, increasing polarity and generating the amphiphilic nature characteristic of triterpene glycosides [6].

Frondoside A is a specific example of this class, isolated from the sea cucumber Cucumaria frondosa. It is a triterpenoid glycoside with a lanostane-derived aglycone and an acetoxy group at C-16. Its carbohydrate chain is structurally complex, consisting of five sugar residues, including xylose as the third unit and a terminal 3-O-methylglucose, with a sulfate group on the first sugar [7] (Figure 3).

Frondoside A contains a nonpolar aglycone core and a polar saccharide chain, giving it an amphiphilic structure (Figure 5). The aglycone is a lanostane-derived triterpenoid with a complex pentacyclic hydrocarbon framework and relatively few polar functional groups, so it remains largely hydrophobic. This nonpolar core originates from cyclization

of 2,3-oxidosqualene into a cucumarioside-type scaffold that is subsequently modified while retaining its lipid-like character. The saccharide chain is highly polar and contains multiple sugar residues added during biosynthetic tailoring [6].

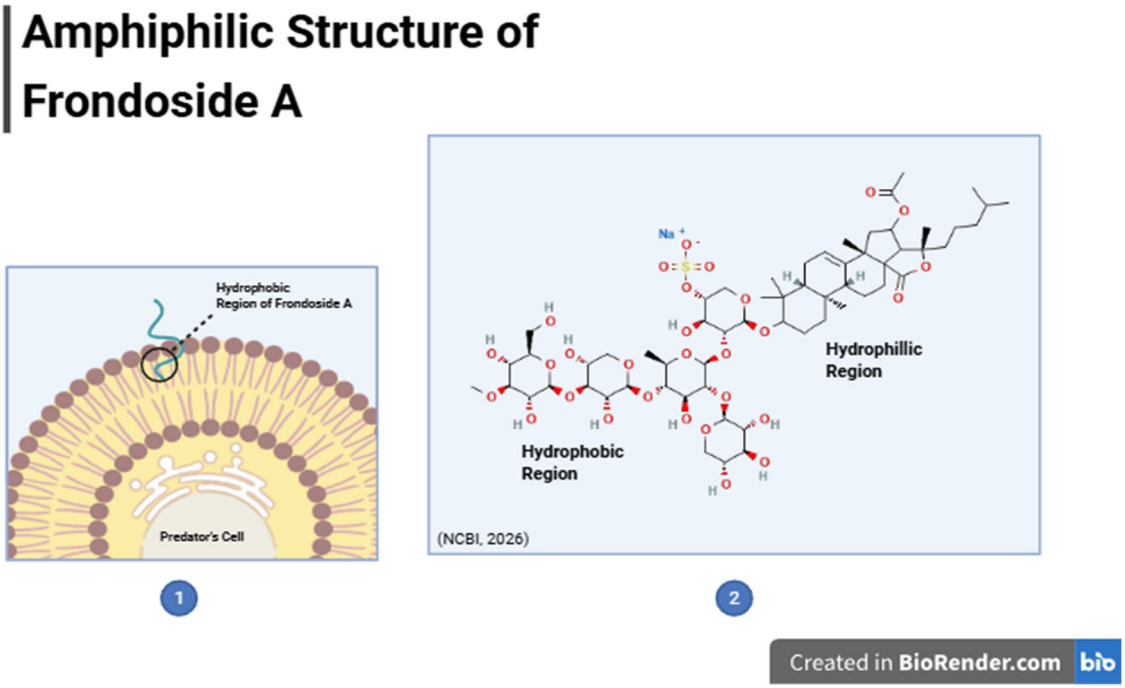

Figure 5. Schematic illustration of Frondoside A's chemical structure. (1) The hydrophobic lanostane-derived aglycone can interact with the lipid bilayer, while the hydrophilic five-sugar chain remains oriented toward the aqueous environment. (2) Frondoside A contains a lanostane-derived aglycone, a C-16 acetoxy group, and a five-sugar chain; the first sugar carries a sulfate group, the third sugar is xylose, and the terminal sugar is 3-O-methylglucose. The two-dimensional structure was obtained from PubChem. Illustration created using BioRender.com.

Penetration of sea cucumber-derived triterpene glycosides into the cell membrane is dependent on their hydrophobic core. Within the membrane, triterpene glycosides can form complexes with 5(6)-unsaturated sterols, which subsequently leads to a disturbance of the membrane's transport properties and cell lysis. This strong membranolytic action provides the saponins with many anticancer properties such as cell apoptosis and cytotoxicity [9,40].

Previous studies have indicated that the lytic activity of various triterpene glycosides may be influenced by their structural characteristics. However, the underlying mechanism of action relating the structural properties of saponins to their biological activity is not yet fully established [41,42]. In the case of Frondoside A, the sulfate group attached to C-4 of the xylose group contributes to enhanced membranolytic activity, whereas triterpene glycosides lacking a sulfate group are associated with lower lytic activity [9]. Additionally, monosulfated Frondoside A has been found to exhibit higher membranolytic activity than saponins containing more sulfate groups such as bisulfated Frondoside B [10] and trisulfated cucumarioside A_7-1 [31].

3.2. Broad-Spectrum Anti-Inflammatory and Antithrombotic Effects

Beyond its direct antiproliferative effects, Frondoside A exhibits anti-inflammatory and antithrombotic activities in non-CRC preclinical models that are relevant to tumour-promoting inflammation. In murine models of thrombosis, Frondoside A suppresses platelet activation and thrombus formation by inhibiting PI3K/Akt/ERK signalling,

although concentration-dependent hemolytic activity on murine erythrocytes has also been observed at higher exposures and warrants careful therapeutic-window evaluation [11]. Concurrently, Frondoside A downregulates NF-κB and MAPK signalling and antagonizes prostaglandin E2 (PGE2) receptors (EP2 and EP4), thereby suppressing inflammatory gene transcription [5,43].

Across activated macrophage and systemic inflammation models, Frondoside A reduces the production of pro-inflammatory mediators including tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and cyclooxygenase enzymes (COX-1 and COX-2), while decreasing endothelial adhesion molecule expression involved in leukocyte recruitment [7,31]. Additional neuroprotective studies in Caenorhabditis elegans and rodent models of neurodegeneration and cerebral ischaemia indicate that Frondoside A attenuates oxidative stress and neuroinflammation [44,45]. Collectively, these anti-inflammatory and antithrombotic actions provide supportive mechanistic context for limiting inflammation-driven tumour progression.

3.3. Immunomodulatory Effects and Cytokine Regulation

A distinguishing feature of Frondoside A is its biphasic, dose-dependent immunomodulatory profile. Whereas micromolar concentrations (0.5–2.5 μM) exert direct cytotoxic effects on malignant cells, sub-cytotoxic doses stimulate innate immune surveillance in mammalian models [7,39]. In foundational murine studies, administration of Frondoside A at low doses (0.2 μg/mouse) significantly enhanced lysosomal activity and phagocytic capacity in peritoneal macrophages and stimulated reactive oxygen species (ROS) generation during pathogen clearance, without inducing acute systemic toxicity [39].

Importantly for tumour immunology, Frondoside A modulates cytokine networks and lymphocyte effector functions through antagonism of PGE2 signalling [7,43]. Tumour-derived PGE2 acts via EP2 and EP4 receptors on innate immune cells to suppress antitumour immunity—specifically by inhibiting dendritic cell secretion of interleukin-12 (IL-12) and suppressing natural killer (NK) cell cytotoxicity and interferon-γ (IFN-γ) production [7,43]. In a syngeneic murine breast cancer model, Ma et al. [43] demonstrated that Frondoside A directly antagonizes EP4 and EP2 receptors, thereby abrogating PGE2-mediated immunosuppression and restoring the capacity of NK cells to secrete IFN-γ and mediate antimetastatic surveillance in vivo [7,43]. Furthermore, transcriptomic profiling of Frondoside A-treated human cells has revealed enrichment of IFN-γ and IFN-α hallmark signalling gene sets alongside KRAS and p53 pathway modulation, while broader holothurian saponin fractions (such as Frondanol A5) suppress chronic intestinal inflammation and pro-tumorigenic cytokine surges in rodent colon carcinogenesis models [10,31]. Although direct measurements of IL-12 and IFN-γ in orthotopic CRC models treated with purified Frondoside A remain to be conducted, these findings suggest that localized colonic release could help reprogram the immunosuppressive CRC microenvironment alongside direct tumour cell killing.

Frondoside A has demonstrated concentration- and time-dependent antiproliferative activity across multiple solid and hematological cancer models, though the depth of evidence varies substantially by tumour type (Figure 6 and Table 2). In direct CRC studies using human cell lines representing distinct molecular subtypes—epithelial adenocarcinoma HT-29 cells (BRAF-mutant, microsatellite-stable) and microsatellite-instable HCT116 and HCT8/S11 cells (KRAS-mutant)—Frondoside A reduced cell viability at low micromolar concentrations in vitro [13]. Notably, 2.5 μM Frondoside A achieved near-complete growth inhibition of HT-29 cells after 72 h, whereas comparable inhibition under identical in vitro conditions required 100 μM of oxaliplatin or 5-fluorouracil (5-FU) [13]. While these results demonstrate broad activity across genetically distinct CRC cell lines in

vitro, they have not yet been corroborated in orthotopic or patient-derived CRC xenograft models.

Broader antitumour efficacy, including in vivo activity, has been reported primarily in non-CRC solid tumour models. In pancreatic ductal adenocarcinoma (AsPC-1 and S2013 cells), Frondoside A inhibited proliferation in vitro and suppressed subcutaneous xenograft growth in athymic mice while enhancing gemcitabine efficacy [8,46]. Similarly, Frondoside A reduced primary tumour growth and spontaneous lung metastasis in murine breast cancer models [43,47], and induced apoptosis in human leukemia cells [48,49]. In A549 lung cancer cells, Frondoside A reduced cell viability and colony growth in vitro and, in combination with butein, showed additive effects on viability, colony growth, and invasion [50].

Across these models, non-malignant immortalized epithelial cells exhibit lower sensitivity to Frondoside A than malignant cells under comparable in vitro conditions [7,47]. This differential sensitivity is hypothesized to reflect malignant cells' heightened dependence on constitutively active survival networks—such as p21-activated kinase 1 (PAK1), PI3K/Akt, and ERK1/2—which Frondoside A suppresses [7]. However, because normal colonic organoid and in vivo mucosal toxicity studies remain limited, Frondoside A should currently be regarded as a promising preclinical candidate whose selectivity and therapeutic index warrant further investigation in physiologically relevant CRC models (Figure 6).

Overall, current primary evidence supports Frondoside A as a highly potent anticancer agent against CRC. Its low-dose efficacy against HT-29 and HCT116 cells, superiority over conventional drugs in vitro, likely tumour selectivity, and compatibility with local colon delivery make it an excellent candidate for further translational development (Figure 6). Incorporation into Frondoside A microcapsules (FA-MCs) could therefore substantially enhance therapeutic outcomes in colorectal cancer treatment.

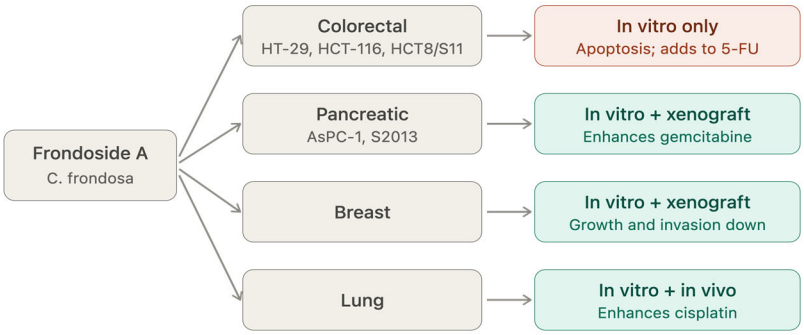

Figure 6. Summary of reported preclinical anticancer effects of Frondoside A across tumour models, compiled from published studies [13,43,46,47,50]. In colorectal cancer (HT-29, HCT116, and HCT8/S11 cell lines), evidence is currently restricted to in vitro studies reporting apoptosis induction, reduced invasion, and enhanced activity of 5-fluorouracil (5-FU) and oxaliplatin. In pancreatic and breast cancer models, efficacy has been demonstrated both in vitro and in vivo, whereas lung cancer evidence is currently in vitro [50].

Table 2. Summary of preclinical anticancer evidence for Frondoside A across tumour models, distinguishing direct colorectal cancer (CRC) findings from mechanisms demonstrated in other malignancies.

Tumour Type	Experimental Model(s)	Effective Dose/Conc.	Reported Effects & Molecular Mechanisms	Evidence Level for CRC
Colorectal Cancer (CRC)	HT-29, HCT116, HCT8/S11 human CRC lines (in vitro only)	0.5–2.5 μM (24–72 h)	Growth inhibition; annexin V apoptosis; caspase-3/7 & PARP cleavage; reduced Matrigel invasion; enhances 5-FU & oxaliplatin [7,13]	Direct CRC evidence (in vitro only)
Intestinal Neoplasia (Extract)	ApcMin/+ mice; HCT116 cells (dietary Frondanol A5)	Dietary extract containing Frondoside A	Reduced intestinal polyp formation; G2/M arrest; enhanced innate immune surveillance [10]	Indirect (multi-component extract)
Pancreatic Cancer	AsPC-1, S2013 lines; athymic mouse xenografts	0.5–2.0 μM in vitro; 10–100 μg/kg/day i.p.	Mitochondrial apoptosis (decreased Bcl-2/Mcl-1, increased Bax, cytochrome c, caspase-9/-3); enhances gemcitabine [8,46,51]	Extrapolated GI epithelial model
Breast Cancer	MDA-MB-231, MCF-7, 4T1; syngeneic & xenograft mice	0.5–2.5 μM in vitro; 10–100 μg/kg/day i.p.	Reduced tumour growth & lung metastasis; EP2/EP4 antagonism; restores NK cell IFN-γ; MMP-2/9 suppression [43,47]	Extrapolated epithelial model
Lung Cancer	A549 lung cancer cells (in vitro); butein combination studies	In vitro (see [50])	Reduced viability and colony growth; additive effects with butein on viability, colony growth, and invasion; synergism on endothelial cell migration [50]	Extrapolated solid tumour model (in vitro)
Leukemia	HL-60, NB4, THP-1, Jurkat human lines (in vitro)	0.3–1.0 μM	Caspase-dependent and caspase-independent (AIF) apoptosis; potentiates conventional antileukemic drugs [48,49]	Extrapolated hematological model

A key feature of Frondoside A is its selectivity toward malignant cells, which is thought to arise from cancer-specific dependencies on these dysregulated signaling networks. Cancer cells often exhibit constitutive activation of Akt and MAPK pathways, making them more vulnerable to agents that disrupt these pathways. In contrast, normal cells rely less heavily on these survival signals, contributing to the observed lower toxicity [7,13,46,47,51]. Furthermore, Frondoside A has been shown to induce both caspase-dependent and caspase-independent cell death, such as apoptosis-inducing factor (AIF), which broadens its ability to overcome resistance mechanisms commonly seen in colorectal cancer.

4. Mechanisms of Action Relevant to Colorectal Cancer (CRC)

Evasion of apoptosis via TP53 loss-of-function, KRAS/BRAF activation, or Bcl-2 family overexpression is a central driver of therapeutic resistance in CRC. In human CRC cell lines (HT-29, HCT116, and HCT8/S11), Attoub et al. [13] demonstrated directly in vitro that Frondoside A triggers concentration- and time-dependent phosphatidylserine externalization (annexin V positivity), executioner caspase-3/7 activation, and poly (ADP-ribose) polymerase (PARP) cleavage.

Detailed upstream mechanistic dissection—performed primarily in pancreatic, breast, and leukemia models—indicates that Frondoside A engages the intrinsic mitochondrial apoptotic pathway [47,48,51]. Specifically, in pancreatic and breast carcinoma cells, Frondoside A disrupts mitochondrial membrane potential, downregulates anti-apoptotic Bcl-2 and Mcl-1 while upregulating pro-apoptotic Bax, and promotes cytosolic release of cytochrome c to trigger apoptosome assembly and initiator caspase-9 activation [47,51]. In leukemia models, Frondoside A also induces caspase-independent cell death via nuclear translocation of apoptosis-inducing factor (AIF) [48]. Furthermore, suppression of pro-survival PAK1, PI3K/Akt, and ERK1/2 signalling—observed in both CRC cell lines [7,13] and pancreatic/breast models [47,51]—lowers the apoptotic threshold of malignant cells (Figure 7). While elevated basal mitochondrial membrane potential and oxidative stress in CRC cells provide a plausible biological rationale for selective mitochondrial destabilization, direct confirmation of Bax/Bcl-2 modulation, ROS generation, and AIF release in CRC tissues in vivo remains an important research priority.

Once the mitochondrial pathway is initiated, Frondoside A drives the proteolytic cleavage of pro-caspase-3 and pro-caspase-7 into their active effector forms [48,51]. The activation of these caspase-3/7 dependent mechanisms results in the cleavage of poly (ADP-ribose) polymerase (PARP), a hallmark of programmed cell death that leads to irreversible DNA fragmentation and cellular disintegration [47,48,51]. Beyond direct mitochondrial interference, Frondoside A has also been shown to inhibit survival signaling pathways like Akt and ERK1/2, further sensitizing carcinoma cells to apoptotic stimuli and making it a potent candidate for natural product-based cancer therapy [7,13,51]. Inhibition of PI3K/AKT signalling has likewise been linked to apoptosis induced by other sea cucumber-derived compounds in breast cancer cells [52].

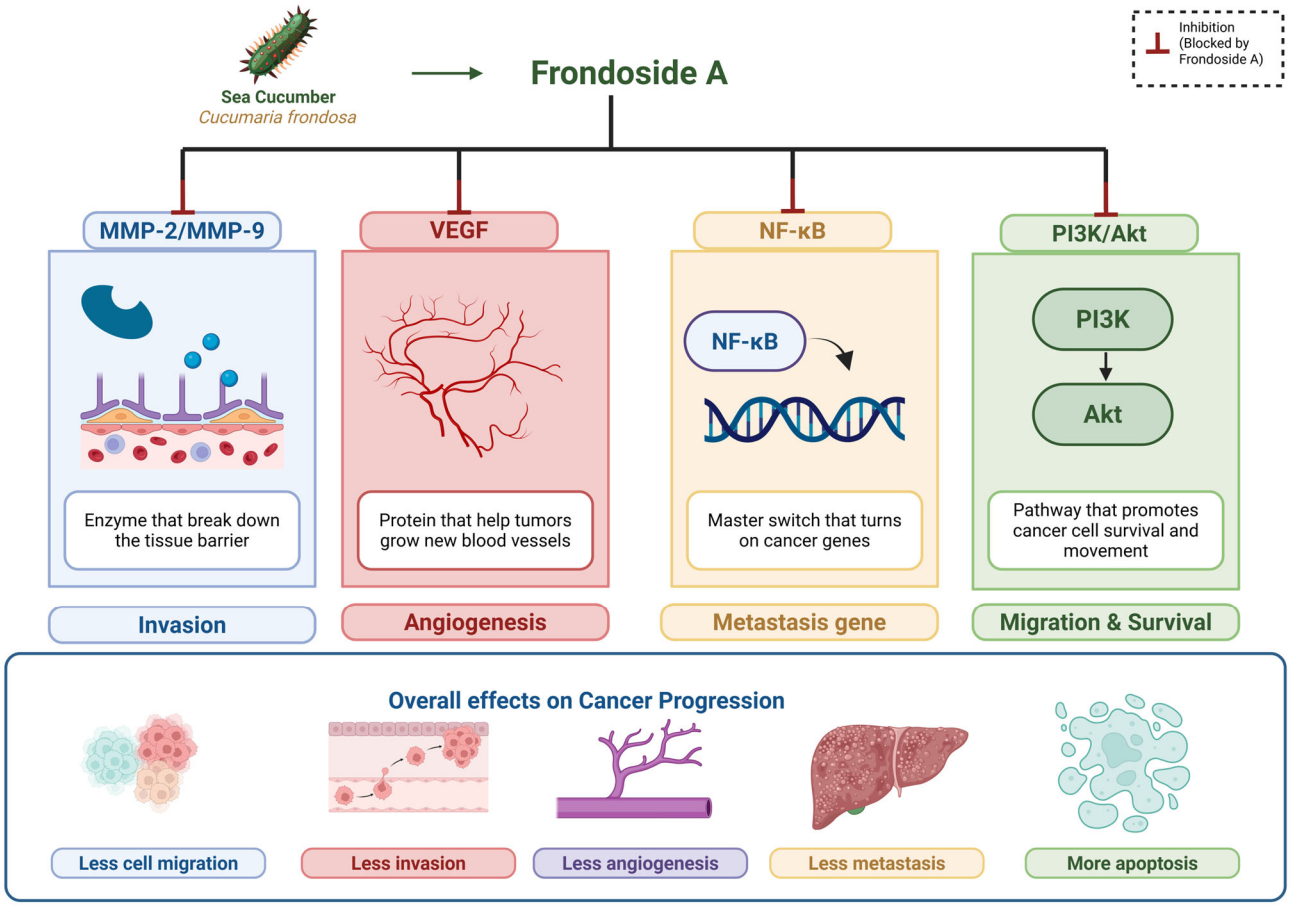

Figure 7. Proposed mechanisms of the anticancer effects of Frondoside A relevant to colorectal cancer progression, integrating direct in vitro CRC findings (caspase-3/7 apoptosis, reduced invasion) with pathways characterized in pancreatic and breast tumour models and lung cancer cells (MMP-2/MMP-9, VEGF, NF-κB, and PI3K/Akt suppression) [7,13,43,46,47,50,51].

Metastasis—predominantly to the liver via the portal venous circulation—is the primary cause of CRC mortality. In direct in vitro CRC studies using HT-29, HCT116, and HCT8/S11 cells, Frondoside A significantly suppressed cellular invasion through extracellular matrix (Matrigel) barriers at concentrations below those required for complete cytolysis [13].

Broader antimetastatic mechanisms have been characterized in vivo using breast carcinoma models [43,47], with supporting in vitro invasion data from lung cancer cells [50]. In murine breast cancer models, Frondoside A significantly reduced spontaneous pulmonary metastases, inhibited directional cell migration, and suppressed matrix metalloproteinase-2 and -9 (MMP-2/MMP-9) activity—proteolytic enzymes that degrade type IV collagen in basement membranes and are clinically associated with CRC hepatic metastasis [43,47]. In A549 lung cancer cells, Frondoside A combined with butein additively reduced invasion and synergistically reduced endothelial cell migration in vitro [50]. Mechanistically, suppression of PI3K/Akt, ERK1/2, NF-κB, and EP2/EP4 prostaglandin receptor signalling is implicated in reversing epithelial–mesenchymal transition (EMT) and reducing stromal invasion [7,43,47]. Confirming whether these anti-angiogenic and MMP-inhibitory effects translate to reduced hepatic metastasis in orthotopic CRC mouse models is a necessary next step.

At the cellular level, Frondoside A has been shown to significantly inhibit cancer cell migration, a key step in the metastatic cascade, by reducing the motility and viability of tumour cells [13,43,47]. This reduction in migratory capacity directly limits the ability of CRC cells to invade surrounding tissues and disseminate to secondary sites such as the liver. Furthermore, sea cucumber-derived compounds have been reported to interfere with tumor cell adhesion and interactions within the tumour microenvironment, processes that are essential for metastatic colonization [10,12]. Additional evidence indicates that these bioactive compounds possess antitumour and anti-metastatic properties mediated by diverse chemical constituents, including saponins, glycosaminoglycans, and phenolic compounds, which collectively contribute to the inhibition of cancer progression and spread [12,31]. Taken together, these findings demonstrate that sea cucumber-derived compounds effectively target key steps in CRC metastasis, particularly by inhibiting cell migration and invasion, thereby reducing the likelihood of liver metastasis.

Acquired resistance to fluoropyrimidines (5-FU and capecitabine) and oxaliplatin frequently limits clinical outcomes in advanced CRC, driven in part by compensatory activation of PI3K/Akt and ERK1/2 survival pathways and upregulation of anti-apoptotic proteins [53]. In human CRC cells in vitro, co-treatment with Frondoside A significantly enhanced the growth-inhibitory and pro-apoptotic effects of both 5-FU and oxaliplatin compared with single-agent exposure [13]. Complementary in vivo evidence from pancreatic cancer xenografts (where Frondoside A enhanced gemcitabine efficacy) [46], together with additive effects with butein in A549 lung cancer cells in vitro [50], supports the broader hypothesis that Frondoside A acts as a chemosensitizer across epithelial malignancies by lowering apoptotic thresholds and blunting stress-induced survival signalling.

It has been hypothesized that combining localized colonic release of Frondoside A via pH-responsive microcapsules (FA-MCs) with systemic fluoropyrimidine regimens could enhance local tumour cytotoxicity and potentially permit lower 5-FU doses, thereby mitigating dose-limiting mucositis and myelosuppression [13,14,16–19,53]. However,

dose reduction remains an untested preclinical hypothesis; rigorous pharmacokinetic, maximum-tolerated-dose, and orthotopic in vivo efficacy studies are required before any clinical dose-sparing claims can be substantiated.

Frondoside A counteracts these resistance mechanisms by suppressing Akt and ERK signaling and promoting mitochondrial-mediated apoptosis, thereby sensitizing cancer cells to 5-FU [13]. This results in enhanced caspase activation, increased PARP cleavage, and greater overall apoptotic cell death compared to either treatment alone. Together, these findings suggest that Frondoside A may function as a chemosensitizer, potentially improving therapeutic outcomes while allowing for reduced 5-FU dosage and toxicity.

5. Challenges in Oral and Systemic Administration

5.1. Gastrointestinal Instability

Anionic polymers containing carboxyl groups on their side chains, such as Eudragit L, S, and F, HPMC-P, and HPMC-AS, use a pH-dependent mechanism to remain insoluble in acidic environments (pH 1.5–3.5) while dissolving or swelling in alkaline environments (pH 7.0–8.0) [54]. In the acidic conditions of the stomach, the high proton concentration keeps these carboxyl groups in a protonated, neutral state, facilitating intermolecular hydrogen bonding and hydrophobic interactions that maintain the polymer as an insoluble barrier [54]. Once the polymer reaches the more neutral or alkaline environment of the intestine, the carboxyl groups deprotonate to form negatively charged carboxylate ions. This change triggers electrostatic repulsion between polymer chains, significantly increasing their hydrophilicity and causing the material to swell or dissolve completely [54]. This chemical transition is essential for preventing gastric degradation of acid-sensitive medications, supporting targeted colonic drug delivery, and enhancing the bioavailability of weakly basic drugs by maintaining them in a soluble state for absorption [54].

5.2. Systemic Toxicity and Off-Target Effects

Intravenous (IV) chemotherapy is widely used in cancer treatment because it allows drugs to rapidly enter systemic circulation and bypass gastrointestinal absorption barriers. However, because IV-administered drugs circulate throughout the entire body, healthy tissues are exposed to toxicity, resulting in systemic toxicity and unwanted side effects. Due to the lack of selective or targeted killing, normal tissue may be damaged alongside tumour cells [21]. Common systemic toxicities associated with IV chemotherapy include myelosuppression, neurotoxicity, liver dysfunction, kidney dysfunction, neutropenia and vomiting [21,22]. In addition, many chemotherapeutic agents are metabolized and eliminated through the liver and kidneys, which may contribute to toxicity buildup and further organ dysfunction [55]. IV administration itself may also introduce procedural complications, including phlebitis, infiltration, extravasation, thrombophlebitis, pulmonary complications and infection that may progress to sepsis [55]. Studies have further shown that IV drug administration carries a higher risk and severity of medication errors compared to other administration routes with incompatibilities contributing to treatment complications [55]. Together, these limitations demonstrate how systemic exposure during IV chemotherapy can damage healthy tissues and negatively impact patient safety and treatment tolerance.

Despite widespread systemic distribution, only a limited fraction of intravenously administered chemotherapy successfully accumulates within tumour tissue. Conventional therapeutic approaches suffer from nonspecific targeting, poor distribution and drug resistance which reduces overall treatment efficiency [56]. One of the primary obstacles in IV chemotherapy is achieving sufficient drug concentration at the tumour site without causing side effects in healthy tissues outside the tumor [23]. Solid tumors often

develop abnormal vasculature, poor vascular organization and elevated interstitial fluid pressure (IFP), which impair drug transport into deeper tumor regions [24]. Increased IFP obstructs convective transport and restricts drug diffusion, resulting in limited penetration of chemotherapeutic agents into primary tumors and metastasis [24]. Studies show that many chemotherapeutic drugs penetrate only approximately 100 μm from blood vessels, meaning only cancer cells located close to the vasculature receive therapeutic drug concentrations [24]. In addition, rapid systemic clearance and accumulation of drugs within healthy tissues further reduce localized drug concentration at the tumour site, contributing to reduced therapeutic efficacy and increased off-target toxicity [24,56]. These limitations have driven increasing interest in localized and targeted drug delivery systems designed to improve tumour-specific drug accumulation while minimizing systemic side effects [57].

5.3. The "Delivery Gap" and Gastrointestinal Variability

The oral delivery of therapeutic agents to the colon is significantly limited by multiple physiological and biochemical barriers within the gastrointestinal tract (GIT), creating what is commonly referred to as the "delivery gap." For effective colon-targeted drug delivery, formulations must remain intact during transit through the stomach and small intestine without premature release, degradation, or absorption [14,15,23]. However, conventional oral drug delivery systems often fail to achieve this due to the harsh acidic environment of the stomach (pH 1–3), enzymatic degradation, and variable gastrointestinal transit times, all of which compromise drug stability and bioavailability before reaching the colon [15]. Furthermore, the anatomical and physiological complexity of the GIT, including differences in pH, enzyme activity, and fluid composition across regions, presents significant challenges in ensuring site-specific drug delivery.

In addition to chemical degradation, physical and biological barriers further limit the ability of drugs to reach the colon intact. The gastrointestinal epithelium and mucus layer act as major physiological barriers that restrict drug permeation and absorption, particularly for hydrophilic and macromolecular compounds [14–19]. Tight junctions between epithelial cells reduce paracellular transport, while the mucus layer acts as a viscous, semipermeable barrier that traps and eliminates foreign particles, thereby limiting drug penetration to the intestinal surface. Moreover, variability in gastric emptying time, intestinal transit, and microbial activity introduces additional unpredictability in drug release and targeting efficiency [14–19]. These factors collectively result in premature drug release or insufficient drug concentration at the target site, preventing effective delivery to the colon. Consequently, advanced colon-targeted drug delivery systems, such as pH-sensitive, enzyme-responsive, and microbiota-activated formulations, are necessary to overcome these barriers and ensure that therapeutic agents reach the colon intact and exert localized effects.

A central barrier is the dynamic and variable pH profile of the gastrointestinal tract. Under healthy fasting conditions, luminal pH transitions from highly acidic in the stomach (pH 1.0–3.0) to weakly acidic or neutral in the proximal small intestine (pH 5.5–6.5), reaching pH 6.8–7.5 in the distal ileum and ascending colon before declining slightly to pH 6.0–7.0 in the transverse and descending colon due to microbial fermentation of dietary fibre into short-chain fatty acids [15,16,54]. However, gastrointestinal pH exhibits substantial inter-individual and disease-state variability. Patients with active inflammatory bowel disease (IBD), colitis-associated dysplasia, or advanced colorectal tumours frequently exhibit altered colonic buffering, accelerated transit, and pronounced luminal acidification (pH 4.5–6.0) driven by mucosal inflammation and local lactate accumulation [15,17,58]. Additionally, prior surgical bowel resection, concurrent proton-pump inhibitor therapy, fed versus fasted states, and antibiotic-induced microbiome shifts can alter both

regional pH thresholds and residence times [14–19]. Consequently, single-trigger pH-responsive coatings engineered to dissolve strictly above pH 7.0 risk incomplete release in an acidified diseased colon or premature release during delayed small-intestinal transit.

A central barrier is the changing pH environment of the gastrointestinal tract. After oral administration, therapeutic agents first encounter the acidic stomach, where acid-sensitive drugs, proteins, peptides, and natural bioactive compounds may degrade before reaching the intestine. Although pH-responsive coatings are often used to delay release, this approach is limited because gastrointestinal pH varies between individuals and disease states. For example, inflammation, diet, gastric emptying rate, and microbial activity can alter pH, meaning that a formulation designed to dissolve at a specific pH may release too early in the small intestine or too late after passing the colon [14–19,54].

Enzymatic degradation is another major obstacle. The stomach and small intestine contain digestive enzymes that can break down many therapeutic molecules before they reach the colon. This is particularly problematic for biologics, peptides, polysaccharides, and other structurally sensitive compounds. While the colon has lower levels of some digestive enzymes than the small intestine, the drug must still pass through the upper gastrointestinal tract first. As a result, conventional oral delivery often leads to premature degradation or absorption before the therapeutic agent reaches the intended colonic site [14–19]. Transit time also creates a major delivery challenge. Gastric emptying and intestinal transit are highly variable, depending on food intake, age, disease state, stress, motility, and individual physiology. Time-dependent delivery systems assume that a drug will reach the colon after a predictable number of hours, but this assumption is unreliable. If transit is faster than expected, the formulation may not release enough drug in the colon; if transit is slower, the drug may be released prematurely in the small intestine. This variability makes it difficult to design oral systems that consistently deliver therapeutic agents intact to the colon across different patients [14–19].

The mucus layer and epithelial barrier further limit colonic delivery. Even if a therapeutic compound reaches the colon, it must diffuse through mucus, avoid being trapped or cleared, and interact with the epithelial surface or local immune environment. Large, hydrophobic, or charged molecules may have limited penetration through the mucus barrier. At the same time, epithelial tight junctions restrict paracellular transport, limiting absorption of many compounds.

These barriers are protective under normal physiology, but they also reduce the ability of orally delivered agents to reach deeper tissue targets in the colon [14–19]. The colonic microbiota presents both an opportunity and a limitation. Many colon-targeted systems are designed to exploit bacterial enzymes that degrade specific polysaccharides or prodrugs, allowing drug release in the colon. However, microbiome composition varies significantly between individuals and can be altered by antibiotics, diet, disease, and inflammation. This means microbiota-triggered release may be inconsistent across patients. Therefore, while the microbiota is useful as a biological trigger, it also introduces unpredictability into colon-targeted delivery systems [14–19,23].

Overall, the delivery gap in colon-targeted oral therapy results from the combined effects of acidic pH, enzymatic degradation, variable transit time, premature small-intestinal absorption, mucus restriction, epithelial barriers, and microbiome variability. Current approaches such as pH-sensitive coatings, time-dependent release systems, prodrugs, and microbiota-responsive carriers have improved colon targeting, but none fully overcome the biological variability of the gastrointestinal tract. This creates an important research gap for natural bioactive compounds such as sea cucumber saponins: although their membrane-interactive properties may offer potential advantages for enhancing permeability or localized delivery, future research must determine whether these compounds

can remain stable, reach the colon intact, and function safely within controlled oral delivery systems.

6. pH-Responsive Marine-Biopolymer Functionalization and Colon-Targeted Delivery

6.1. Marine Polysaccharides as Functional Delivery Materials

Marine polysaccharides provide the principal biopolymer link between Frondoside A and colon-targeted formulation. Chitin is an abundant structural polysaccharide in crustacean shells and other marine biomass; controlled deacetylation converts chitin into chitosan, a more soluble and processable cationic polymer. Chitosan contains protonatable amino groups that support mucoadhesion, ionic interactions, film formation, and polyelectrolyte complexation. Its performance depends on marine source, purification, molecular weight, degree and pattern of deacetylation, crystallinity, residual protein and mineral content, and batch consistency. These variables influence solubility, charge density, mechanical strength, biodegradation, biological interactions, and the reproducibility of microcapsules [25,33,35,59].

Alginate, an anionic polysaccharide commonly recovered from brown macroalgae, is composed of mannuronic- and guluronic-acid residues and can be ionically crosslinked with divalent cations to form hydrogels and beads [35]. Alginate can entrap Frondoside A, whereas a chitosan coating can reduce porosity and create a compact polyelectrolyte complex under gastric conditions. At higher intestinal pH, changes in polymer ionization, swelling, and matrix permeability may facilitate release [27]. Other marine polysaccharides—including carrageenans from red algae, ulvan from green algae, and fucoidan from brown algae—may provide sulfate groups, gel-forming capacity, or biological activity, but their inclusion should be based on demonstrated formulation function rather than marine origin alone [60]. Sea-cucumber sulfated polysaccharides and glycosaminoglycans are relevant bioactives, yet they should not be conflated with Frondoside A or with a validated carrier unless direct loading and release data are available.

Functionalization may involve ionic crosslinking, chitosan–alginate complexation, multilayer coating, grafting of pH-responsive groups, or combination with microbiota-degradable polysaccharides. Candidate systems should be compared using polymer provenance and purity, molecular characteristics, fabrication method, particle size, surface charge, Frondoside A loading and encapsulation efficiency, gastric stability, release threshold and kinetics, retained bioactivity, storage stability, mucosal interaction, and batch-to-batch reproducibility [27,33,35,59,60]. Chitosan–alginate systems are especially relevant to the present review because they can combine marine-derived raw materials with tunable protection and release; however, their value for Frondoside A remains a testable formulation hypothesis rather than an established therapeutic platform.

6.2. Principles of pH-Triggered Release: Luminal Colonic pH vs. Acidic TME

Saponins are amphipathic compounds composed of a hydrophilic sugar moiety, known as the glycone, and a lipophilic steroidal or triterpenoid moiety called the sapogenin or aglycone [20]. These components are linked by an O-linked glycosidic bond, which may be hydrolyzed under acidic conditions or by enzymatic or microbial activity. Hydrolysis of the glycosidic bond separates the sugar group from the sapogenin, resulting in structural transformation of the molecule [20]. This process represents a potential mechanism for the degradation of triterpene glycosides and may influence their stability, metabolism, and biological activity under physiological or environmental conditions [20].

As the system transitions into more neutral or slightly alkaline environments in the distal small intestine and proximal colon (pH ~6.8–7.5), ionizable functional groups

undergo deprotonation (or shifts in polyelectrolyte charge balance), increasing polymer hydrophilicity and electrostatic repulsion to drive swelling, pore formation, and controlled payload release [61,62]. Crucially, however, the pH transitions governing gastrointestinal transit and those characterizing the colorectal tumour microenvironment (TME) operate in opposite directions and must be distinguished in formulation design [58,62,63]. Macroscopic colon targeting relies on an alkaline shift from the stomach (pH 1.5–3.5) to the distal ileum/colon (pH 6.8–7.5) to trigger swelling of an outer enteric or alginate–chitosan microcapsule shell [27,54]. Once released into the colonic lumen or mucosal interface, drug carriers encountering solid tumour tissue face an acidic extracellular TME (pH 6.2–6.8, and endosomal/lysosomal pH 5.0–5.5) generated by aerobic glycolysis (the Warburg effect) and poor vascular perfusion [58,63]. Simple pH-induced capsule swelling in the colonic lumen increases regional mucosal exposure but does not automatically confer selective release inside an acidic TME. Achieving secondary TME-specific release requires hierarchical or dual-responsive architectures—such as releasing acid-labile aliphatic polycarbonate or pH-responsive block-copolymer nanoparticles from a macroscopic enteric capsule, where mild TME acidosis (pH ~6.5) triggers side-chain protonation, micellar disassembly, or accelerated drug release directly at the tumour cell surface [63,64].

As the system transitions into more alkaline environments such as the small intestine or colon (pH ~7.0–8.0), these functional groups become deprotonated, increasing polymer hydrophilicity and causing electrostatic repulsion between polymer chains. This leads to polymer swelling, pore formation, or complete dissolution, allowing controlled release of the encapsulated drug. Mechanistically, this process is driven by changes in polymer charge density and osmotic pressure, which alter the structural integrity of the delivery matrix [61,62]. In colorectal cancer, this enables site-specific drug delivery, ensuring that compounds like Frondoside A are released preferentially in the colon, improving therapeutic efficacy while minimizing degradation and systemic toxicity.

6.3. Encapsulation Technologies and Biopolymer Selection

Insights from emerging antibody–drug conjugates (ADCs) and targeted nanomedicines in gastrointestinal oncology underscore that pairing potent cytotoxic or signalling-inhibitory payloads with tumour-selective surface recognition (e.g., targeting CEACAM5, MET, or Claudin-family antigens) markedly widens the therapeutic window in gastrointestinal adenocarcinomas [65]. For an amphiphilic, membrane-permeabilizing saponin such as Frondoside A—which carries a risk of off-target hemolysis or mucosal irritation if released indiscriminately—encapsulating the payload within a dual-stage carrier (an outer pH/microbiota-responsive alginate–chitosan microcapsule protecting an inner ligand-decorated nanoparticle) represents a rational strategy to combine macroscopic colonic protection with receptor-mediated tumour cell internalization [27,63–67].

While passive pH- and microbiota-triggered mechanisms govern regional delivery to the colonic lumen, integrating active targeting ligands onto secondary nano- or microcarriers can enhance cellular selectivity within the colorectal tumour microenvironment [65–67]. Colorectal carcinoma cells overexpress several surface receptors relative to healthy colonocytes, offering opportunities for ligand-functionalized marine-biopolymer systems: (i) CD44 receptor targeting, in which hyaluronic acid (HA)—a natural glycosaminoglycan with high affinity for CD44 overexpressed on CRC stem-like and invasive cells—is electrostatically layered onto cationic chitosan carriers to promote receptor-mediated endocytosis following outer enteric-shell dissolution [33,66]; (ii) folate receptor-α (FR-α) and epidermal growth factor receptor (EGFR) targeting, achieved by conjugating folic acid or anti-EGFR ligands onto chitosan or alginate-modified nanocarriers to enhance intracellular uptake and bypass efflux-mediated resistance [33,65,66]; and (iii) epithelial cell adhesion molecule (EpCAM) or integrin $\alpha v\beta 3$ targeting using EpCAM-binding

aptamers or RGD peptides to anchor carriers to malignant epithelial cells and angiogenic tumour vasculature [66,67].

6.4. Active Receptor-Mediated Targeting Strategies

Encapsulation technologies for colon-targeted delivery should be evaluated according to polymer origin, production and purification method, molecular characteristics, fabrication process, Frondoside A loading, encapsulation efficiency, particle size, surface properties, gastric stability, release threshold, release kinetics, and compatibility with the bioactive compound. Chitosan–alginate systems are attractive because electrostatic complexation can produce a compact barrier under gastric conditions and a more permeable matrix after transition to intestinal conditions [27]. However, chitosan molecular weight and degree of deacetylation, alginate mannuronic-to-guluronic acid ratio, crosslinking-ion concentration, and coating architecture strongly influence swelling and release [27,33,35,59]. Chitin itself is less soluble and is generally converted to chitosan before pharmaceutical processing, although chitin-derived nanomaterials may provide reinforcement or surface functionality [25]. Carrageenan, fucoidan, ulvan, pectin, dextran, and resistant starch offer complementary gel-forming or microbiota-responsive properties, whereas synthetic enteric polymers provide more sharply tunable dissolution thresholds [60]. Evidence from generic payloads cannot establish the behaviour of Frondoside A; direct comparative studies are therefore required to determine loading stability, simulated gastrointestinal release, retained anticancer activity, mucosal interaction, and formulation reproducibility.

6.5. The Colorectal Tumour Microenvironment

The colorectal tumour microenvironment (TME) is a complex and dynamic ecosystem composed of cancer cells, immune cells, stromal fibroblasts, extracellular matrix (ECM), cytokines, and abnormal vasculature that collectively regulate tumour growth, invasion, metastasis, and therapeutic response [68]. Chronic inflammation is a defining feature of colorectal cancer, with elevated levels of pro-inflammatory cytokines, chemokines, and growth factors promoting tumour progression, angiogenesis, and immune evasion [69]. In addition, colorectal tumours exhibit increased production of reactive oxygen species (ROS), which contribute to oxidative stress, genomic instability, epithelial-to-mesenchymal transition (EMT), and metastatic progression [70]. The tumour microenvironment is also characterized by an acidic extracellular pH resulting from altered tumour metabolism, which influences cancer-cell proliferation, invasion, and resistance to therapy [58]. These pathological characteristics create an opportunity for localized drug-delivery systems, particularly pH-responsive formulations that remain stable during passage through the upper gastrointestinal tract before releasing their therapeutic payload in the colon. By exploiting these features of the colorectal microenvironment, colon-targeted delivery systems may enhance local drug accumulation while minimizing systemic toxicity. This strategy is particularly relevant to Frondoside A, whose pro-apoptotic, anti-inflammatory, and anti-metastatic properties could be maximized through localized release within the tumour microenvironment, thereby improving therapeutic efficacy and reducing off-target effects.

7. Translational Outlook and Future Directions

Colon-targeted, pH-responsive delivery systems enhance the therapeutic efficacy of anticancer agents by preserving drug integrity during gastrointestinal transit and allowing localized release to cells within the tumour microenvironment. Studies have shown that nanocarriers enhance the apoptotic activity of encapsulated bioactive compounds compared with free drugs [71]. For instance, pH-responsive artesunate polymer prodrugs

demonstrated enhanced induction of apoptosis-associated proteins and reactive oxygen species generation in a colon-cancer xenograft model relative to the free drug [71].

The pH-responsive behaviour of alginate–chitosan microcapsules arises from reversible electrostatic interactions between oppositely charged polymers. Under acidic gastric conditions, protonation of chitosan and suppression of alginate ionization result in a compact, low-permeability network that restricts enzyme diffusion [27,29,72]. At intestinal pH, deprotonation of chitosan and re-ionization of alginate disrupt the polyelectrolyte complex, leading to swelling, increased porosity, and controlled release of encapsulated bioactives [27,29,72]. Therefore, controlled and precise release via specialized microcapsules may enhance the apoptotic effect of drugs targeting colorectal cancer.

The diverse biological activities of Frondoside A suggest several promising preclinical avenues for translational investigation. Within colorectal oncology, its ability to promote caspase-dependent apoptosis, inhibit invasion, and suppress pro-survival PI3K/Akt and ERK1/2 signalling indicates potential utility as a chemosensitizing adjunct to standard fluoropyrimidine- or oxaliplatin-based regimens [7,13]. While preclinical in vitro data raise the hypothesis that localized colonic co-delivery via pH-responsive microcapsules could enhance antitumour efficacy and potentially allow lower systemic chemotherapy doses, validating this hypothesis requires comprehensive pharmacokinetic, mucosal safety, and orthotopic in vivo efficacy studies [7,73].

This targeted "smuggling" of intact drugs to tumour tissues significantly enhances therapeutic efficacy by increasing intracellular drug concentration and maximizing apoptotic effects. Targeted DDS exploit mechanisms such as receptor-mediated uptake, endocytosis, and intracellular trafficking to ensure that drugs are delivered directly into tumour cells while minimizing exposure to healthy tissues [66,74]. The cited study reports that targeted delivery systems use strategies such as the enhanced permeability and retention effect and ligand–receptor interactions to achieve selective tumour accumulation and controlled drug release [66]. This localized accumulation enables higher drug concentrations within cancer cells, which in turn enhances cytotoxic activity and promotes apoptosis. Furthermore, as illustrated in Figure 1 of the cited study [67], nanoscale carriers can be absorbed through the gastrointestinal epithelium and transported systemically to reach distant organs, including tumours, where they release their payload in a controlled manner. Collectively, these findings demonstrate that improving bioavailability through targeted delivery not only increases drug stability and tumour specificity but also maximizes apoptotic effects by ensuring efficient intracellular drug delivery.

Clinical Translation Potential

The diverse biological activities of Frondoside A suggest several promising clinical applications beyond its experimental anticancer effects. One of the most significant applications is its potential use as an adjunctive therapy for colorectal cancer, where it may complement existing chemotherapeutic regimens. Experimental studies demonstrate that Frondoside A enhances apoptosis, inhibits tumour cell proliferation and metastasis, and suppresses pro-survival signaling pathways such as PI3K/Akt and ERK. These complementary mechanisms indicate that Frondoside A could function as a chemosensitizer, improving the efficacy of standard agents such as 5-fluorouracil while potentially allowing lower therapeutic doses and reducing treatment-associated toxicity. Furthermore, its reported selectivity toward malignant cells suggests a more favorable therapeutic index than conventional cytotoxic drugs, making it an attractive candidate for incorporation into combination treatment strategies. With the development of pH-responsive, colon-targeted delivery systems, Frondoside A could achieve localized drug release within the colorectal tumour microenvironment, maximizing therapeutic efficacy while minimizing systemic exposure [7,73].

Beyond oncology, the broad pharmacological profile of Frondoside A supports potential applications in several other clinical settings. Its ability to inhibit inflammatory mediators, including NF-κB, MAPK signaling, tumour necrosis factor-α (TNF-α), and cyclooxygenase enzymes, suggests therapeutic potential for chronic inflammatory disorders such as inflammatory bowel disease, where localized anti-inflammatory treatment is desirable. In addition, its antiplatelet and anticoagulant properties may have implications for preventing cancer-associated thrombosis, a common complication in patients with advanced malignancies. Preclinical evidence demonstrating neuroprotective effects through the reduction in oxidative stress and neuroinflammation also suggests potential applications in neurodegenerative disorders, including Alzheimer's disease and ischemic brain injury. Although these therapeutic indications remain in the preclinical stage, the multifunctional biological activity of Frondoside A highlights its potential as a versatile marine-derived therapeutic agent with applications extending beyond colorectal cancer [7,73].

8. Conclusions

Frondoside A is a biologically active marine triterpene glycoside with preclinical relevance to colorectal cancer, whereas chitin, chitosan, alginate, and related marine polysaccharides provide the biopolymer platform needed to translate that bioactivity into a controllable oral formulation. This review therefore positions the compound and the carrier as distinct but complementary components: Frondoside A is the therapeutic payload; marine polysaccharides are produced, characterized, and functionalized to protect it during gastrointestinal transit; and localized colonic delivery is the intended biomedical application. Evidence supports the effects of Frondoside A on apoptosis, proliferation, invasion, inflammatory signalling, and responses to selected chemotherapeutic agents, but clinical superiority and tumour selectivity have not been established.

Translation will require standardized sourcing, extraction, purification, and analytical specifications for both Frondoside A and its biopolymer carriers. For chitin and chitosan, molecular weight, degree of deacetylation, residual impurities, and batch variability should be reported; for alginate, composition, viscosity, and crosslinking behaviour require equivalent attention. Formulation studies should quantify loading capacity, encapsulation efficiency, gastric protection, pH- and microbiota-dependent release, retained bioactivity, local tissue exposure, systemic absorption, storage stability, and safety. pH-responsive encapsulation may increase colonic exposure, but regional colon targeting should not be equated with selective release in an acidic tumour microenvironment. Until compound-specific formulations are validated, the most defensible conclusion is that Frondoside A-loaded marine-polysaccharide microcapsules represent a promising preclinical platform at the intersection of marine-biopolymer production, functionalization, and biomedical application.

Author Contributions: Conceptualization, A.R.; validation, A.R.; writing—original draft preparation, D.D., Z.C., E.M., A.J., N.I., M.O., B.W., C.D., L.Z. and A.R.; writing—review and editing, A.R. and D.D.; visualization, D.D., Z.C., E.M., A.J., N.I., M.O., B.W. and C.D.; supervision, A.R.; project administration, A.R.; funding acquisition, A.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding; all expenses were covered by CCCR's internal research funds.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study.

https://doi.org/10.3390/md24120430

Conflicts of Interest: The authors declare no conflicts of interest.

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