

Bioactive Peptides from Sea Cucumbers and Sea Urchins: Therapeutic Roles and Mechanistic Insights

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Abstract

Marine organisms, especially those in the Echinozoa class like sea urchins and sea cucumbers, have received significant attention for their bioactive peptides that exhibit a wide range of therapeutic potential. This review presents recent research findings related to the extraction, identification, and bioactivity of such peptides. Antioxidant peptides from Echinozoa could protect against oxidative damage by reducing the levels of ROS and increasing the generation of antioxidant enzymes. This might be helpful for developing new therapeutic drugs to cure neuropathic pain. Peptides with anticancer properties isolated from species such as sea urchins have been observed to trigger apoptosis and suppress cancer cell growth through the PI3K/AKT signaling pathway. Peptides that combat fatigue boost endurance by stimulating the NRF2 and AMPK pathways, enhancing energy metabolism and lowering fatigue indicators in animal studies. Antidiabetic peptides from sea cucumber regulate blood glucose through their influence on the IRS/Akt and AMPK pathways, enhancement of insulin responsiveness, and acceleration of wound healing in diabetic models. ACE-inhibitory peptides from sea cucumbers and sea urchins have also exhibited an antihypertensive activity by inhibiting the conversion of angiotensin I to angiotensin II. Due to their membranolytic mode of action, sea urchin-derived antimicrobial peptides-strongylocins and centrocins-express a wide range of activities against Gram-positive and Gram-negative bacteria. Other sea cucumber peptides that improve memory can become an experimental therapy for the improvement of cognitive ability. Although these discoveries sound promising, the main barriers toward clinical use are problems of peptide stability, their bioavailability, and lack of efficient delivery. Addressing these difficulties is critical for increasing the therapeutic effectiveness of Echinozoa-derived peptides in both nutraceutical and pharmaceutical applications.

Keywords: Bioactive peptides, Echinozoa, Sea urchins, Sea cucumbers, Antioxidant, Anticancer, Antidiabetic, Antimicrobial, ACE inhibitory, Memory-enhancing

Introduction

Marine ecosystems are unique resources for pharmaceuticals since marine organisms possess bioactive qualities with substantial promise in biomedical and pharmaceutical disciplines [1]. The extreme conditions of marine ecosystems, including high pressure, salinity, and temperature variations, have compelled marine organisms to develop specialized metabolic pathways. This results in the production of diverse chemical structures with potent biological activities [2]. Among these bioactive compounds, marine-derived peptides have attracted considerable attention because of their distinctive structural properties and potential applications in health-promoting activities [3,4].

Bioactive peptides, typically comprise fewer than 20 amino acids or with molecular weights below 10 kDa [5,6]. These compounds confer a range of health benefits, including reduction of blood pressure [4], cholesterol levels [7], and inflammation [8], as well as inhibition of cancer progression [9], modulation of the immune system [10], and exhibition of antibacterial properties [11]. Antioxidant properties include free radical scavenger and metal ion chelation activity, which also confer additional health benefits [12]. The main advantages of peptides are that they are naturally occurring, biocompatible with the human body, and thus are generally toxicologically safe, mostly with fewer side effects than other drugs [13]. In addition, bioactive peptides act selectively in target tissues to minimize toxicity, besides being effective at low dosages or low concentration. On the other hand, most of the synthetic chemical compounds used in pharmaceuticals have a tendency to accumulate in the body, which may give rise to various problems and adverse effects [14,15].

In addition to their health-promoting characteristics, these peptides' distinct structural features, structural diversity, and broad range of

biological activities make them ideal for pharmacological, nutraceutical, and cosmeceutical applications [16-18]. These peptides are used in pharmaceutical formulations as well as health-promoting dietary mixtures. In cosmetics, polypeptides provide antioxidant, anti-inflammatory, anti-wrinkle, skin-lightening, and wound-healing benefits [19,20]. In the food sector, peptides function as sweeteners, colorants, thickeners, anti-caking agents, emulsifiers, flavor enhancers, and acidity regulators [21]. The distinctive biological properties of marine-derived peptides have generated significant interest in product development within the pharmaceutical, cosmetic, and nutraceutical industries [22,23].

Bioactivity of the peptides is influenced by various structural and compositional elements. The structure of a peptide, therefore, is an important determinant for its bioactivity. Molecular dimensions, charge distribution, amino acid sequence, and hydrophobic or hydrophilic nature are some of the factors which have a direct impact on functional and bioactive properties of peptides [10,24]. In general, the overall conformation of the molecule may be affected by processes like epimerization and may thus alter the bioactivity of the peptide itself. Notably, amino acid composition of peptides affects their resistance to digestive enzymes and intestinal peptidases, hence their bioavailability and bioactivity. High concentrations of acidic and hydrophobic amino acids in peptides have demonstrated increased bioavailability and retained antioxidant activity [25]. Furthermore, the position of certain amino acids in a peptide sequence is important for binding with receptors and inducing functional responses [26]. How these factors influenced the activity of peptides can be observed in **Table 1** which summarized the identified peptide sequences derived from sea cucumbers and sea urchins.

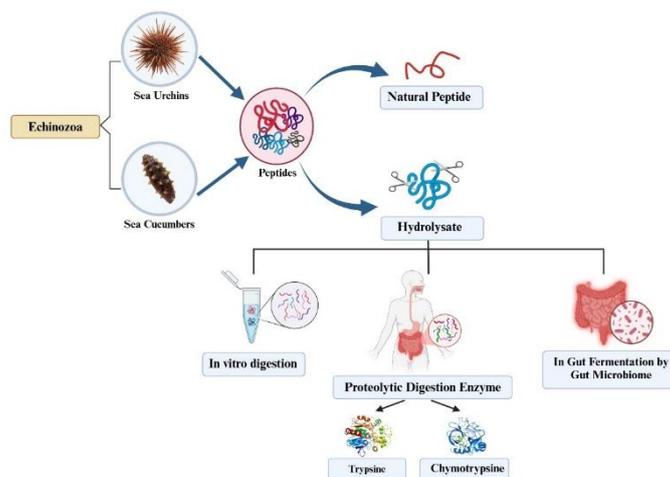


Figure 1 Types of peptides comprises from natural peptides and hydrolysate.

Table 1 Summary of data Echinozoa peptides from various species.

Echinozoa	Species	Peptide sequens	Biological activity	References
	<i>Acaudina molpadioides</i>	MEGAQEAQGD	ACE-inhibitor	[52]
	<i>Acaudina molpadioides</i>	FLAP	Antioxidant	[52]
	<i>Actinopyga lecanor</i>	GVSGLH	Antioxidant	[52]
	<i>Cucumaria frondose</i>	GPPGPQWPLDF	Antioxidant	[58]
	<i>Cucumaria frondose</i>	GPGMMGP	Antioxidant	[58]
	<i>Cucumaria frondose</i>	APDMAFPR	Antioxidant	[58]
	<i>Acaudina leucoprocta</i>	QIKNITRSRGDGEYQCKEHRM	Antifatigue	[57]
	<i>Acaudina leucoprocta</i>	EACPERASQQI	Antifatigue	[57]
Sea Cucumber	<i>Acaudina leucoprocta</i>	NIDQNMQQGIQ	Antifatigue	[57]
	<i>Acaudina leucoprocta</i>	MTMSFYVYVSLGNFMKILGF[95]	Antifatigue	[57]
	<i>Apostichopus japonicus</i>	TYPE	Antioxidant & Anti-aging	[94]
	<i>Apostichopus japonicus</i>	FHLPI	Antioxidant & Anti-aging	[94]
	<i>Apostichopus japonicus</i>	PIWL	Antioxidant & Anti-aging	[94]
	<i>Stichopus japonicus</i>	GPEPTGPTGAPQWLR	Antioxidant	[46]
	<i>Holothuria scabra</i>	FNDLGAW	Antioxidant UV-B Protective	[95]
	<i>Holothuria scabra</i>	KFGEGK	Antioxidant UV-B Protective	[95]
	<i>Holothuria tubulosa</i>	ASHLGHHALDHLK	Antimicrobial	[142]
	<i>Echinus esculentus</i>	XNPFKKIAHRXCYPKNE	Antimicrobial	[146]
Sea Urchin	<i>Echinus esculentus</i>	RXACTVUAQ	Antimicrobial	[146]
	<i>Strongylocentrotus droebachiensis</i>	SGIHAGQRGCSAL	Antimicrobial	[145]

The bioactive peptides from marine species can be obtained either by direct isolation or enzymatic hydrolysis. Bioactive peptides are naturally present in various marine organisms such as algae, sponges,

cnidarians, and mollusks. Due to the aquatic environment, these peptides have acquired some unique characteristics, making them strong bioactive compounds [27]. Peptides become physiologically

active when they get digested by proteolytic enzymes such pepsin and trypsin, which occur during digestion or microbial fermentation [28,30]. This method may be used on many different kinds of marine sources with high protein content, including macroalgae and microalgae [29] (**Figure 1**). The intestinal digesting process not only changes peptide profiles, but it may also improve certain bioactivities as antioxidant activity and bile acid-binding capacity [31], ACE inhibitory and antihypertensive characteristics [32,33], and anti-fatigue benefits [34]. Hydrolysate protein is widely used in the food industry because of its high nutritional content, bioactivity, and beneficial properties, which help to generate health-promoting products with antioxidant, antibacterial, anticancer, and antihypertensive effects [35,36]. Despite the promising therapeutic potential of marine-derived peptides, their transition from laboratory research to commercial markets has been limited. Though some peptides and their derivatives have entered pharmaceutical and nutraceutical fields, most of them are still in various stages of clinical and preclinical trials. However, given their remarkable bioactivities, more drugs derived from marine peptides are foreseen to enter the market in the coming years to address various health challenges [20].

Echinozoa, notably sea cucumbers and sea urchins, are excellent sources of bioactive peptides due to their high protein content and ability to survive in many different kinds of marine habitats. Despite ongoing study in this area, researchers still have a limited understanding of the bioactive peptides formed from these species, as well as their modes of action and prospective uses. Bridging this knowledge gap might open up opportunities for using these marine resources in therapeutic and industrial applications.

The main objective of this review is to investigate the potential of sea urchins and sea cucumbers as sustainable sources of bioactive peptides. This study covers the current state of knowledge on bioactive peptides produced from these species, as well as their structural and functional attributes in relation to health benefits. It also discusses various alternative manufacturing processes, including natural extraction and enzymatic hydrolysis, and brings into focus industrial uses and also the hurdles in

commercialization. Such critical issues, when covered by this review, would help close the information gap and create a platform for further research and development of Echinozoa-derived bioactive peptides for medicinal and commercial applications.

Peptides of echinozoa

Sea cucumbers and sea urchins, both members of Echinozoa, are rich sources of bioactive molecules because of their unique nutritional profiles and diverse biochemical compounds. These marine invertebrates contain a variety of bioactive substances, including peptides, triterpene glycosides, polysaccharides, phenols, and lipids [37]. Their high content of proteins combined with a low content of fat makes these organisms ideal substrates for the production of bioactive peptides besides providing valued nutritional and health benefits. Indeed, peptides produced by these organisms have been reported showing a wide panorama of biological activities including antioxidant, anti-diabetic, ACE inhibitory, immunomodulatory anti-cancer properties, and neuroprotective among others [38,39] (**Figure 2**).

Sea cucumber and sea urchin bioactive peptides have been identified and isolated from gonads, body wall, internal organs, and aquaparyngeal bulb of echinoderms [40]. The body wall dermis is composed of collagenous connective tissue, hence it provides a rich supply of physiologically active peptides in sea cucumbers [41]. Sea urchins are known to contain high PUFA content and, thus, have been reported to possess comparable potential to sea cucumbers [42]. Both echinoderms, sea cucumbers and sea urchins, are a rich source of various bioactive peptides with cumulative therapeutic potential. In fact, bioactive peptides demonstrate diverse biological activities owing to their contribution to the health benefits of marine invertebrates. Cumulative reports have shown that sea cucumbers and sea urchins have strong antioxidant [43], antidiabetic [44], anticancer [45], immunomodulatory [46], angiotensin-converting enzyme (ACE) inhibitor [47], anti-fatigue [48], anti-aging [49], neuroprotective [12], antithrombotic [50], wound healing [51] and micromineral chelating [52] properties.

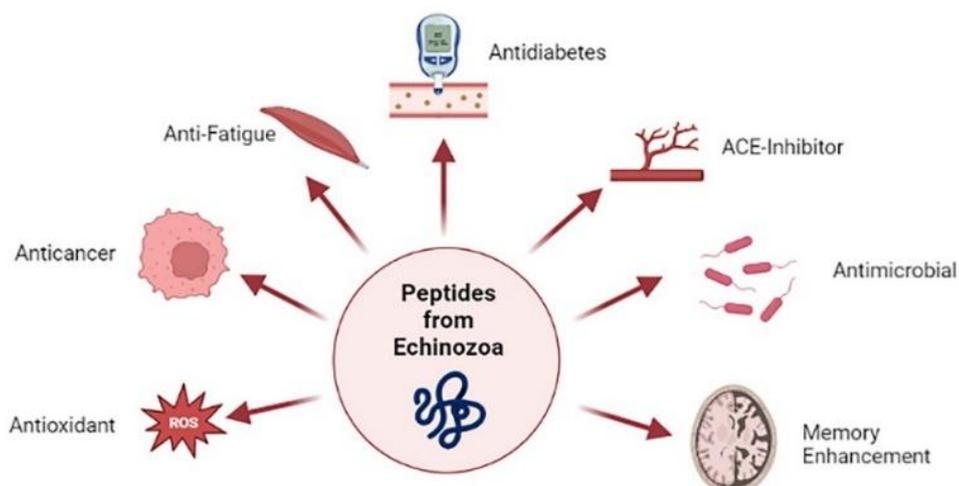


Figure 2 The benefit of Echinozoa peptides for human health.

Both naturally obtained from Echinozoa isolation and peptides derived from hydrolysis will be interacting in the body through their target proteins to provide the activity [30]. That peptides modulate several physiological responses in the body is brought about by the binding with particular receptors. Interactions here play crucial roles in cell signaling, immune function, and neurological processes. Thus, the peptides targeting the receptors for pro-angiogenic growth factors like FGF, PDGF, and VEGF may favorably influence the process of angiogenesis implicated in several pathologies. Other peptides, on the other hand, can also bind to their receptors in some unexpected ways with novel physiological results [53]. For example, the identification of peptides that could selectively interact with specific receptors, such as EphA4-binding receptors in the nervous system, may open new directions for targeted therapy and diagnostic applications.

More detailed information concerning the specific bioactivities of peptides derived from sea cucumbers and sea urchins, including their action mechanisms and pharmaceutical/nutraceutical applications, will be further elaborated in the following sections in an attempt to profoundly understand their biological potential.

Isolation of peptides from echinozoa

Representative species in the subgroup Echinozoa, such as sea cucumbers and sea urchins, can be optimally developed for extraction and hydrolysis techniques to isolate bioactive peptides with considerable health

benefits and functional properties. Generally, the steps involved are pretreatment, enzymatic hydrolysis, purification, and drying. According to Mildenerger *et al.* [54], each of these steps requires optimization for maximum yield, preserving bioactivity, and ensuring safety.

Preparation entails washing, removal of internal organs, and dry wringing of the bodies of the specimens as an initial process in extracting sea cucumbers. This eliminated impurities, making the raw material suitable for extraction. The dried sea cucumber tissue was ground into a fine powder (200 - 300 mesh) to increase its surface area and improve enzyme accessibility during hydrolysis. During the enzymatic hydrolysis phase, proteolytic enzymes, generally at a concentration of 10 - 20 g per 1,000 g of powder, are introduced to degrade proteins into smaller bioactive peptides. This reaction typically occurs for 30 - 60 min before it is stopped to avoid excessive degradation. After hydrolysis, ultrafiltration was employed to isolate the target low-molecular-weight peptides from the larger molecules and contaminants. The purified peptide solution was spray-dried at room temperature, resulting in a stable and concentrated powdered form of the bioactive peptides. A final vacuum drying treatment at 70 - 80 °C was applied to enhance purity and stability [55].

Although no specific protocols of extraction have been comprehensively reviewed for sea urchins, protocols used for sea cucumbers can be adapted. A pH-shift method can be applied for the isolation of proteins in sea urchins, where proteins are solubilized at different

pH and then precipitated. This method has successfully extracted proteins from several tissues in sea urchins. After isolation, hydrolysis was done using some food-grade commercial enzymes such as alcalase and flavourzyme. These enzymes hydrolyze proteins into smaller peptides, which are further assayed for functional properties. In the case of sea cucumbers also, further ultra-filtration and drying is done in order to have the bioactive peptides in stable form [56].

The source of proteins, enzymatic treatment, and processing conditions would be the key factors that would affect the bioactivity of peptides from Echinozoa. The different amino acid compositions and sequences, and thus their bioactivities, may be due to different types of marine tissue. The collagen-rich body walls of sea cucumbers and PUFA-rich tissues of sea urchins are the most appropriate substrates to produce bioactive peptides with better functional properties [57]. Selection and optimization of enzymatic treatments are of equal importance. The types of enzymes used, their concentration, and the order of the reactions determine the type of peptides that are produced. Different enzymes produce peptides with certain bioactivities such as antiproliferative, antimicrobial, antihypertensive, antiglycemic, antitumor, and antioxidative activities. The use of appropriate combinations of enzymes, concentrations, and hydrolysis conditions is very important to optimize the production of peptides responsible for particular health benefits [58]. Extraction conditions should be strictly controlled to preserve the integrity and bioactivity of peptides. The main factors affecting the quality of the final product are temperature, pH, and time of reaction. Too much heat and/or prolonged processing can destroy these sensitive bioactive compounds, reducing their therapeutic potential. Some of the purification techniques whose optimization is of utmost importance

in the effective separation and recovery of bioactive peptides while retaining functional properties include ultrafiltration and chromatography [59].

Roles of echinozoa peptides and its mechanism

Sea cucumbers and sea urchins are of wide bioactive properties, hence adding to their potential in the therapeutic field of several disease treatments. Sea urchins have shown remarkable anti virus [42], antifungal [60], antiparasitic [61,62], anti inflammatory [63,64], anti lipidemic [42], antidiabetic [65,66], anticardiotoxic [67] and hepatoprotective [42] as well as gastroprotective activities, which, to date, have been attributed to compounds such as naphthoquinones, PUFAs, and pigments like echinochrome A. Similarly, antioxidant [67-69] anticancer [54,69], antidiabetic [67,70,71], antimicrobial [67,70,72], anti obesity [70], anti thrombotic [73,74] and anti-inflammatory [67,75] activities are well documented in sea cucumbers due to their high composition of triterpene glycosides, polysaccharides, saponins, and lipids. However, while these studies demonstrate the pharmacological potential of Echinozoa, most of the available studies have focused on the broader chemical compounds and extracts rather than the isolation and characterization of specific peptides. Most research on their bioactivities has been limited to organic extracts or enzymatic hydrolysates, without any in-depth exploration into peptide sequences or mode of action. Such is a serious gap in literature, while peptides from these marine species would likely be responsible for much of the health benefits seen and remain unexploited. This gap is foreseen to be filled by further research that should focus on the isolation, identification, and functional analysis of bioactive peptides opening new perspectives for therapeutic applications (**Table 2**).

Table 2 Summary of bioactive compounds and therapeutic activities of sea cucumbers and sea urchins, with emphasis on non-peptide components.

Echinozoa	Biological activity	Extract/compound	Spesies	References
Sea urchin	Antifungal	Whole body dichloromethate extract.	<i>Diadema antillarum</i>	[60]
	Antiparasitic	Extracts obtained with PBS from the spine and test.	<i>Echinometra mathaei</i>	[61]

Echinozoa	Biological activity	Extract/compound	Species	References
		Crude extract and fractionated naphthoquinones pigments from spines and test.	<i>Evechinus chloroticus</i>	[62]
	Antiinflammatory	Diterpenoid isolated from the gonads.	<i>Stomopneustes variolaris</i>	[63]
		Lyophilized gonads.	<i>Mesocentrotus nudus</i>	[64]
	Antilipidemic	Echinochrome-A isolated from test and spines.	<i>Paracentrotus lividus</i>	[42]
		Methanolic extracts obtained from the soft tissues (gonad, mouth and intestine) Echinochrome-A isolated from test and spines.	<i>Echinometra mathaei</i>	[61]
	Antidiabetic	Aqueous extracts from viscera and spines.	<i>Scaphechinus mirabilis</i>	[65]
		Echinochrome-A	<i>Scaphechinus mirabilis</i>	[66]
	Anticardiotoxic	Thermostable fractions of coelomic fluid.	<i>Tripneustes depressus</i>	[66]
	Antivirus	Echinochrome-A isolated from test and spines.	<i>Paracentrotus lividus</i>	[42]
	Hepatoprotective	Echinochromes	<i>Paracentrotus lividus</i>	[42]
	Gastroprotective	Organic/ aqueous extracts, enzymatic hydrolysates, and polysaccharides.	<i>Apostichopus japonicas</i> , <i>Actinopyga lecanora</i> ,	[67]
Sea cucumber	Antioxidant	Organic extract, and extracts triterpene glycosides.	<i>Holothuria parva</i> ,	[68]
			<i>Apostichopus japonicus</i> , <i>Neothyonidium magnum</i>	[69]
	Anticancer	Organic extract fucoidan fucosylated chondroitin sulphate enzymatic hydrolysates	<i>Stichopus japonicus</i> ,	[69]
			<i>Isostichopus badiionotus</i> , <i>Cusumaria frondose</i> , <i>Holothuria forskali</i> , <i>Parastichopus tremulus</i> .	[54]
	Antiinflammatory	Fucosylated polysaccharide sulfate.	<i>Holothuria mexicana</i> ,	[75]
			<i>Isostichopus badiionotus</i> , <i>Pearsonothuria graeffei</i> , <i>Holothuria polii</i> .	[67]
	Antithrombotic	Organic extract	<i>Holothuria parva</i> ,	[73]
			<i>Stichopus variegatus</i> .	[74]
	Antimicrobial	Peptide , saponin, and fatty acids	<i>Acaudina molpadioides</i> ,	[70]
			<i>Holothuria thomasi</i> <i>Stichopus japonicas</i>	[67]

Echinozoa	Biological activity	Extract/compound	Species	References
				[72]
	Antidiabetic	Phospholipid, cerebrosides, fucosylated chondroitin sulfate, and vanadium	<i>Cucumaria frondose</i> , <i>Acaudina molpadioides</i> , <i>Apostichopus japonicas</i> .	[67] [70]
	Antiobesity	Cerebrosides, and phosphatidylcholine.	<i>Acaudina molpadioides</i> .	[71] [70]

Antioxidant and antiaging

Oxidative stress arises due to an imbalance in the generation and neutralization capacity of ROS that leads to cell damage and degeneration. In oxidative stress, the free radicals have a reaction with proteins, lipids, and DNA by causing cellular dysfunctions, which may lead to the induction of apoptotic cell death and a variety of chronic diseases. This could be countered through neutralizing free radicals, chelation of pro-oxidant metal ions, inhibition of lipid peroxidation, and modulation of the activity of oxidative enzymes through the action of antioxidants such as bioactive peptides and protein hydrolysates [76,77].

The mechanisms of action in antioxidant peptides are scavenging of free radicals by the neutralization of the reactive oxygen/nitrogen species by donation of hydrogen atoms or electrons through processes termed hydrogen atom transfer, HAT, and single electron transfer, SET [78]. Besides, peptides may chelate transition metal ions, reduce the metal-catalyzed oxidative damage, and inhibit lipid peroxidation by stabilizing the lipid membranes. The most interesting thing is that some peptides can even activate endogenous antioxidant enzymes like superoxide dismutase (SOD), glutathione peroxidase (GSH-Px),

and catalase (CAT), thus increasing the body's self-defense against oxidative stress [79] (**Figure 3**).

The structure–activity relationship (SAR) of antioxidant peptides is quite complicated, and many factors need to be considered: molecular weight, amino acid content, sequence, and the positioning of certain residues of peptides. Peptides with molecular weights below 1,400 Da showed stronger activity as antioxidants because they can interact more with free radicals [80]. Aromatic and positively charged amino acids like cysteine, tryptophan, histidine, and tyrosine increase radical scavenging properties through electron donation [81]. Hydrophobic amino acids, especially proline, could show higher interaction of peptides with the lipid-based radicals and thus improve the antioxidant activity [82]. Moreover, the position and sequence of certain amino acids within a peptide also determine the extent of antioxidant activity. For example, the substitution of leucine with isoleucine internally, but not at the N- or C-terminal, enhances antioxidant activity [83]. The electronic, hydrogen-bonding, and steric effects of amino acid residues, especially in tripeptide sequences, also impact antioxidant activity. The peptide chain's steric structure and chemical interactions at both termini might help or hamper reactive oxygen species neutralization [81].

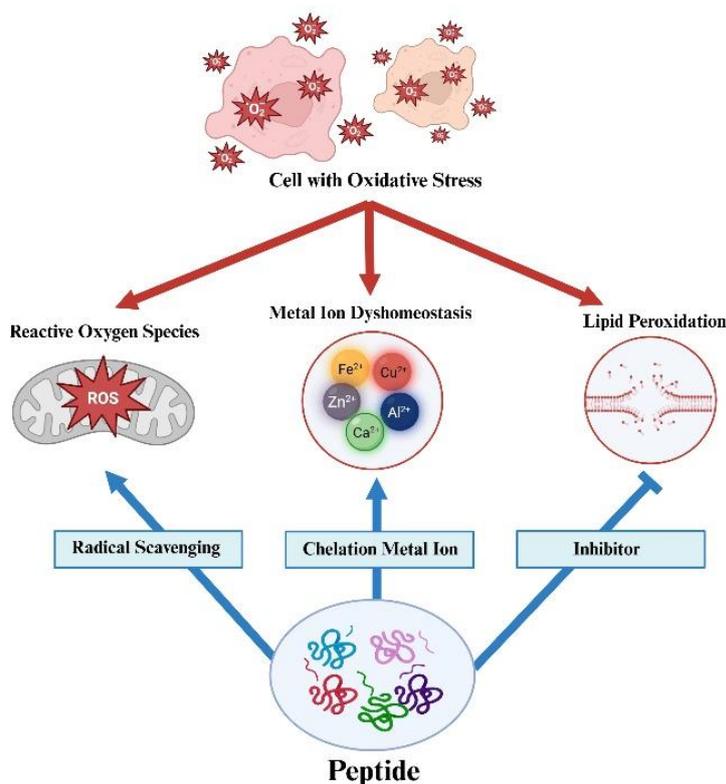


Figure 3 Antioxidant mechanism of peptides derived from sea urchins and sea cucumbers.

The antioxidant activity of peptides is influenced by their structural characteristics such as amino acid composition, sequence, and size [84]. Certain amino acids, particularly tryptophan, tyrosine, cysteine, and homocysteine, have shown antioxidant abilities at physiological concentrations [85]. The antioxidant capacity of peptides is closely related to their molecular mass, hydrophobic amino acid residues, acidic amino acids, and specific antioxidant amino acids. Interestingly, the interaction of peptides with phenolic compounds can affect their antioxidant capacity profile, potentially increasing the cellular antioxidant activity [86]. This suggests that the presence of specific amino acids may be the key to enhancing antioxidant activity [87]. These structural traits demonstrate the difficulty of creating and discovering antioxidant peptides with optimal functions

Several studies have demonstrated potent antioxidant activity of peptides derived from sea cucumbers. Peptide hydrolysates from *Apostichopus japonicus* (AjPH) have been shown to significantly reduce ROS levels and enhance antioxidant defense

mechanisms in *Caenorhabditis elegans*, highlighting their potential in combating aging and cancer-related oxidative stress [88]. Peptides of *Acaudina leucoprocta* have been reported to increase longevity in *C. elegans*. It enhanced the lifespan of *C. elegans* by up to 31.46 %, mainly due to increased resistance to oxidative stress, reduced accumulation of age-related pigments, and activation of the DAF-16/DAF-2/SOD-3/OLD-1/PEPT-1 signaling pathway that regulates antioxidant defenses and longevity [89]. In another study, peptides from *Holothuria leucospilota*, prepared by enzymatic hydrolysis using alcalase and flavourzyme, showed potent antioxidant activities by scavenging superoxide radicals and reducing mitochondrial oxidative damage [90]. These peptides prevent also glutathione depletion and mitophagy of neuroblastoma cells, which further illustrate their neuroprotective actions. Other works have pointed out that peptides from sea cucumber can enhance cell viability after UV-B treatment. This work also has shown that hydrolysates can increase the survival rate of HaCaT cells (keratinocyte cell lines) [91]. Interestingly, peptides digested with trypsin show

strong antioxidant activities compared to peptides digested with other enzymes [41]. Bioactive peptides from sea urchins also possess excellent antioxidant activity. Two peptides, SnP7 (AAVPSGASTGIYEALRLR) and SnP10 (NPLLEAFGNAK), with molecular weights of approximately 1,173.34 and 1,805.03 Da, respectively, isolated from the purple sea urchin (*Strongylocentrotus nudus*), reduce ROS levels and upregulate the expression of antioxidant enzymes superoxide dismutase-3 (SOD-3) and heat shock protein-16.2 (HSP-16.2) in oxidation-stressed nematodes [86]. These peptides also induced nuclear translocation of DAF-16 and expression of stress-related genes, further enhancing their antioxidant potential (Table 3).

It was demonstrated in the *in silico* experiment that tryptophan-rich peptides of sea urchin origin have remarkable antioxidant abilities, simply because of this amino acid's peculiar chemical nature that assists the peptides in giving a more active neutralization response toward ROS [86]. The hydrolysates of protein from *Holothuria leucospilota* reduce ROS production and mitochondrial superoxide level, hence supporting mitochondrial health and preventing oxidative stress-related damages [92]. Other than anti-oxidative activities, peptides from *Apostichopus japonicus* showed anti-aging activity: Gradual decrease of pigmentation was identified along with the potential for life span extension. These effects are likely closely related to their unique physicochemical properties, as was confirmed by the *in silico* analysis [93].

Table 3 Peptides derived from sea cucumbers and sea urchins exhibit antioxidant activity.

Species	Peptides	Outcomes	Activities	References
<i>Acaudina leucoprocta</i>	CFH	Improve learning memory and cognitive impairment	Antioxidant	[90]
<i>Stichopus variegates</i>	SVH-PF, SVH-CAH-PF	Up-regulate Klotho expression, activating SOD and GSH-Px; inhibit lipid peroxidation and protein oxidation	Antioxidation, antiaging	[85]
<i>Apostichopus japonicus</i>	AjPH(GF2, GF3)	Increase the survival rate; reduce ROS level; delay physiological aging	Antioxidant, antiaging	[88]
<i>Apostichopus japonicus</i>	TP-WW-620, TP-WW-621, TP-WW-623	Protect cells against hydrogen peroxide; reduce the oxidative stress induced through the depletion of cellular glutathione; decrease mitochondrial superoxide levels; alleviate mitophagy in human neuroblastoma cells	Antioxidant	[88]
Sea cucumber	SCP-1, SCP-2	Improve exercise performance in mice; reduce metabolism accumulation and muscle injury and enhanced glycogen storage in mice; inhibit oxidative stress and enhance energy metabolism in mice; modulate oxidative stress and mitochondria function-related protein	Antifatigue, oxidative stress, mitochondrial	[106]

Species	Peptides	Outcomes	Activities	References
		expression in the skeletal muscles		
<i>Strongylocentrotus nudus</i>	SnP7, SnP10	Reduce reactive oxygen species (ROS) level and the expression of superoxide dismutase-3 (SOD-3) and heat shock protein-16.2 (HSP-16.2) in oxidation-damaged nematodes; induce DAF-16 nuclear translocation and the expression of stress-related genes, such as sod-3	Antioxidant	[86]

Sea cucumbers and sea urchins have demonstrated promising anti-aging properties because of their peptides and other bioactive compounds. These marine invertebrates possess potent antioxidant and anti-inflammatory activities, both of which are crucial for slowing down the aging process and protecting against age-related diseases [90]. Research has explored the relationship between oxidative stress and aging in various sea urchin species of different lifespans. Studies on short-lived *Lytechinus variegatus*, long-lived *Strongylocentrotus franciscanus*, and intermediate-lived *Strongylocentrotus purpuratus* revealed that negligible senescence in longer-lived species is linked to sustained antioxidant capacity and stable proteasome enzyme activity, which together reduce the accumulation of oxidative damage over time [46]. The insulin/IGF-1 signaling (IIS) pathway is a key pathway associated with aging. Inhibiting the IIS signaling pathway can extend lifespan and delay age-related diseases in animal models. The key molecules in the IIS pathway include DAF-2, AGE-1, and DAF-16. Autophagy gene cascade signaling, acting downstream of the IIS, can modulate autophagy, affect aging, and regulate anti-aging activity [94]. Moreover, peptides of *Acaudina leucoprocta* have been reported increase heat resistance and mobility in nematodes, which further justifies their use in healthy aging. More interestingly, antioxidant peptides prepared from oyster *Pinctada fucata* have been reported to prevent photoaging of the skin. Such peptides retard wrinkle formation, maintain skin elasticity, and regulate lipid peroxidation by preserving the activities of antioxidant enzymes like SOD, GSH-Px, and CAT [90]. Though not on sea cucumbers and sea urchins, this

current research indicates the promising avenue of anti-aging in a marine-derived peptide.

Anticancer properties

Bioactive peptides have received great attention as promising candidates for anticancer therapies due to their excellent specificity, good cellular permeability, robust target engagement, and acceptable safety profile. Their multifaceted modes of action can allow them to target cancer cells with a minimal deleterious effect on healthy organs. Such peptides can kill cancer cells through membrane disruption, inhibition of intracellular signaling pathways, or interference with the cell cycle [76]. Besides, the anticancer peptides (ACPs) can be designed to preferably interact with cancer-specific receptors or proteins by acting as agonists or antagonists. They can also be used for diagnostic imaging agents or even theranostic agents with combined therapeutic and diagnostic functions [95].

Numerous studies have documented the anti-cancer properties of bioactive peptides derived from sea cucumbers. Sea cucumber-derived compounds, including peptides, have been reported to possess anticancer activity [96]. The cytotoxic activity of peptides derived from sea cucumber has been reported for *Isostichopus badionotus* hydrolysates, and ultrafiltered fractions showed cytotoxic activity against colorectal HT-29 cells, marking the first such report for sea cucumber peptides [97]. Likewise, organic extracts of *Stichopus horrens* exhibited cytotoxic activities against A549 lung cancer and TE1 esophageal cancer cells with IC₅₀ values of 15.5 and 4.0 µg/mL, respectively [98]. In addition, sphingoid bases from sea

cucumber cerebrosides induce apoptosis in DLD-1, WiDr, and Caco-2 colon cancer cells, suggesting that these compounds could be a good bioactive dietary food component to suppress colon cancer [99]. In this direction, other cyclic peptides, geodiamolides A, B, H and I, extracted from the sponge *Geodia corticostylifera* expressed their anti-proliferative activities against the 2 human breast carcinoma cell lines T47D and MCF7 by inducing disorganization in actin filaments. In another study, MCF-7 tumor cells were treated with sea cucumber peptides. The results showed that sea cucumber peptides could activate the apoptotic pathway via PI3K/AKT signalling [44]. Peptides from *H. leucospilota* may block several proteins that regulate the cell cycle in multiple cell lines including NIH-3T3, HaCaT, and 16HBE. These peptides may also activate the apoptotic pathway and induce apoptosis in these 3 cell lines. Moreover, such peptides could inhibit the migration of cancerous cells and may have applications in the design of anticancer compounds [100]. Showing more evidences of anticancer activities, ESC-AQ fraction, the aqueous fraction from edible sea cucumber *Holothuria edulis*, exerted a robust cytotoxic action on human leukemia cells HL-60. In fact, by using flow cytometric analysis, the ESC-AQ was confirmed to induce the apoptotic body formation of HL-60 cells [101]. This is an upregulation mechanism for the pro-apoptotic proteins Bax and caspase-3, while the anti-apoptotic protein Bcl-xL is downregulated. The mechanistic studies have also pointed out that peptides from sea cucumbers could activate the PI3K/AKT signaling pathway, which is in charge of cellular growth, death, and metabolism. This system, once being activated by peptides from sea cucumbers, suppresses PTEN, leading to a change in glucose metabolism and energy generation which promotes the death of tumor cells. This route is blocked by these peptides, reducing the ability of tumor cells to wildly proliferate and survive [39].

Although less studied, peptides derived from sea urchin have also shown potential anticancer activities. *In silico* analysis of peptides from the species *Arbacia lixula* indicated that these peptides may inhibit proteins

involved in NSCLC, including EGFR, PI3K, BRAF V600E, and JAK3 [102]. This suggests their potential to target multiple signaling pathways critical for the survival and proliferation of cancer cells. Indeed, peptides obtained from *S. nudus* SnP7: AAVPSGASTGIYEALELR; SnP10: NPLLEAFGNAK have shown *in vitro* induction of apoptosis and retardation in the development of cancer. Peptides down-regulated ROS levels by up-regulation of stress-responding gene expression such as SOD-3 and HSP-16.2, thereby showing dual potential in alleviation of oxidative stress and induction of death of cancerous cells [86].

Based on the cumulative research conducted to date, bioactive peptides from sea cucumbers and sea urchins have been found to exhibit anticancer properties through several interrelated mechanisms. One of the major pathways involves the induction of apoptosis, where peptides upregulate pro-apoptotic proteins such as Bax and caspase-3, while downregulating anti-apoptotic proteins such as Bcl-xL, leading to programmed cell death in cancer cells [101]. These peptides further disrupt important cancer signaling pathways, including PI3K/AKT and STAT3, critical for cancer cell survival, proliferation, and metabolic pathways. Suppression of such pathways interferes with glucose metabolism and triggers programmed cell death to affect cancer growth [103]. Indeed, some peptides also prevent the migration and invasion capability of cancer cells by disrupting the structures, thereby reducing metastatic occurrences, which is evident in *in vitro* experiments carried out with peptides obtained from *Holothuria leucospilota* [104]. Another important process is the modulation of oxidative stress. By decreasing the levels of ROS in cancerous cells, these peptides induce oxidative stress in malignant cells while normal cells are protected, thereby making the environment unfavorable for tumor survival and thus leading to the death of cancerous cells [86]. Collectively, these processes demonstrate the great therapeutic potential of Echinozoa-derived peptides in cancer prevention and treatment (**Figure 4**).

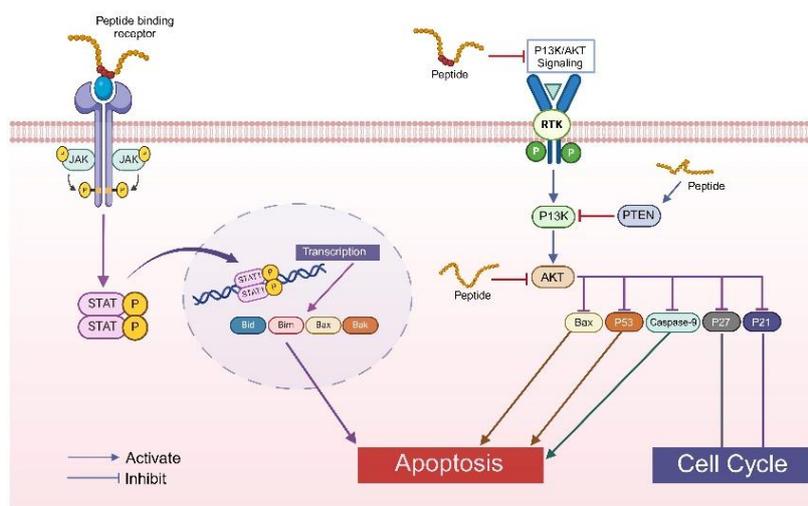


Figure 4 Anticancer mechanism of peptides derived from sea urchins and sea cucumbers.

Antifatigue

Bioactive peptides derived from sea cucumbers have shown promising anti-fatigue effects, primarily by improving energy metabolism, enhancing antioxidant capacity, and regulating key signaling pathways. In animal studies, sea cucumber peptides have demonstrated the ability to significantly improve exercise performance and alleviate fatigue symptoms. Research indicates that peptides can prolong exhaustion time during swimming tests and increase forelimb grip strength in mice. Hence, 2 peptides with different degrees of hydrolysis from *A. leucoprocta* were tested in mice, and both peptides significantly enhanced endurance and reduced fatigue [105]. Notably, the peptide with a higher degree of hydrolysis produced stronger antifatigue effects, as evidenced by prolonged exhaustive swimming time, increased forelimb grip strength, reduced metabolite accumulation (such as blood urea nitrogen (BUN) and lactate acid), and elevated muscle glycogen and energy metabolism levels.

These antifatigue effects are closely associated with the activation of the nuclear factor erythroid 2-related factor 2 (NRF2) and AMP-activated protein kinase (AMPK) signaling pathways. AMPK pathway activation potently improves energy production by enhancing peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α), a principal regulator of mitochondrial biogenesis. Subsequently, NRF1 and TFAM are activated, further enhancing mitochondrial function and energy metabolism. Thus,

this increased mitochondrial activity enhances energy metabolism, reducing fatigue. Further, sea cucumber peptides may stimulate mRNA and protein expressions involved in genes of lipid catabolism, mitochondrial biogenesis, and gluconeogenesis, further contributing to enhanced energy use during exercise. With the heightened quantity and activity, there is higher efficiency in the production of ATP, enhanced oxidative phosphorylation, both events summing up to alleviate fatigue. Moreover, the NRF2-Keap1 pathway plays an important role in decreasing oxidative stress through the activation of antioxidant defenses and further supports antifatigue properties of peptides described above [106].

Recent research has also put forward the role of bioactive peptides in modulating metabolic factors related to physical endurance [107]. For instance, peptides from sea cucumbers have been reported to modulate enzymes involved in glycogen storage and fatty acid oxidation, thus supplying energy during prolonged exercises. Such peptides improve physical performance and delay the onset of fatigue by enhancing mitochondrial function and reducing oxidative damage. Considering antioxidant properties and metabolic regulation, the fact that studies related to peptides from sea urchins are few is highly promising. Further research will be required in order to fully investigate the antifatigue properties of peptides from sea cucumbers and sea urchins and their applications relating to sports nutrition and functional food products.

Antidiabetic

Bioactive peptides have emerged as highly promising antidiabetic agents, as they demonstrate the capacity to modulate key enzymes and pathways involved in glucose metabolism and insulin signaling.

The antidiabetic properties of these peptides are manifested through multiple mechanisms, including the inhibition of carbohydrate-digesting enzymes, modulation of the insulin pathway, and regulation of oxidative stress and inflammatory processes (**Figure 5**).

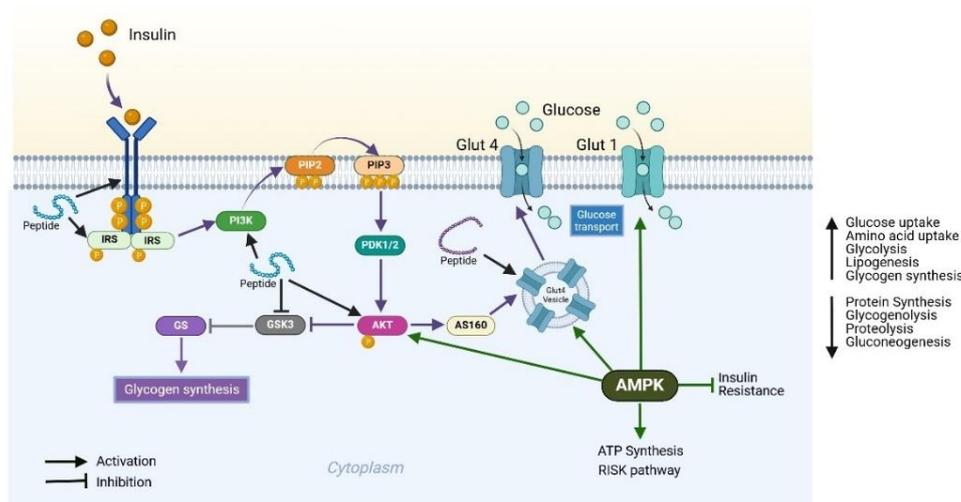


Figure 5 Antidiabetic mechanism of peptides derived from sea urchins and sea cucumbers.

These peptides act to regulate the level of blood sugar by inhibiting the action of enzymes α -amylase and α -glucosidase, which breaks down carbohydrate foods into glucose. Inhibition of these enzymes reduces the gut absorption rate, hence preventing sharp blood glucose elevation, the same as pharmacological anti-diabetic agents, acarbose [108]. Apart from that, these peptides may influence GLP-1 activity by inhibiting DPP-IV and, therefore, play an important role in enhancing insulin secretion and promoting blood glucose homeostasis [108,109]. Some bioactive peptides have been reported to exhibit improved insulin sensitivity and the promotion of insulin production, just like conventional drugs prescribed for this purpose, such as metformin [110].

Sea cucumbers contain a variety of bioactive substances, including peptides, polysaccharides, and lipids, all of which contribute to their strong anti-diabetic capabilities [50,53]. For example, gonadal protein hydrolysates from *Cucumaria frondosa* have been demonstrated to reduce blood glucose and glycated hemoglobin (HbA1c) levels in diabetic rats. Treated mice also consumed less water, a common sign of diabetes, indicating successful glucose management.

Histological analysis revealed that these peptides protected liver tissue, most likely by modulating glucose metabolism via the activation of the IRS/Akt signaling pathway and the AMP-activated protein kinase (AMPK) pathway, both of which regulate lipid metabolism [105]. Further, peptides derived from sea cucumbers demonstrated positive effects in wound healing on a diabetic model by reducing the pro-inflammatory cytokines like IL-6, IL-8, and TNF- α , reactive oxygen species, while increasing the albumin, prealbumin, nitric oxide, and vascular endothelial growth factor, thus aiding the tissue and vascular repair mechanisms [110]. These findings shed light that peptides of sea cucumber play a dual role not only in the control of blood glucose but also in wound healing, an important part of treating diabetes.

On the molecular level, bioactive peptides from Echinozoa may increase insulin receptor sensitivity, thus enhancing the efficiency of insulin signaling. The IR undergoes autophosphorylation upon binding of insulin, leading to the activation of IRS. This will initiate a variety of downstream pathways such as PI3K/Akt and MAPK pathways that are necessary for glucose uptake and metabolic regulation [111]. The activated PI3K

pathway initiates a phosphorylation cascade via PDK1, which activates the Akt/PKB proteins. Akt enhances the translocation of glucose transporters GLUT4 to the cell membrane, thereby increasing the rate of glucose uptake in both muscle and fat cells. Meanwhile, activation of the MAPK pathway occurs via the GRB2/SOS/Ras/MAPK cascade, phosphorylating ERK1/2; these events support the promotion of cell proliferation and differentiation to further support metabolic health. The AMPK pathway further interacts with NRF2 to regulate energy metabolism and oxidative stress. Phosphorylated AMPK enhances the expression of PGC-1 α , facilitating mitochondrial biogenesis and enhancing the oxidation of lipids. This enhances energy balance and glucose utilization. Activation of the NRF2-Keap1 complex further diminishes oxidative stress, which is one of the major factors contributing to insulin resistance [107]. Peptides from Echinozoa have also demonstrated enzyme inhibition, regulation of insulin pathways, and reduction in oxidative stress—all of which enhance their potential as a natural antidiabetic drug. The ability to target multiple pathways gives some advantage over single-target conventional medications; hence, they are an exciting candidate for incorporation into functional foods and nutraceutical products in the prevention and control of diabetes [109,112]. However, further study is required to completely understand their mechanisms of action, enhance bioavailability, and assess effectiveness and safety in clinical settings.

ACE-inhibitor

Bioactive peptides from sea cucumbers and sea urchins have shown considerable potential as natural ACE inhibitors with possible applications in hypertension treatment. ACE regulates blood pressure by converting angiotensin I to angiotensin II, which is a potent vasoconstrictor, and also degrading bradykinin, which is a vasodilator [113,114]. ACE inhibition prevents the synthesis of angiotensin II while bradykinin levels are maintained, causing vasodilation, a reduction in blood volume and, finally, a reduction in blood pressure [115]. This inhibition is often competitive, such as for some dipeptides generated from milk proteins, but the inhibitory mechanisms of marine-derived peptides include non-competitive and mixed inhibition [116].

Numerous investigations have revealed strong ACE-inhibitory peptides derived from sea cucumbers.

The peptide NAPHMR, obtained from the gonad hydrolysate of *Stichopus japonicus*, had the most significant ACE-inhibitory action, with an IC₅₀ of 260.22 \pm 3.71 μ M [117]. Three non-competitive inhibitory peptides—DDQIHIF, HDWWKER, and THDWWKER - extracted from the gonads of *Apostichopus japonicus* shown ACE-inhibitory activity with IC₅₀ values of 333.5, 583.6 and 1291.8 μ mol/L, respectively [118]. The ACE-inhibitory action of these peptides is affected by the type of sea cucumber and the particular circumstances of enzymatic hydrolysis employed. Hydrolysates from *Holothuria atra* generated with alcalase exhibited the highest ACE inhibition, with an IC₅₀ value of 0.32 mg/mL [119]. Collagen hydrolysates from sea cucumbers have been shown to reduce ACE activity and improve vascular function, contributing to their antihypertensive properties [120]. Bioactive peptides from *Stichopus vastus* also exhibited significant ACE-inhibitory activity and could be potentially used in functional foods and nutraceuticals for the management of hypertension [121].

Studies on revealing the mechanism of ACE-inhibitory peptides from sea cucumbers exhibits that the peptides bind to the active site of the ACE enzyme, block the conversion of angiotensin I to angiotensin II and inhibit bradykinin breakdown. The binding contact comprises hydrogen bonds, hydrophobic interactions, and coordination with the enzyme's Zn⁺-binding motif, which is important for ACE function. *In silico* molecular docking studies indicate that the NAPHMR peptide blocks substrate access by interacting with ACE's S2 pocket and Zn²⁺-binding site via hydrogen and π -bond interactions [117]. Similarly, the peptides DDQIHIF, HDWWKER, and THDWWKER from *A. japonicus* were discovered to stabilize within the ACE protein cavity, boosting their inhibitory impact via strong contacts with the enzyme's active region [118]. Interestingly, these peptides show non-competitive inhibition, attaching to places other than the active site, changing the enzyme's structure and decreasing its activity. In contrast, peptides from *Holothuria atra* hydrolysates demonstrated a mixed inhibitory pattern, indicating that marine-derived peptides can affect ACE activity via a variety of ways [119].

Aside from direct ACE inhibition, certain peptides may alter larger signaling cascades important to vascular

health. ACE inhibitors have been demonstrated to stimulate casein kinase 2 (CK2), which phosphorylates the ACE serine residue, activating the MKK7/JNK pathway. This, in turn, phosphorylates c-Jun, boosting the activity of activator protein 1 (AP-1) and encouraging the production of cyclooxygenase-2 (COX-2), a critical enzyme in regulating vascular inflammation [106]. Additionally, ACE inhibitors can cause MYH9 phosphorylation, which stabilizes ACE in the plasma membrane and influences blood vessel tension [122].

The ACE-inhibitory peptides found in sea cucumbers and sea urchins not only contribute to maintaining blood pressure but also provide other cardiovascular advantages by controlling oxidative stress, inflammation, and vascular remodeling. Their multifaceted approach, along with their natural nature, makes them promising candidates for the creation of antihypertensive functional foods and nutraceutical products. Furthermore, peptides such as Muracein A from *Nocardia orientalis* are highly selective for ACE while having little impact on other zinc-dependent enzymes, indicating the possibility for designing focused medicines with low side effects [123]. Further structure-activity relationship studies, improvement in bioavailability, and determination of their long-term safety and efficacy in clinical trials would be required. Investigating additive effects with existing anti-hypertensive drugs may provide novel opportunities for combination therapy.

Antimicrobial

In this regard, bioactive peptides obtained from sea cucumbers and sea urchins showed very important antibacterial activity and hence are interesting candidates for the development of new therapies against microbial diseases. Antimicrobial peptides (AMPs) represent key components of innate immunity in marine invertebrates and usually show broad-spectrum activity against Gram-positive and Gram-negative bacteria and fungus [124]. Being of natural origin, their multi-targeting modes make them very effective options for conventional antibiotics in view of the development of resistance.

Several novel AMPs have been discovered in sea urchins. Notably, strongylocins 1 and 2 isolated from the green sea urchin (*Strongylocentrotus droebachiensis*) have substantial antibacterial action against a variety of

bacterial infections [125]. These defensin-like peptides are cationic and disrupt bacteria membranes. In another study, peptide fractions with high antibacterial activity were isolated from the brown sea urchin *S. nudus* [126]. Further investigation of the red sea urchin *Pseudocentrotus depressus* led to the identification of amino acids like arginine, glutamine, phenylalanine, and threonine that assist in the antimicrobial defense of the organism [127]. Centrocins 1 and 2 are isolated from *S. droebachiensis* and *Echinus esculentus*, respectively, 2 other potent antimicrobial peptides found in sea urchin. Centrocins contain a heavy and light chain, and their antibacterial activities appear to reside primarily in the heavy chains. Their antibacterial activity is connected to the C-terminal amino acids (Gly and Arg), which play an important role in breaking bacterial membranes [128,129]. Centrocin 1, which is stored in phagocyte vesicles, is activated to generate phagolysosomes upon bacterial infection, showing its critical involvement in the sea urchin immune system even during the larval pluteus stage [130].

Sea cucumbers have been demonstrated as well to have high levels of AMPs. The methanolic extract of *Holothuria parva*'s intestinal tract demonstrated significant antifungal and antibacterial activity, with minimum inhibitory concentrations (MICs) of 0.09 mg/mL against *Saccharomyces cerevisiae* and 0.04 mg/mL against *Staphylococcus epidermidis* [130]. This extract showed less efficacy against more resistant bacterial strains, including *Candida albicans*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae*, thus displaying selective activity against microbes. Lysozymes from sea cucumbers have been reported with high antibacterial activities against Gram-positive and Gram-negative bacteria. Notably, the C-terminal peptide (SjLys-C) from the species *Stichopus japonicus* was identified and appeared to enhance the activity after heating treatment [131]. Antimicrobial peptides - The coelomic fluid of *Cucumaria frondosa* possesses antimicrobial peptides. This peptide showed an approximate molecular weight of 6,000 Da and proved to be efficient at pH 5. [132]. Furthermore, peptides derived from sea cucumber hemolytic lectins have been transformed into new AMPs, CGS19 and CGS20, which exhibit significant anti-MRSA efficacy via 2 mechanisms: compromising membrane integrity and blocking the folate biosynthesis pathway [119].

Furthermore, peptides derived from *Holothuria tubulosa* have shown inhibitory efficacy against *Listeria monocytogenes*, a common foodborne pathogen. These peptides exert inhibitory action on bacterial growth and limit biofilm formation, a key event in persistent infections and antibiotic resistance [133]. The molecular dynamics simulations highlighted that the tertiary structure and its amphipathic characteristics confer on these peptides the capability of interacting effectively with bacterial membranes, thus compromising their integrity.

The mechanism of antimicrobial actions of AMPs from sea cucumbers and sea urchins primarily exert their antimicrobial effects by targeting the bacterial membrane, leading to cell death [134]. These peptides are typically cationic, allowing them to bind strongly to the negatively charged bacterial membranes. Once attached, they disrupt membrane integrity through mechanisms such as the barrel-stave, toroidal, and carpet models, resulting in pore formation or membrane disintegration [135,136]. In addition to disrupting membranes, certain AMPs enter microbial cells and interfere with intracellular activities such as DNA replication, protein synthesis, and enzyme function. This dual action, which combines membrane disruption and intracellular targeting, not only improves antimicrobial

activity but also lowers the risk of bacteria acquiring resistance [137]. *In silico* studies have revealed the molecular connections between these peptides and bacterial targets. Molecular docking simulations indicated that sea cucumber peptides may establish hydrogen bonds and hydrophobic interactions with bacterial membrane components, increasing their antibacterial activity [138]. These computational methods have also been utilized to create tailored peptides with greater stability and selectivity (Figure 6).

In addition to these mechanisms, antibiotic-resistant biofilms also pose a huge problem in the treatment of bacterial diseases. Antibiofilm activities have been reported for sea cucumber and sea urchin peptides, which might become potential drugs for chronic infections. For example, coelomocyte lysates from the sea urchin *Paracentrotus lividus* and sea cucumber *Holothuria tubulosa* contain AMPs that inhibit biofilm formation in *Staphylococcus epidermidis* and *Staphylococcus aureus* at sub-MIC levels [125]. e, Holothuroidin 2 (H2) and its derivative H2d, isolated from *H. tubulosa*, have shown strong antibiofilm action against *Listeria monocytogenes* [134]. This feature is particularly useful in food safety and medical device coatings, where biofilm avoidance is vital.

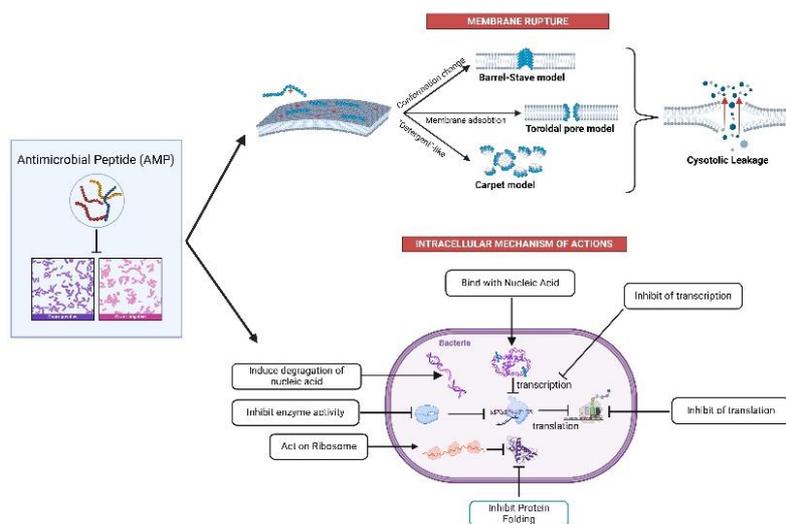


Figure 6 Antimicrobial mechanism of peptides derived from sea urchins and sea cucumbers.

Memory enhancement

Bioactive peptides produced from sea cucumbers can also enhance memory function and modify the

hippocampus in an animal model of memory impairment. If left untreated, this memory disorder may develop into Alzheimer disease (AD). Based on this

work, mice fed peptides derived from sea cucumbers exhibited modest memory improvement. This memory improvement was demonstrated by analyzing the behavior of the mice, such as by placing the mice in a labyrinth. The brain damage in mice treated with sea cucumber peptides improves; this behavioral trial shows that these peptides have the power to increase and minimize damage to the brain and memory. Measuring acetylcholine levels in the hippocampus, besides other capabilities, these peptides can also increase acetylcholine levels in the hippocampus. Because acetylcholine is not degraded by acetylcholinesterase, high content means that information may be transmitted, thus improving mouse memory [139,140]. It assessed the neuroprotective function of sea cucumber ovum peptide-derived NDEELNK and found its neuroprotective effect by improving acetylcholine levels and reducing AChE activity in PC12 cells. This enhances superoxide dismutase, reduces reactive oxygen species, improves energy metabolism in scopolamine-exerted PC12 cells, and downregulates various signaling proteins. The confirmation study by *in silico* showed the interaction of AChE involves hydrophobic and hydrogen bonds, bioactive peptides crucial for their activity. The interactions observed through molecular docking confirmed that the binding cavity wall amino acid residues interacted with the active site of NDEELNK peptide from sea cucumber ovum enhance the AChE inhibition effect.

Current challenges and future prospective

Current challenges for bioactive peptides

The main problem with bioactive peptides for drug development is related to stability, delivery, and bioavailability. Oral administration is poor due to the gastrointestinal tract because peptides are easily digested by enzymes and have low permeability across the intestinal epithelium [141,142] (**Figure 7**). Thus, their oral bioavailability is usually low, which calls for other modes of delivery or strategies to protect them. Bioactive peptides can be absorbed and may exert multi-bioactive functions. Depending on the amino acid sequence, their function can be identified by protein selection, enzymatic hydrolysis, the isolation, and the purification of these amino acids. The final listing was for the biological activities, the peptide sequences, and the functional properties [143]. Microencapsulation is one of the effective techniques to enhance biopeptides' stability and bioavailability due to environmental factors. Sensitive compounds were enclosed in small capsules in order to minimize the risks and increase their efficiency [144,145]. Microencapsulation can protect the peptides from digestion enzymes, improve the solubility, and increase the dissolution rate of the bioactive compounds. In one study, lutein microparticles with smaller particle sizes showed higher apparent solubility and dissolution rates compared to bigger particles and their physical mixtures [48].

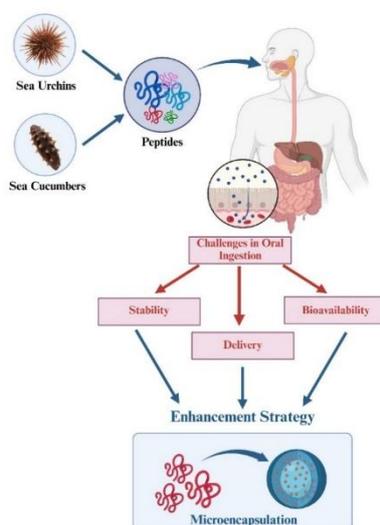


Figure 7 Challenge in the development of bioactive peptides for drug discovery.

The challenge with *in vitro*-based bioactive peptides is that bioactive peptides tested *in vitro* are not always active *in vivo*. This may be due to the digestion or non-absorption of bioactive peptides. This protein is susceptible to extensive hydrolysis in the gastrointestinal tract by stomach, intestine, and brush border peptidases. Consequently, many of the peptides produced do not reach the reabsorption stage in the duodenum and jejunum [146,147]. This suggests that various gastrointestinal proteases, such as pepsin, trypsin, chymotrypsin, brush border, and serum peptidases, can affect peptide bonds through endogenous cleavage, resulting in conformational changes and loss of biological function. Most of the blood pressure entering the duodenum and jejunum is not absorbed into the bloodstream. Digestion resistance and absorption resistance, 2 major aspects of bioactive peptide function, should be evaluated prior to further characterization of bioactive peptides in the blood [148].

The challenges in the separation and characterization of bioactive peptides are commonly solved using various chromatographic techniques. However, the isolation and characterization of bioactive peptides is highly problematic and constrained. Bioactive peptides frequently contain short sequences (2 - 6 amino acids). This is a significant obstacle to the current proteomics technology [149]. Small peptides may not be ideal for MS detection because they are frequently simply loaded and it is challenging to acquire adequate fragments [150]. Peptides of less than 6 amino acids are generally not recognized by high-fidelity bioinformatics tools, and peptides are typically recognized by distinct protein sequences, rendering the MS/MS database search methods obsolete. This is caused by AChE inhibitory peptides, which are typically short peptides.

However, small peptides require specialized separation techniques. Traditional RP-HPLC and SEC methods for separating bioactive peptides are unsuitable for small peptides [151]. Another issue in peptide purification and fractionation is related to scale. Peptide properties such as molecular weight, net charge, and hydrophobicity are important parameters to be considered in separation processes for industrial applications [152]. Solid-phase methods and techniques like RP-HPLC are the most commonly used methods in

research (laboratory scale), but the problem is that they are expensive.

While generally said to be nontoxic, the studies that are emerging indicate some peptides may cause allergic reactions or are toxic [153]. This therefore means that development must predominantly consider safety assessment. Besides, therapeutic peptides production has its challenges in ensuring consistent quality between batches—an issue that influences their efficacy and safety profiles [147]. Various strategies, including chemical modification, nanocarrier encapsulation, and cell-penetrating peptides, are being developed to enhance stability and bioavailability [154]. The integration of computational tools and artificial intelligence in peptide research offers promising avenues for addressing these challenges, with the potential to accelerate the development of effective peptide-based therapeutics.

Conclusions

Bioactive peptides obtained from Echinozoa, particularly sea cucumbers and sea urchins, have enormous therapeutic potential due to their various biological activity. These peptides have antioxidant, anticancer, antidiabetic, antifatigue, ACE inhibitor, antibacterial, and memory-enhancing effects. Their mechanisms of action include scavenging free radicals, altering critical metabolic pathways, causing apoptosis in cancer cells, controlling blood glucose levels, and decreasing angiotensin-converting enzyme (ACE) activity to control hypertension. Notably, sea cucumber peptides have been shown to increase insulin sensitivity, mitochondrial function, and activate crucial signaling pathways such as NRF2 and AMPK, all of which contribute to their antifatigue and antidiabetic benefits. Similarly, sea urchin peptides have showed potential in inhibiting cancer growth and fighting microbial infections by disrupting membranes and targeting intracellular pathways. Despite these potential bioactivities, there are significant challenges in extracting, purifying, and characterizing these peptides. Peptide breakdown during digestion, limited bioavailability, and challenges in detecting small peptide sequences all limit their medicinal utility. To ensure efficacy and safety, these problems must be addressed through the development of enhanced

extraction procedures, new delivery systems, and rigorous *in vivo* validation.

Future study should focus on overcoming these limitations by refining manufacturing procedures, investigating synergistic effects with current medicines, and performing clinical trials to fully fulfill Echinozoa-derived peptides' medicinal potential. Their inclusion into functional foods, nutraceuticals, and pharmaceutical items might provide new approaches to controlling chronic illnesses and increasing overall human health. These marine-derived peptides show enormous potential as natural, effective medicines in modern medicine, since they bridge the gap between research and application.

Acknowledgements

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