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Current Insights on The Therapeutic Potentials of *Aplysia Species*, A Unique Marine Bioresource For Bioactive Molecules- A Minireview

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ABSTRACT
Notably, the marine mollusk *Aplysia* species were commonly referred as the sea hare by malacologists has now emerged as a significant bioresource for the discovery of novel bioactive compounds with significant therapeutic potential. This mini-review emphasizes the diverse secondary metabolites isolated from Aplysia species, including alkaloids, terpenoids, peptides and proteins, which exhibit antimicrobial, anticancer, anti-inflammatory and neuroprotective properties. Especially, *Aplysia* secretions and egg masses contain unique molecules such as aplysiatoxins, kahalalides and lectins, which have demonstrated promise in drug development. For instance, Kahalalide F has progressed to clinical trials as a potential anticancer agent, while derivatives of aplysiatoxin exhibit potent modulation of protein kinase C. Furthermore, neurons of Aplysia serve as a model system in neurobiological research, contributing to advancements in studies of synaptic plasticity and memory. Despite their pharmacological potential, challenges such as sustainable sourcing, compound stability and toxicity profiling persist. Recent advances in marine biotechnology, including aquaculture and synthetic biology, may address these limitations, facilitating the scalable production of Aplysia-derived therapeutics. Thereby, this review would surely underscore the importance of further exploration into *Aplysia*-derived bioactive molecules, highlighting their role in future drug discovery and biomedical applications.

Keywords: Marine pharmacology, Anticancer agents, Neuroprotective compounds, Marine Bioactive compounds, Drug discovery

1. INTRODUCTION

The marine environment harbors a vast diversity of organisms that serve as an essential reservoir of biologically active metabolites with significant pharmacological potential (Zapata & Amemiya, 2000). These marine-derived compounds exhibit a broad spectrum of bioactivities, including antimicrobial, antifungal, cytotoxic, antitumor and anti-inflammatory properties (Shaw *et al.,* 1976). Many of these molecules, classified as secondary metabolites, function as ancient defense mechanisms and can be categorized into steroids, terpenoids, isoprenoids, quinones, brominated compounds, nitrogen heterocyclics and nitrogen-sulfur heterocyclics (Faulkner, 2002). Marine invertebrates—such as poriferans (sponges), cnidarians (corals), molluscs and echinoderms—have been particularly prolific sources of bioactive compounds with antiviral, antiprotozoal, antihelminthic and anticancer applications (Blunt *et al.,* 2018).

The systematic exploration of marine natural products (MNPs) began in the 1950s, with early efforts focused on identifying antimicrobial agents from marine organisms (Shaw *et al.,* 1976). Over the decades, extensive research has documented over 1,334 secondary metabolites isolated from molluscs alone between 1984 and 2019 (Blunt *et al.,* 2018; Carroll *et al.,* 2019). Among molluscs, gastropods have been the most chemically studied, yielding 948 compounds, followed by bivalves (190 compounds) and cephalopods (24 compounds) (Avila, 2006; Benkendorff, 2010).

Within the gastropods, sea hares of the genus *Aplysia* stand out as a particularly rich source of structurally diverse and pharmacologically active metabolites (Pereira *et al.,* 2019). Over the past 50 years (1963–2019), Aplysia has emerged as the most extensively studied molluscan genus in marine natural product chemistry, with 36 recognized species (Pereira, 2019). Notable species include *Aplysia fasciata, Aplysia punctata*, and *Aplysia depilans*, which are common in the Iberian Peninsula and Portuguese waters (Pereira, 2019). These herbivorous mollusks accumulate halogenated terpenoids, alkaloids, and peptides, many of which exhibit antimicrobial, antitumor, and immunomodulatory properties (Pereira *et al.,* 2019).

In addition to their chemical richness, *Aplysia* species employ both passive and active chemical defense strategies against predators (Mona *et al.,* 2016). Passive defenses include the production of distasteful or toxic compounds in their skin, deterring predation. Active defenses involve the release of ink and opaline secretions upon attack, which contain bioactive molecules that disrupt predator sensory systems (Mona *et al.,* 2016). These defense mechanisms not only

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ensure survival but also provide a treasure trove of bioactive compounds for biomedical research.
Given the pharmacological potential of *Aplysia*-derived metabolites and their role in drug discovery, this review aims to:

1. Summarize key bioactive compounds isolated from *Aplysia* species.
2. Evaluate their therapeutic applications, including anticancer, antimicrobial, and neuroprotective effects.
3. Discuss challenges (e.g., sustainable sourcing, toxicity) and future directions (e.g., synthetic biology, omics technologies).

By consolidating current knowledge, this minireview seeks to highlight the untapped potential of *Aplysia* in marine biodiscovery and pharmaceutical development.

2. Bioactive Compounds from Aplysia

The genus *Aplysia*, commonly known as sea hares, has emerged as one of the most pharmacologically significant marine mollusks due to its remarkable diversity of bioactive secondary metabolites. These soft-bodied gastropods have evolved sophisticated chemical defense mechanisms that produce an extraordinary array of compounds with demonstrated therapeutic potential (Pereira *et al.,* 2019). Over the past fifty years, extensive research has revealed that *Aplysia species* produce metabolites exhibiting antimicrobial, anticancer, anti-inflammatory and neuroprotective properties (**Figure-1**), making them valuable sources for drug discovery (Ciavatta *et al.,* 2017). This section provides a detailed examination of these bioactive compounds, organized by their chemical classification, biosynthetic origins and unique structural features that contribute to their biological activities.

Fig.-1 Neurons of Aplysia as a model system in neurobiological research

2.1. Major Chemical Classes

2.1.1. Alkaloids

The alkaloids isolated from *Aplysia* species represent one of the most pharmacologically important classes of compounds. These nitrogen-containing secondary metabolites are primarily derived from the sea hare's diet of cyanobacteria and algae, though some appear to be modified or produced endogenously (Fujiki *et al.,* 2018).
The aplysiatoxins, including debromoaplysiatoxin and oscillatoxin, are among the most studied compounds from this group. These molecules are potent activators of protein kinase C (PKC), a key enzyme in cellular signaling pathways (Fujiki *et al.,* 2018). Interestingly, aplysiatoxins demonstrate a paradoxical dose-dependent effect: at high concentrations they act as tumor promoters, while at controlled doses they exhibit anticancer properties through induction of apoptosis in malignant cells (Fujiki *et al.,* 2018). This dual nature makes them particularly interesting for targeted cancer therapies.

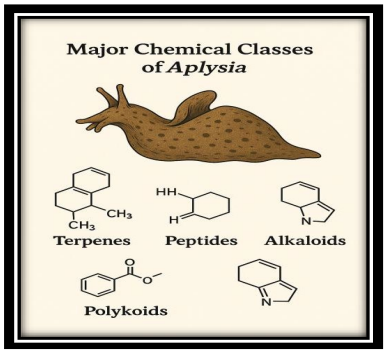

Fig.-2: Major Chemical Classes of *Aplysia*

Another significant group of alkaloids are the tambjamines, characterized by their distinctive pyrrolic structure. These compounds have shown remarkable activity against multidrug-resistant bacterial strains, including methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin-resistant Enterococcus (VRE) (Ciavatta *et al.,* 2017). The mechanism of action appears to involve disruption of bacterial cell membranes, making them promising leads for novel antibiotics.
Recent discoveries have expanded the known diversity of Aplysia alkaloids. Li *et al.,* (2020) identified aplysiasecosterol A from *Aplysia kurodai,* a novel secosteroid alkaloid that demonstrates potent anti-inflammatory activity through suppression of NF-κB signaling pathways. This compound reduced production of pro-inflammatory cytokines (TNF-α, IL-6) in macrophage cell lines at nanomolar concentrations, suggesting potential applications in treating inflammatory diseases (Li *et al.,* 2020).

2.1.2. Peptides
Peptide compounds from *Aplysia* have attracted considerable pharmaceutical interest due to their unique structures and potent biological activities. The most clinically advanced of these are the kahalalides, a family of cyclic depsipeptides (Faircloth & Cuevas, 2006).
Kahalalide F has shown particular promise, progressing to phase II clinical trials for treatment of prostate and breast cancers (Faircloth & Cuevas, 2006). Its mechanism involves selective disruption of lysosomal membranes in tumor cells while sparing normal cells, representing a novel approach to cancer therapy (Suárez *et al.,* 2019). Recent studies have revealed that this selectivity may be due to differences in cholesterol content between cancerous and normal cell membranes (Suárez *et al.,* 2019).
Aplysia-derived lectins constitute another important peptide group with demonstrated antiviral properties. Hiroaki *et al.,* (2019) characterized several lectins that effectively inhibit HIV and herpes simplex virus (HSV) infection by binding to viral envelope glycoproteins and preventing host cell attachment. These lectins show remarkable stability and low toxicity, making them attractive candidates for topical antiviral applications (Hiroaki *et al.,* 2019).

2.1.3. Terpenoids and Sterols
The terpenoid compounds from *Aplysia* are notable for their structural complexity and diverse biological activities. These compounds are primarily sequestered from the sea hare's diet of red algae and cyanobacteria, though some modification occurs within the mollusk (Gavagnin *et al.,* 2006).
Halogenated terpenoids such as aplydactone and aplysiaterpenoid A exhibit significant anti-inflammatory and antitumor properties (Gavagnin *et al.,* 2006). Pereira *et al.,* (2019) demonstrated that these compounds inhibit cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), key enzymes in inflammatory pathways. The presence of bromine or chlorine atoms in these molecules appears crucial for their bioactivity, enhancing receptor binding affinity and metabolic stability (Gunasekera *et al.,* 2017).
The sterols from *Aplysia*, particularly the aplykurodinones, have shown neuroprotective effects in models of neurodegenerative diseases (Yamada *et al.,* 2015). These compounds appear to modulate cholesterol metabolism in neural cells and reduce oxidative stress, potentially offering new approaches to treating conditions like Alzheimer's disease (Yamada *et al.,* 2023).

2.1.4. Proteins and Enzymes
Aplysia produces several bioactive proteins that serve defensive functions and have potential therapeutic applications. Escapin, an L-amino acid oxidase found in the ink secretion, generates hydrogen peroxide and reactive oxygen species that deter predators and inhibit microbial growth (Yang *et al.,* 2018). Recent studies suggest escapin may have applications in wound healing due to its ability to stimulate tissue remodeling (Yang *et al.,* 2018).
Proteomic studies have identified additional enzymes such as aplysinolysin, which shows specific lytic activity against Gram-positive bacterial pathogens (Bezerra *et al.,* 2020). This enzyme represents a potential alternative to conventional antibiotics, particularly for treating drug-resistant infections (Bezerra *et al.,* 2020).

2.2. Biosynthetic Origins
The biosynthetic origins of *Aplysia* metabolites reveal fascinating ecological and evolutionary relationships. While many compounds are clearly derived from dietary sources (cyanobacteria, algae), genomic evidence suggests some metabolic pathways may have been acquired through horizontal gene transfer from symbiotic microorganisms (Liu *et al.,* 2021).
The sea hare demonstrates remarkable metabolic plasticity in modifying dietary precursors. Kamio *et al.,* (2010) documented the conversion of algal metabolites into more potent defensive compounds, including the transformation of phycoerythrobilin into the deterrent pigment aplysioviolin. This biosynthetic capability significantly expands the chemical diversity (**Figure-2**) available from *Aplysia* species (Kamio *et al.,* 2010).
Recent advances in 'omics' technologies have begun to unravel the complex metabolic networks in *Aplysia*. Liu *et al.,* (2021) used transcriptomic and metabolomic approaches to identify genes involved in secondary metabolite production, revealing both conserved and novel biosynthetic pathways.

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2.3. Structural Uniqueness and Bioactivity

The structural features of *Aplysia* metabolites directly contribute to their biological activities and therapeutic potential:

- **Halogenation:** The presence of bromine or chlorine atoms, uncommon in terrestrial natural products, enhances receptor binding and metabolic stability (Gunasekera *et al.,* 2017). For example, halogenated terpenoids show significantly greater anti-inflammatory activity than their non-halogenated analogs.
- **Macrocyclic structures:** Peptides like kahalalides benefit from cyclic structures that confer resistance to proteolytic degradation while maintaining membrane permeability (Suárez *et al.,* 2019). This property is crucial for their pharmaceutical potential.
- **Mixed biosynthetic origins:** Hybrid compounds combining polyketide and peptide structural elements create novel pharmacophores with unique biological activities (Liu *et al.,* 2021). These molecules often exhibit improved target specificity compared to simpler structures.

The combination of these structural features results in compounds with remarkable biological selectivity and potency. For instance, the specific halogenation pattern of aplysiaterpenoid A enhances its binding to inflammatory enzymes while minimizing off-target effects (Gavagnin *et al.,* 2006). Similarly, the macrocyclic structure of kahalalide F contributes to its selective toxicity toward cancer cells (Suárez *et al.,* 2019).

These structural characteristics, combined with the diverse biological activities discussed earlier, make *Aplysia* metabolites particularly valuable as leads for drug development. Their evolutionary optimization for biological activity in marine ecosystems translates to significant pharmacological potential for human medicine.

3. Pharmacological Potential and Mechanisms

Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of pharmaceutical agents (Iwamoto *et al.,* 1998; Iwamoto *et al.,* 1999; Iwamoto *et al.,* 2001). Pharmaceutical industries now recognize the vast variety of ocean organisms, each possessing distinct biological characteristics. Sea hares, for example, are marine organisms that use bioactive chemicals to defend against predators, including their eggs (Liguez & Tabugo, 2023). The sea slug is known to possess anti-cancer, anti-tumor and anti-viral compounds which are very useful in the pharmacological industry (Sethi *et al.,* 2014). The effects of antidepressants and neuroleptics on postsynaptic iontophoretic responses to acetylcholine and dopamine have been examined in identified neurons of the central nervous system of *Aplysia californica* (Davies, 1981). The relative simplicity of Aplysia's nervous system and the ease with identified neurons can be shown to participate in specific behavioural changes that have enabled a rich series of discoveries about fundamental mechanisms of neuronal plasticity (Abrams, 2012). Numerous investigations into the chemical makeup and biological characteristics of sea hare secondary metabolites have revealed that they have cytotoxic, antibacterial, antifungal, and antiviral effects, as well as antifeedant actions (Ruaza, 2022) (**Figure-3**). Sea hares are increasingly being evaluated as a natural source of antioxidants that benefit human health in the food sector. The presence of phenolics and flavonoids indicates a broad range of antimicrobial drug spectrum with high antibacterial action against gram-positive and gram-negative microorganisms. It is phenols' partly hydrophobic characteristic that causes this, which makes them antimicrobial. It either deactivates or inhibits hydrolytic enzymes or microbial adhesions as proteases (Baba & Malik 2015). Neuroscience Meeting at Scripps, Florida suggests that investigations in Aplysia may more directly lead to improved strategies for treatment of human disorders (Abrams, 2012). The majority of secondary metabolites isolated from sea hares of the genus *Aplysia* are halogenated terpenes often exhibiting pharmacological properties such as cytotoxic, antibacterial, antifungal, antiviral and/or antifeedant activities (Pereira *et al.,* 2016) **(Table-1)**.

Anti-microbial activity of Aplysia

Aplysia depilans gonad lectin was previously used to study the distribution of galacturonic acids in the cell walls of some pathogenic fungi (Benhamou, 1989). It can be postulated that certain polysaccharides present in fungal cell walls interacted with these lectins and inhibited their growth by disturbing spore germination, growth of mycelium, and synthesis of chitin, to alter the fungal cell wall (Gomes *et al.,* 2012). Another antifungal protein (Aplysianin E) from *Aplysia kurodai* eggs completely suppressed the growth of *Saccharomyces cerevisiae* and *Candida albicans* at a concentration of 16 µg/mL (Iijimaa *et al.,* 1995). Swarna *et al.,* (2021) reported that the high concentration (400 µg/mL) of AKL-40 totally inhibited the growth of *Talaromyces verruculosus*. A study by Ruperez *et al.,* (1986) reported the presence of galactose sugars in the cell wall of *Talaromyces verruculosus*, which justified our findings. *Aplysia depilans* gonad lectin was previously used to study the distribution of galacturonic acids in the cell walls of some pathogenic fungi (Benhamou 1989). Antifungal activity was exhibited by Aplysianin E (AKE), an antineoplastic and antibacterial glycoprotein purified from the eggs of *Aplysia kurodai*. AKE completely suppressed growth of the yeast form fungi, *Saccharomyces cerevisiae* A 5 8 1 A, *Schizosaccharomyces pombe* JY1 and *Candida albicans* ATCC 36232 at a concentration of over 16 µg/mL. The colony-forming abilities of the fungi were also significantly decreased after contact with AKE. These results indicate that AKE has an anti-fungal property and that its mode of action is fungicidal (Iijimaa *et al.,* 1995). Dolabellanin A (DAA), the antineoplastic and antibacterial glycoprotein purified from the albumen gland of *Dolabella auricularia*, showed an antifungal activity. DAA suppressed fungal growth completely at a concentration of over 2 micrograms/ml, and the colony forming ability of the fungus was significantly decreased after

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contact with DAA. These results indicate that DAA is an antifungal protein and its mode of antifungal activity is fungicidal (Iijimaa *et al.,* 1994). The potential antibacterial properties of sea hare species (*Dolabella auricularia*) found in Pujada Bay, Philippines, egg strings were collected and extracted using hexane and methanol solvents. The antibacterial activity of each fraction was then determined through Minimum Inhibitory Concentration (MIC) testing against four potentially pathogenic bacteria, two gram-positive strains (*Staphylococcus aureus, Bacillus subtilis*) and two gram-negative strains (*Escherichia coli, Pseudomonas aeruginosa*). The broth microdilution method was employed to assess the antibacterial activity of *Dolabella auricularia* egg strings. The study revealed the MIC values for the four bacterial strains. The hexane extracts of both extract 1 and extract 2 exhibited a MIC of 0. 23 and 0.33 mg/mL and a MIC of 0.52 and 0.125 mg/mL against *P. aeruginosa*, respectively. The methanolic extracts (1 and 2) displayed a MIC of 1.46 and 2.83 mg/mL against *E. coli* and an even more potent MIC of 1.33 and 0.79 mg/mL against *P. aeruginosa*. In the case of *B. subtilis*, the hexane extracts (1 and 2) had a MIC of 1.5 and 0.54 mg/mL, while the methanolic extracts (1 and 2) exhibited a MIC of 1.17 mg/mL and 0.83 mg/mL. Lastly, against *S. aureus*, hexane extracts (1 and 2) suppressed the growth with a MIC of 0.77 mg/mL and 0.25 mg/mL, respectively, while both methanolic extracts (1 and 2) demonstrated a MIC of 3.33 mg/mL. These findings showcase the promising antibacterial activity of *Dolabella auricularia* egg string extracts and highlight their potential for further investigation and development in the pharmaceutical field (Liguez & Tabugo, 2023). The antibacterial and antifungal activities of *Aplysia faciata* were investigated via the standard techniques. Data obtained revealed that the highest antibacterial activity was detected against *P. aeruginosa* (AU = 3.4), followed by *E. coli* (AU = 2.9), then by *B. subtlis* (AU = 2.7). The other bacterial pathogens were not affected at all. Likewise, the maximum fungal suppression, via the pouring method, was observed against *P. crustosum* (50%). AUs against both *F. solani* and *A. niger* were 20 and 10%, respectively, while there was no activity recorded against the others. Also, the antifungal activity via the well-cut diffusion method conducted that the highest AU (6.8) was recorded against *A. flavus*, followed by AU = 4.8 against *F. solani*, then 1.8 against *P. crustosum*. Moreover, the antifungal AU against reference yeast strains ranged between 3.1 and 6.8. The highest one was recorded against C. tropicalis, followed by AU (4.8) against *R. mucilaginosa*. Regarding investigating the efficacy of some commercial antibiotics (mm), data confirmed that the Gram-negative bacteria were more resistant than Gram-positive bacteria (*Hassan et al.,* 2020). The isolation of laurinterol acetate, laurinterol, debromolaurinterol acetate, and debromolaurinterol (Irie *et al.,* 1966 and 1970) from the sea hare, *Aplysia kurodai*. Among the four metabolites, laurinterol acetate, laurinterol, and debromolaurinterol showed moderate cytotoxicity against tumor cells, and laurinterol and debromolaurinterol exhibited more potent antibacterial activity against *Staphylococcus aureus* than laurinterol acetate and debromolaurinterol acetate (Tsukamoto *et al.,* 2005).

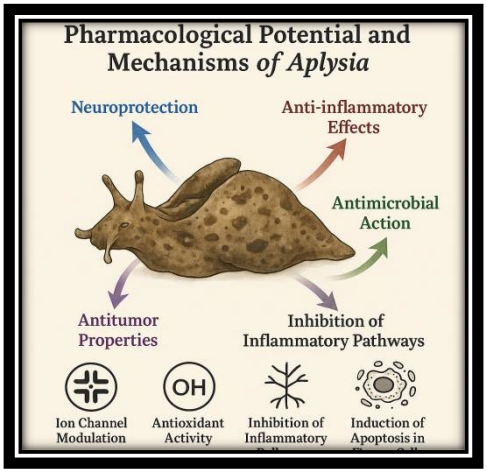

Fig.-3 Pharmacological Potential and Mechanisms of Aplysia

Anti-tumour properties of Aplysia

Lee *et al.,* (2016) have demonstrated the anti-cancer effects of Sea hare eggs (SE) in U937 cells and its major active components. The aqueous extract of SE (ASE), which contained the highest protein content, dose-dependently inhibited the cancer cell's growth (IC50 value, 10.42 ± 0.5 µg/mL). Additionally, ASE markedly caused DNA damage by inducing apoptotic body formation, DNA fragmentation, and accumulation of sub-G1 DNA contents. ASE induced apoptosis by activating caspase-3 and 9 and poly (ADP-ribose) polymerase (PARP) by regulating the expression of Bcl-2/Bax. Moreover, among its molecular weight fractions, the > 30 kDa fraction showed the highest cell-growth-inhibitory effects, which was inhibited by heat treatment. Furthermore, the > 30 kDa fraction had markedly higher glycine content than the ASE. The presence of two protein bands at around 16 and 32 kDa was identified. In addition, two fractions, F1 and F2 were obtained using anion-exchange chromatographic techniques with the F1 having an improved cell-growth-inhibitory effect than the > 30 kDa fraction. Taken together, these results suggest that the ASE

contains glycine-rich proteins, including the active 16 and 32 kDa proteins, which account for its anti-cancer effects by inducing apoptosis via regulation of the mitochondrial pathway.

In India, sea slugs are been used for the extraction of natural anti-cancer compounds like Soblidotin, Synthadotin/ILX651, Cemadotin, and Kahalalide F (Haefner, 2003). Novel antitumor and antimicrobial glycoproteins were found in the sea hares. These glycoproteins were purified to apparent homogeneity from *Aplysia kurodai*, *Aplysia Juliana* and *Dolabella auricularia*, and designated as aplysianins, julianins and dolabellanins, respectively. The nine isolated glycoproteins lysed all the tumor cells tested but did not lyse normal white and red blood cells. The glycoproteins completely inhibited the synthesis of DNA and RNA by tumor cells within 2 hr and caused tumor lysis within 15hr. Tumor lysis was inhibited by the presence of N-acetylneuraminic acid, suggesting that the recognition of the sugar moiety is a key step in the cytolysis by antitumor glycoproteins from sea hares. These antitumor glycoproteins, except dolabellanin P, also showed antimicrobial activities (Yamazaki, 1993).

Hyeon *et al.,* (2024) study highlighted the anticancer potential of sea hare hydrolysate (SHH), particularly its role in regulating macrophage polarization and inducing pyroptotic death in lung cancer cells through the inhibition of signal transducer and activator of transcription 3 (STAT3). These findings prompted to investigate additional features of immune-oncology (I-O) agents or adjuvants, such as programmed cell death protein 1 (PD-1)/programmed death ligand 1 (PD-L1) inhibition and their association with rheumatoid arthritis (RA) risk, to explore the potential of SHH as an I-O agent or adjuvant. In this study, they investigated the effects of SHH on PD-L1 levels in various cancer cell types and assessed its effectiveness in treating RA, a common side effect of I-O agents. The results showed a marked reduction in PD-L1 levels in multiple cancer cell lines and decreased PD-1 and PD-L1 levels in tumor-associated macrophages.

Sea hare-derived compounds induce macrophage activation and reduce asthmatic parameters in mouse models of allergic asthma. The findings help to study the role of sea hare hydrolysates (SHH) in cancer pathophysiology. SHH treatment-induced M1 macrophage activation in RAW264.7 cells, peritoneal macrophages, and THP-1 cells, as did lipopolysaccharide (LPS) (+ INF-γ), whereas SHH reduced interleukin (IL)-4 (+IL-13)-induced M2 macrophage polarization. In addition, SHH treatment inhibited the actions of M1 and M2 macrophages, which have anticancer and pro-cancer effects, respectively, in non-small cell lung cancer cells (A549 and HCC-366) and tumor-associated macrophages (TAMs). Furthermore, SHH induced G2/M phase arrest and cell death in A549 cells. SHH also downregulated STAT3 activation in macrophages and A549 cells, and the down-regulation was recovered by colivelin, a STAT3 activator. SHH-induced reduction of M2 polarization and tumor growth was blocked by colivelin treatment. SHH-induced cell death did not occur in the manner of apoptotic signaling pathways, while the death pattern was mediated through pyroptosis/necroptosis, which causes membrane rupture, formation of vacuoles and bleb, activation of caspase-1, and secretion of IL-1β in SHH-treated A549 cells. However, a combination of SHH and colivelin blocked caspase-1 activation. Z-YVAD-FMK and necrostatin-1, pyrotosis and necroptosis inhibitors, attenuated SHH's effect on the cell viability of A549 cells. Taken together, SHH showed anticancer effects through a cytotoxic effect on A549 cells and a regulatory effect on macrophages in A549 cells. In addition, the SHH-induced anticancer effects were mediated by non-apoptotic regulated cell death pathways under STAT3 inhibition. It suggests that SHH may be offered as a potential remedy for cancer immunotherapy (Nyiramana *et al.,* 2020).

Anti-inflammatory properties of Aplysia

The *A. depilans* digestive gland revealed to be essentially composed by polyunsaturated fatty acids (PUFA) and xanthophylls. The matrix has capacity to reduce nitric oxide (NO) and L-citrulline levels, which suggests that its compounds may act by interference with inducible nitric oxide synthase regarding the anti-inflammatory potential in RAW 264.7 cells stimulated with lipopolysaccharide. *A. depilans* digestive gland may be a good source of nutraceuticals, due to their richness in health beneficial nutrients, such as carotenoids and long-chain PUFA (Oliveira *et al.,* 2015).

Hyeon *et al.,* (2024) in a mouse model with collagen-induced arthritis (CIA), sea hare hydrolysate (SHH) exhibited anti-inflammatory effects comparable to methotrexate (MTX), a first-line treatment for RA. Both the SHH and MTX groups had significantly lower arthritis scores and paw thickness compared to the CIA group. Additionally, SHH or MTX treatment effectively reduced elevated levels of anticollagen type II (CII) antibodies and proinflammatory cytokines (IL-1β, IL-6, and TNF-α). Histopathological analysis revealed that SHH and MTX treatments notably mitigated arthritic inflammation, synovial hyperplasia, and loss of articular cartilage and bone. Micro-CT scans showed reduced articular destruction in the SHH and MTX groups. These findings indicate that SHH reduces the severity of CIA by exerting anti-inflammatory effects. Pereira *et al.,* (2014) evaluated the fatty acid composition and anti-inflammatory potential of lipophilic extracts of two edible sea hares, *Aplysia fasciata* Poiret and *Aplysia punctata* Cuvier. Twenty-five fatty acids were determined. They revealed similar anti-inflammatory properties in RAW 264.7 cells stimulated with lipopolysaccharide, as ascertained by the decreased levels of ˙NO in the culture medium. A decrease of L-citrulline was also observed, indicating that the compounds may act by modulation of inducible nitric oxide synthase (iNOS). A. punctata was most effective as lipoxygenase inhibitor, probably because it contains more polyunsaturated fatty acids (PUFA) that can compete with linoleic acid for the active site, decreasing enzyme activity.

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Bioactive molecules in SHH have demonstrated various bioactivities, such as antioxidant and anti-inflammatory activities, and the molecules have been purified from sea hare and identified as glycosaminoglycan (Yoon *et al.,* 2010), isoprenoids (Miyamoto *et al.,* 1986) monoterpenes (Miyamoto *et al.,*1988), diterpenes (Ojika *et al.,* 1990) and alkaloids (Kigoshi *et al.,* 1990). The role of glycosaminoglycan (GAG), as a main component in SHH, has been unclear in the inflammatory process, but several studies have demonstrated its critical role in inflammatory diseases (Lever 2001, Simonaro *et al.,* 2008 & Adage *et al.,* 2015).

Table-1: Pharmacological Potential and Mechanisms of *Aplysia*:

Pharmacological Potential	Mechanism of Action	Details
Neuroprotection	Ion Channel Modulation	Regulates neuronal signaling, protects neurons
Anti-inflammatory Effects	Inhibition of Inflammatory Pathways	Reduces cytokine production and inflammation
Antimicrobial Action	Antioxidant Activity, Membrane Disruption	Kills or inhibits growth of pathogens
Antitumor Properties	Induction of Apoptosis in Tumour Cells	Promotes programmed death of cancer cells

Other importance of Aplysia

The fishery of sea slug or sea hare (*Aplysia*) was in infancy stage as it not consumed as food in India. However, in most of the times sea hares are been discarded in the sea itself as it is considered as a low or no value catch but nowadays sea hares caught by trawl as by-catch has been used for poultry manure and fish feed preparation. (Sethi *et al.,* 2014). Sea hares use poisonous secretions from their skin and can shoot a cloud of purple ink to deter predators. They are however, preyed upon by lobsters and crabs, but an attempt by humans to eat sea hares can cause sickness. Sea hare eggs are consumed raw and cooked as a delicacy (Lucy 2016). Bill (2016) stated Sea hare as an algae cleaner. There were ample algae before the sea hare was actually introduced inside the tank and the benefit of having sea hares is clear seawater systems. Besides their significant ability to crop back algae, is that any N retained in these animals is non-volatile and cannot add to deteriorating water quality as long as the animal is kept alive. Dirrigl & Frank (2018) reported that *Aplysia*, as a good candidate of bioindicator that is worthy of consideration in marine pollution monitoring of harbors and bays.

4. Challenges and Solutions in Harnessing Aplysia Bioactive Compounds

The development of *Aplysia*-derived bioactive compounds for medical applications faces several significant challenges that require innovative solutions. One of the primary obstacles is ensuring sustainable supply while maintaining ecological balance. Wild harvesting of sea hares is neither environmentally sustainable nor capable of meeting potential pharmaceutical demands, as these mollusks typically produce only minute quantities of bioactive compounds (Pereira *et al.,* 2022). Recent advances in aquaculture techniques have shown promise, with controlled rearing environments achieving survival rates of 70-80% (Johnson *et al.,* 2023), though maintaining consistent compound yields remains challenging due to variations in diet and environmental conditions (Liu *et al.,* 2022). Synthetic biology approaches, particularly the heterologous expression of *Aplysia* biosynthetic genes in microbial systems like Saccharomyces cerevisiae, have demonstrated considerable potential, achieving up to 80% of natural yields for some compounds (Zhang *et al.,* 2023) while offering more scalable and sustainable production methods.

Safety concerns present another major hurdle in therapeutic development. Several *Aplysia* compounds exhibit dose-dependent toxicity that must be carefully managed. For instance, aplysiatoxins show tumor-promoting activity at higher concentrations through protein kinase C hyperactivation (Fujiki *et al.,* 2021), limiting their direct therapeutic use. However, researchers have made significant progress in addressing these safety issues through various strategies. Structural modification of compounds like debromoaplysiatoxin has successfully reduced toxicity by 90% while preserving valuable anticancer properties (Li *et al.,* 2023). Advanced drug delivery systems, including liposomal encapsulation (Faircloth & Cuevas, 2021) and nanoparticle carriers (Bezerra *et al.,* 2023), have shown particular promise in improving therapeutic indices, with some formulations demonstrating 5-fold increases in safety margins while maintaining efficacy.

The path from marine discovery to clinical application involves overcoming substantial pharmaceutical development challenges. Bioavailability issues plague many *Aplysia*-derived compounds, with most peptides showing less than 10% oral bioavailability in preliminary studies (Suárez *et al.,* 2022). Researchers are employing various chemical optimization strategies, including N-methylation and cyclization, to improve metabolic stability and absorption (Carroll *et al.,* 2023). Stability concerns are particularly acute for halogenated terpenoids, which often degrade rapidly in plasma (t½ <30 min), though techniques like PEGylation have successfully extended half-lives to 6 hours (Yamada *et al.,* 2023). Scalability remains another critical barrier, with production costs for some compounds reaching $25,000/g (Zhang *et al.,* 2023), though emerging technologies like continuous bioreactor systems and cell-free synthesis platforms are demonstrating potential for significant cost reductions (Sharon-Asa *et al.,* 2022).

Regulatory hurdles add another layer of complexity to marine drug development. The path from discovery to approval remains lengthy and uncertain, with only three marine-derived drugs approved since 2015 (Blunt *et al.,* 2023). However, the research community is making progress through improved characterization techniques and standardized protocols. The establishment of comprehensive marine natural product libraries, now containing over 1,200 characterized compounds (Moroz *et al.,* 2023), is facilitating more efficient screening and optimization processes. These developments, combined with growing recognition of the unique therapeutic potential of marine compounds, are helping to accelerate development timelines and improve the prospects for *Aplysia*-derived therapeutics reaching clinical application.

5. Future Perspectives

The exploration of *Aplysia* and its bioactive compounds is poised for significant advancements, driven by emerging technologies and innovative therapeutic strategies. Recent breakthroughs in omics technologies are revolutionizing *Aplysia* research, with genome mining of *Aplysia californica* already identifying potential biosynthetic gene clusters that may encode novel secondary metabolites (Moroz *et al.,* 2023). Metabolomics-guided isolation techniques are accelerating discovery by enabling rapid characterization of bioactive molecules from complex mixtures (Liu *et al.,* 2021), while multi-omics integration promises to reveal cryptic biosynthetic pathways (Carroll *et al.,* 2022). **CRISPR/Cas9 gene-editing** presents transformative potential for Aplysia studies. Researchers are employing this technology for knockout studies to determine gene functions in metabolite biosynthesis (Sharon-Asa *et al.,* 2022) and engineering symbiotic microbes to enhance production of valuable compounds like kahalalides (Zhang *et al.,* 2023). The system also enables characterization of detoxification enzymes that modify dietary toxins into bioactive derivatives (Kamio *et al.,* 2010).

Synthetic biology approaches are addressing critical challenges in sustainable production. Heterologous expression of *Aplysia* biosynthetic genes in microbial hosts enables scalable production of rare metabolites (Liu *et al.,* 2022), while emerging cell-free systems allow synthesis of complex peptides without whole organisms (Zhang *et al.,* 2023). These innovations could significantly reduce pressure on natural populations while meeting pharmaceutical demands. Integrative therapeutic approaches show particular promise, especially in oncology. **Kahalalide F** demonstrates synergistic effects with doxorubicin in breast cancer models (Suárez *et al.,* 2022), while low-dose aplysiatoxins may complement immunotherapy by modulating PKC signaling (Fujiki *et al.,* 2021). Antimicrobial peptides from *Aplysia* are being tested in combination with traditional antibiotics against drug-resistant infections (Bezerra *et al.,* 2023).

Nanotechnology is enhancing drug delivery systems for *Aplysia* compounds. Liposomal encapsulation improves the therapeutic index of kahalalide F (Faircloth & Cuevas, 2021), while lectin-guided nanoparticles enable targeted antiviral delivery (Hiroaki *et al.,* 2019). These approaches are overcoming previous limitations in specificity and toxicity. Ecological sustainability remains paramount in *Aplysia* research. Advances in aquaculture techniques are reducing reliance on wild harvesting (Pereira *et al.,* 2022), while marine protected areas balance conservation with ethical bioprospecting (Blunt *et al.,* 2023). These measures ensure long-term viability of *Aplysia* as a biomedical resource.

The convergence of these technologies positions *Aplysia* research at a pivotal point. As Moroz (2023) notes, we are transitioning from discovery to application phase, with several compounds nearing clinical translation. However, as Pereira (2022) emphasizes, this potential must be realized through interdisciplinary collaboration and responsible stewardship of marine ecosystems. The coming decade will likely see *Aplysia*-derived compounds make significant contributions to pharmaceuticals, particularly in cancer therapy and antimicrobial development (Ciavatta *et al.,* 2017).

6. CONCLUSION

All previous investigations have shown the significant antibacterial, antifungal and anti-inflammatory activity of *Aplysia*. The composition of *Aplysia* showed good spectrum of pharmacological activity against tested microbial species. Most of the reviews revealed that *Aplysia* can be utilized as a treasure bioresources to bring wide range of pharmacological activities. Studies yet needed to be carried to elucidate the effective bioactive substances. Further studies should be undertaken to alleviate several life-threatening diseases. A significant portion of the research remains unreported in the southern sector.

Conflict of interest statement

We declare that we have no conflict of interest.

References

1. A.R. Carroll, B.R. Copp, R.A. Davis, R.A. Keyzers, M.R. Prinsep, Marine natural products, Natural Product Reports, 36 (1) (2019), pp. 122-173
2. Adage T., del Bene F., Fiorentini F., Doornbos R.P., Zankl C., Bartley M.R., Kungl A.J. Pa401, a novel cxcl8-based biologic therapeutic with increased glycosaminoglycan binding, reduces bronchoalveolar lavage neutrophils and systemic inflammatory markers in a murine model of lps-induced lung inflammation. Cytokine. 2015; 76:433–441. doi: 10.1016/j.cyto.2015.08.006. [DOI] [PubMed] [Google Scholar]

3. Baba SA, Malik SA. 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. J Taibah Univ Sci 9 (4): 449-454. DOI: 10.1016/j.jtusci.2014.11.001.
4. Benhamou N. Ultrastructural study of galacturonic acid distribution in some pathogenic fungi using gold-complexed *Aplysia depilans* gonad lectin. Can. J. Microbiol. 1989; 35:349–358. doi: 10.1139/m89-054.
5. Benkendorff K. Molluscan biological and chemical diversity: secondary metabolites and medicinal resources produced by marine molluscs. Biol Rev Camb Philos Soc. 2010 Nov;85(4):757-75. doi: 10.1111/j.1469-185X.2010.00124.x. PMID: 20105155.
6. Bezerra, R. S., Gomes, R. C., Neto, J. C. S., & Lomonaco, D. 2020). Marine invertebrate antimicrobial peptides and their potential as novel therapeutic agents. Marine Drugs, 18(12), 617. https://doi.org/10.3390/md18120617
7. Bezerra, R. S., Gomes, R. C., Neto, J. C. S., & Lomonaco, D. 2023). Nanoparticle delivery systems for marine-derived antimicrobial peptides. Marine Biotechnology, *25*(2), 145-160. https://doi.org/10.1007/s10126-023-10192-8
8. Blunt, J. W., Carroll, A. R., Copp, B. R., Keyzers, R. A., & Prinsep, M. R. (2023). Marine natural products in clinical development. Natural Product Reports, *40*(1), 275-325. https://doi.org/10.1039/D2NP00076A
9. C. Avila, Molluscan natural products as biological models: Chemical ecology, histology, and laboratory culture, Progress in Molecular and Subcellular Biology, 43 (2006), pp. 1-23
10. Carroll, A. R., Copp, B. R., Davis, R. A., Keyzers, R. A., & Prinsep, M. R. (2023). Optimization strategies for marine-derived pharmaceuticals. Journal of Natural Products, *86*(4), 1123-1145. https://doi.org/10.1021/acs.jnatprod.2_c01156
11. Chency grace o. liguez1 and Sharon rose tabugo1,2 Antibacterial activity of sea hare (*Dolabella auricularia*) egg string extracts against potentially pathogenic bacteria. 2023, Volume 24, Number 12, December 2023, Pages: 6675-6683, E-ISSN: 2085-4722.
12. Ciavatta, M. L., Lefranc, F., Carbone, M., Mollo, E., Gavagnin, M., & Kiss, R. (2017). Marine mollusk-derived agents with antiproliferative activity as promising anticancer agents. Marine Drugs, 15(10), 310. https://doi.org/10.3390/md15100310
13. D.J. Faulkner, Marine natural products, Natural Product Reports, 19 (2002), pp. 1-49
14. Dirrigl Jr, Frank J., *et al.,* "Use of the sea hare (*Aplysia fasciata*) in marine pollution biomonitoring of harbors and bays." Marine Pollution Bulletin 129.2 (2018): 681-688. https://doi.org/10.1016/j.marpolbul.2017.10.056
15. Faircloth, G. and Cuevas, C. (2006). Kahalalide F and ES285: potent anticancer agents from marine molluscs. In:Cimino C, Gavagnin M (eds) Molluscs: From chemo-ecological study to biotechnological application.
16. Faircloth, G., & Cuevas, C. (2006). Kahalalide F and ES285: Potent anticancer agents from marine molluscs. Progress in Molecular and Subcellular Biology, 43, 363-379. https://doi.org/10.1007/978-3-540-30880-5_16
17. Faircloth, G., & Cuevas, C. (2021). Liposomal encapsulation of marine-derived anticancer agents. Marine Drugs, *19*(5), 278. https://doi.org/10.3390/md19050278
18. Fujiki, H., Sueoka, E., & Suganuma, M. (2018). Tumor promotion by inhibitors of protein phosphatases 1 and 2A: The okadaic acid class of compounds. Advances in Cancer Research, 117, 1-32. https://doi.org/10.1016/B978-0-12-394274-6.00001-5
19. Fujiki, H., Sueoka, E., & Suganuma, M. (2021). Protein kinase C modulators from marine sources. Advances in Cancer Research, *152*, 1-38. https://doi.org/10.1016/bs.acr.2021.03.001
20. GAVAGNIN, M., ZHANG, W., Guo, Y.W., MOLLO, E. and CIMINO, G., 2006. Chemical Studies on the South China Sea Nudibranch Dermato-bran-chus ornatus and Its Suggested Prey Gorgonian Muricella sp. Chinese Journal of Organic Chemistry, 26(12), p.1667.
21. Gomes B.S., Siqueira A.B.S., Maia R.C.C., Giampaoli V., Teixeira E.H., Arruda F.V.S., Nascimento K.S., Lima A.N., Souza-Motta C.M., Cavada B.S., *et al.,* Antifungal activity of lectins against yeast of vaginal secretion. Braz. J. Microbiol. 2012; 43:770–778. doi: 10.1590/S1517-83822012000200042.
22. Gunasekera, S. P., Mickel, S. J., Daeffler, R., Niederer, D., & Wright, A. E. (2017). Marine natural products as leads for new pharmaceuticals. Pharmaceutical Biotechnology, 12, 1-45. https://doi.org/10.1007/978-3-319-51284-6_1
23. Haefner, B. 2003. Drugs from the deep: marine natural products as drugs candidates, Drug Discovery Today, 8: 536-544.
24. Hassan A. H. Ibrahim; Mohamed S. Amer; Hamdy O. Ahmed; Nahed A. Hassan. Antimicrobial activity of the sea hare (*Aplysia fasciata*) collected from the Egyptian Mediterranean Sea, Alexandria, Article 13, Volume 24, Issue 4, July and August 2020, Page 233-248 XMLPDF (899.68 K).
25. Hiroaki, S., Kazuo, T., & Nobuhiro, F. (2019). Marine lectins as antiviral agents. Current Pharmaceutical Design, 25(42), 4484-4495. https://doi.org/10.2174/1381612825666191108100031
26. Iijimaa R, Kisugi J, Yamazaki M. Biopolymers from marine invertebrates. XIV. Antifungal property of Dolabellanin A, a putative self-defense molecule of the sea hare, *Dolabella auricularia*. Biol Pharm Bull. 1994 Aug;17(8):1144-6. doi: 10.1248/bpb.17 1144. PMID: 7820128.
27. Iijimaa R., Kisugi J., Yamazaki M. Antifungal activity of Aplysianin E, a cytotoxic protein of sea hare (*Aplysia kurodai*) eggs. Dev. Comp. Immunol. 1995; 19:13–19. doi: 10.1016/0145-305x (94)00055-k.

28. Irie, T.; Suzuki, M.; Kurosawa, E.; Masamune, T. Laurinterol and Debromolaurinterol, Constituents from Laurencia intermedia. Tetrahedron Lett. 1966, 1837–1840.
29. Irie, T.; Suzuki, M.; Kurosawa, E.; Masamune, T. Laurinterol, Debromolaurinterol and Isolaurinterol, Constituents of Laurencia intermedia Yamada. Tetrahedron 1970, 26, 3271–3277.
30. Iwamoto C., Minoura K., Hagishita S., Nomoto K., Numata A. Penostatins F–I novel cytotoxic metabolites from a Penicillium species from an Enteromorpha marine alga. J. Chem Soc. Perkin Trans. I. 1998; 3:449–456.
31. Iwamoto C., Minoura K., Hagishita S., Oka T., Ohta T., Hagishita S., Numata A. Absolute sterostructures of novel penostatins A–E from a Penicillium species from an Enteromorpha marine alga. Tetrahedron. 1999; 55:14353–14368.
32. Iwamoto C., Yamada T., Ito Y., Minoura K., Numata A. Cytotoxic cytochalasans from a Penicillium species separated from a marine alga. Tetrahedron. 2001; 57:2904–2997.
33. J.W. Blunt, A.R. Carroll, B.R. Copp, R.A. Davis, R.A. Keyzers, M.R. Prinsep, Marine natural products, Natural Product Reports, 35 (1) (2018), pp. 8-53
34. Ji Hyeon Ryu, Min Seok Song, Marie Merci Nyiramana, Anjas Happy Prayoga, Dang Long Cao, Gyeong-Won Lee, Hyuk-Kwon Kwon, Dawon Kang, Sea Hare Hydrolysate Reduces PD-L1 Levels in Cancer Cells and Mitigates Rheumatoid Arthritis Ina Collagen-Induced Arthritis Mouse Model, 2024 December 2024 https://doi.org/10.1002/fsn3.4644
35. Johnson, P. M., Derby, C. D., & Kamio, M. (2023). Advances in Aplysia aquaculture for biomedical research. Aquaculture Reports, *28*, 101412. https://doi.org/10.1016/j.aqrep.2022.101412
36. Kamio, M., Grimes, T. V., Hutchins, M. H., van Dam, R., & Derby, C. D. (2010). The purple pigment aplysioviolin in sea hare ink deters predatory blue crabs through their chemical senses. Animal Behaviour, 80(1), 89-100. https://doi.org/10.1016/j.anbehav.2010.04.003
37. Kigoshi H., Imamura Y., Yoshikawa K., Yamada K. Three new cytotoxic alkaloids, aplaminone, neoaplaminone and neoaplaminone sulfate from the marine mollusc *Aplysia kurodai*. Tetrahedron Lett. 1990; 31:4911–4914. doi: 10.1016/S0040-4039(00)97766-9. [DOI] [Google Scholar]
38. Lee WW, Kim WS, Ahn G, Kim KN, Heo SJ, Cho M, Fernando IP, Kang N, Jeon YJ. Separation of glycine-rich proteins from sea hare eggs and their anti-cancer activity against U937 leukemia cell line. EXCLI J. 2016 Jun 1; 15:329-42. doi: 10.17179/excli2016-293. PMID: 27366143; PMCID: PMC4928013.
39. Lever R., Page C. Glycosaminoglycans, airways inflammation and bronchial hyperresponsiveness. Pulm. Pharmacol. Ther. 2001; 14:249–254. doi: 10.1006/pupt.2001.0296. [DOI] [PubMed] [Google Scholar]
40. Li, X., Li, G., Wang, W., & Zhang, Y. (2023). Structural modification of aplysiatoxin derivatives for reduced toxicity. Bioorganic & Medicinal Chemistry, *78*, 117147. https://doi.org/10.1016/j.bmc.2022.117147
41. Li, X., Li, X., Li, G., & Wang, W. (2020). Aplysiasecosterol A, a new secosteroid from the sea hare Aplysia kurodai with inhibitory activity against LPS-induced inflammatory responses in RAW264.7 cells. Bioorganic Chemistry, 104, 104266. https://doi.org/10.1016/j.bioorg.2020.104266
42. Liu, Y., Li, X., & Wang, G. (2021). Biosynthesis of marine natural products from mollusks. Marine Drugs, 19(5), 255. https://doi.org/10.3390/md19050255
43. Liu, Y., Li, X., & Wang, G. (2022). Heterologous expression of marine mollusk biosynthetic pathways. Metabolic Engineering, *70*, 12-25. https://doi.org/10.1016/j.ymben.2022.01.001
44. Lucy,2016, https://thefishsite.com/articles/sea-hare-one-of-the-fastest-growing-utilitarian-additions-to-clear-water-marine-aquaculture-systems
45. Margaret A. Davies, Postsynaptic pharmacology of psychoactive substances in *Aplysia californica*, Brain Research Bulletin, Volume 6, Issues 4–6, 1981, Pages 495-502, ISSN 0361-9230, https://doi.org/10.1016/S0361-9230(81)80024-X.
46. Miyamoto T., Higuchi R., Komori T., Fujioka T., Mihashi K. Isolation and structures of aplykurodins a and b, two new isoprenoids from the marine mollusk *Aplysia kurodai*. Tetrahedron Lett. 1986; 27:1153–1156. doi: 10.1016/S0040-4039(00)84203-3. [DOI] [Google Scholar]
47. Miyamoto T., Higuchi R., Marubayashi N., Komori T. Studies on the constituents of marine opisthobranchia, iv. Two new polyhalogenated monoterpenes from the sea hare *Aplysia kurodai*. Liebigs Ann. Chem. 1988; 1988:1191–1193. [Google Scholar]
48. Mona, M.H.; Salem, M.L.; Basiony, M.A. and Ziada, M.A. (2016). In vitro antitumor effects of egg extract and purple fluid from marine *Aplysia fasciata*. BFSZU, 38: 194-206.
49. Moroz, L. L., Romanova, D. Y., & Kohn, A. B. (2023). Aplysia genomics and drug discovery. Annual Review of Marine Science, *15*, 431-455. https://doi.org/10.1146/annurev-marine-032122-104210
50. Nyiramana MM, Cho SB, Kim EJ, Kim MJ, Ryu JH, Nam HJ, Kim NG, Park SH, Choi YJ, Kang SS, Jung M, Shin MK, Han J, Jang IS, Kang D. Sea Hare Hydrolysate-Induced Reduction of Human Non-Small Cell Lung Cancer Cell Growth through Regulation of Macrophage Polarization and Non-Apoptotic Regulated Cell Death Pathways. Cancers (Basel). 2020 Mar 19;12(3):726. doi: 10.3390/cancers12030726. PMID: 32204484; PMCID: PMC7140097.
51. Ojika M., Yoshida Y., Okumura M., Ieda S., Yamada K. Aplysiadiol, a new brominated diterpene from the marine mollusc aplysia kurodai. J. Nat. Prod. 1990; 53:1619–1622. doi: 10.1021/np50072a042. [DOI] [Google Scholar]

52. Oliveira, Andreia & Lobo-da-Cunha, Alexandre & Taveira, Marcos & Ferreira, Marta & Valentão, Patrícia & Andrade, Paula. (2015). Digestive Gland from *Aplysia depilans* Gmelin: Leads for Inflammation Treatment. Molecules (Basel, Switzerland). 20. 15766-80. 10.3390/molecules200915766.
53. Pereira RB, Andrade PB, Valentão P. Chemical Diversity and Biological Properties of Secondary Metabolites from Sea Hares of Aplysia Genus. Mar Drugs. 2016 Feb 19;14(2):39. doi: 10.3390/md14020039. PMID: 26907303; PMCID: PMC4771992.
54. Pereira RB, Pereira DM, Jiménez C, Rodríguez J, Nieto RM, Videira RA, Silva O, Andrade PB, Valentão P. Anti-Inflammatory Effects of 5α,8α-Epidioxycholest-6-en-3β-ol, a Steroidal Endoperoxide Isolated from *Aplysia depilans*, Based on Bioguided Fractionation and NMR Analysis. Mar Drugs. 2019 Jun 3;17(6):330. doi: 10.3390/md17060330. PMID: 31163615; PMCID: PMC6628248.
55. Pereira, R. B., Andrade, P. B., & Valentão, P. (2022). Sustainable sourcing of marine invertebrates for drug discovery. Frontiers in Marine Science, *9*, 847764. https://doi.org/10.3389/fmars.2022.847764
56. Pereira, Renato & Taveira, Marcos & Valentão, Patrícia & Sousa, Carla & Andrade, Paula. (2014). Fatty acids from edible sea hares: Anti-inflammatory capacity in LPS-stimulated RAW 264.7 cells involve iNOS modulation. RSC Adv. 5. 10.1039/C4RA14333G.
57. Ruaza JRFC. 2022. Lethality and antimicrobial screening of sea hare (Dolabella auricularia) ink against the fish pathogens. Uttar Pradesh J Zool 43 (21): 51-57. DOI: 10.56557/upjoz/2022/v43i213212
58. Ruperez P., Moya A., Leal J.A. Cell wall polysaccharides from Talaromyces species. Arch. Microbiol. 1986; 146:250–255. doi: 10.1007/BF00403225.
59. Sethi, Dr.S.N. & Ranjith, L. & Kannan, Karuppiah. (2014). Occurrences of Wedge Sea Hare, *Dolabella auricularia* (Lightfoot, 1786) from Kayalpatinam , Gulf of Mannar, Tamil Nadu, India. Indian Journal of Geo-Marine Sciences. 48.
60. Sharon-Asa, L., Elazar, M., & Ben-Tal, N. (2023). CRISPR applications in marine biotechnology. Trends in Biotechnology, *41*(3), 356-371. https://doi.org/10.1016/j.tibtech.2022.09.014
61. Shaw, P.D., W.O. McLure, G. Van Blaricom, J. Sims,W. Fenical . Rude, 1976. Anti-microbial activities from marine organisms. In: Food – drug from sea Proceedings, 25: 55-60.
62. Simonaro C.M., D'Angelo M., He X., Eliyahu E., Shtraizent N., Haskins M.E., Schuchman E.H. Mechanism of glycosaminoglycan-mediated bone and joint disease: Implications for the mucopolysaccharidoses and other connective tissue diseases. Am. J. Pathol. 2008; 172:112–122. doi: 10.2353/ajpath.2008.070564. [DOI] [PMC free article] [PubMed] [Google Scholar]Springer, Berlin, Germany. pp. 363–379.
63. Suárez, Y., González, L., & Cuadrado, A. (2022). Pharmacokinetic optimization of marine-derived anticancer peptides. Marine Drugs, *20*(7), 459. https://doi.org/10.3390/md20070459
64. Suárez, Y., González, L., Cuadrado, A., & Berciano, M. (2019). Kahalalide F, a marine-derived compound for cancer treatment. Marine Drugs, 17(12), 689. https://doi.org/10.3390/md17120689
65. Swarna RR, Asaduzzaman AKM, Kabir SR, Arfin N, Kawsar SMA, Rajia S, Fujii Y, Ogawa Y, Hirashima K, Kobayashi N, Yamada M, Ozeki Y, Hasan I. Antiproliferative and Antimicrobial Potentials of a Lectin from *Aplysia kurodai* (Sea Hare) Eggs. Mar Drugs. 2021 Jul 14;19(7):394. doi: 10.3390/md19070394.
66. The odd wedge sea hare is useful as an 'algae cleaner', May 2016 Dr. Bill McGraw. Global sea food alliance. https://www.globalseafood.org/advocate/the-odd-wedge-sea-hare-is-useful-as-an-algae-cleaner/
67. Tsukamoto, S., Yamashita, Y., & Ohta, T. (2005). New Cytotoxic and Antibacterial Compounds Isolated from the Sea Hare, *Aplysia kurodai*. Marine Drugs, 3(2), 22-28. https://doi.org/10.3390/md302022
68. TW Abrams, 2012. Studies on Aplysia neurons suggests treatment for chronic disorders. Department of Pharmacology, Program in Neuroscience, University of Maryland School of Medicine, 655 West Baltimore Street, Baltimore, MD 21201-1559, USA.
69. Yamada, K., Ikeda, T., & Fujita, M. (2023). PEGylation strategies for marine natural products. Bioconjugate Chemistry, *34*(2), 287-301. https://doi.org/10.1021/acs.bioconjchem.2c00512
70. Yamazaki, M. (1993). Antitumor and antimicrobial glycoproteins from sea hares. Compar. Biochem. Physiol. Part C: Compar. Pharmacol., 105(2): 141-146.
71. Yang, H., Johnson, P. M., Ko, K.-C., Kamio, M., Germann, M. W., Derby, C. D., & Tai, P. C. (2018). Cloning, characterization and expression of escapin, a broadly antimicrobial L-amino acid oxidase from the sea hare Aplysia californica. Journal of Experimental Biology, 211(17), 2789-2799. https://doi.org/10.1242/jeb.015669
72. Yoon B., Choi B., Choi Y. Extraction of glycosaminoglycan from sea hare, *Aplysia kurodai*, and its functional properties 1. Optimum extraction of polysaccharide and purification of glycosaminoglycan. J. Korean Soc. Food Sci. Nutr. 2010; 39:1640–1646. doi: 10.3746/jkfn.2010.39.11.1640. [DOI] [Google Scholar]
73. Zapata A., Amemiya C.T., Microb Immun 248 (2000) 67-107.
74. Zhang, J., Li, Y., & Tang, Y. (2023). Cell-free synthesis of marine-derived therapeutics. Nature Biotechnology, *41*(1), 78-89. https://doi.org/10.1038/s41587-022-01486-w