REVIEW

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AN OVERVIW OF SEA CUCUMBER: CHEMISTRY & PHARMACOLOGY OF ITS METABOLITES

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ABSTRACT: Sea cucumbers are echinoderms belonging to the class Holothuroidea, are marine invertebrates that are found in benthic areas and deep seas. They are used as food, & medicine for various diseases as well. According to the American Cancer Society, although it has been used in traditional Asian medicine for a variety of ailments, "there is little reliable scientific evidence to support claims that sea cucumber is effective in treating cancer, arthritis, and other diseases."Sea cucumbers are harvested from the environment, and are increasingly farmed via aquaculture. The effectiveness of its metabolites against hypertension, asthma, rheumatism, impotence and constipation is pharmacologically potent. Several unique pharmacological activities namely anti-angiogenic, anti-cancer, anticoagulant, anti-hypertension, anti-inflammatory, antimicrobial, etc. have been ascribed to chemical compounds extracted from different sea cucumber species. They have an impressive profile of valuable nutrients such as Vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin), and minerals, especially calcium, magnesium, iron and zinc. These medicinal benefits of sea cucumbers can be attributed to the presence of appreciable amounts of bioactive compounds, especially the triterpene glycosides, chondroitin sulfates, glycosaminoglycan, etc. Cancer is the second leading cause of death worldwide & the rapid development of resistance to cancer drugs, leads to increase in the demand for new anti-cancer drugs, particularly from natural products. This review not only discusses about the life, habit, use of sea cucumber but also chemistry & pharmacology of its metabolites, such as, Frondoside A against several diseases like cancer, inflammation, & several other diseases.

KEYWORDS: Sea cucumbers, anti-cancer, Vitamin A, triterpene glycosides, Frondoside A, etc.

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INTRODUCTION:

Sea cucumbers are *Echinoderms* from the class *Holothuroidea*. They are marine invertebrates with a leathery skin and an elongated body containing a single, branched gonad. Sea cucumbers are found on the sea floor worldwide.[1*] Various species of sea cucumbers are harvested & grown in a large scale artificially due to their food value & pharmaceutical uses. [2*]

BRIEF HISTORY: Sea cucumbers, commonly known as ***trepang***, ***beche-de-mer***, or ***gamat***, have long been utilized in the sea food and folk medicine systems of Asia and Middle East. Sea cucumbers& its metabolites have been well recognized as a tonic and traditional remedy in various traditional literature for their effectiveness against hypertension, asthma, rheumatism, cuts and burns, impotence and constipation. Several unique biological and pharmacological activities namely anti-angiogenic, anticancer, anticoagulant, anti-hypertension, anti-inflammatory, antimicrobial, antioxidant, antithrombotic, antitumor, and wound healing have been ascribed to chemical compounds extracted from different sea cucumber species. [2*, 4*]

Nutritionally, sea cucumbers have an impressive profile of valuable nutrients such as Vitamin A, Vitamin B1 (thiamine), Vitamin B2 (riboflavin), Vitamin B3 (niacin), and minerals, especially calcium, magnesium, iron and zinc. These medicinal benefits and pharmacological functions of sea cucumbers can be attributed to the presence of appreciable amounts of bioactive compounds, especially the *triterpene glycosides (saponins), chondroitin sulfates, glycosaminoglycan, sulfated polysaccharides, sterols (glycosides and sulfates), phenolic, peptides, and lectins.*[1*, 3*]

A classic example is **Frondoside A** is a natural triterpene glycoside extracted from the sea cucumber, *Cucumaria frondosa*, which has been used as a traditional remedy, recently, the extract was found to have potential anti-tumor properties.[4]

Sea cucumber

Fig. 1:A Sea Cucumber (***Actinopygaechinitess***), displaying its feeding tentacles and **tube feet.**[1]

Scientific classification

Kingdom:	Animalia
Phylum:	Echinodermata
Subphylum:	Echinozoa
Class:	**Holothuroidea** Blainville, 1834

Orders

Orders:

Apodida Brandt, 1835

Aspidochirotida Grube, 1840

Dendrochirotida Grube, 1840

Elasipodida Théel, 1882

Molpadida Haeckel, 1896 [1]

OBJECTIVES:

Due to the growth of Modern Technology & demand for medicinal products from natural source, I choose this subject for Marine Drugs collected & isolated from the metabolites of sea cucumber. As far my knowledge, previously no comprehensive review article as such has ever been

published covering the detailed Physiology, Chemistry of its metabolites, Medicinal & Pharmacological aspects of sea cucumbers. This review is an attempt to mainly compile an inclusive report covering the description of high-value components and bio-actives as well as biological and medicinal properties of these multipurpose marine invertebrates, as one of the potential sources for functional foods and nutraceuticals.

Fig. 2: Marketed Product from Sea Cucumber extract(Cucumaria Frondosa)[2*]

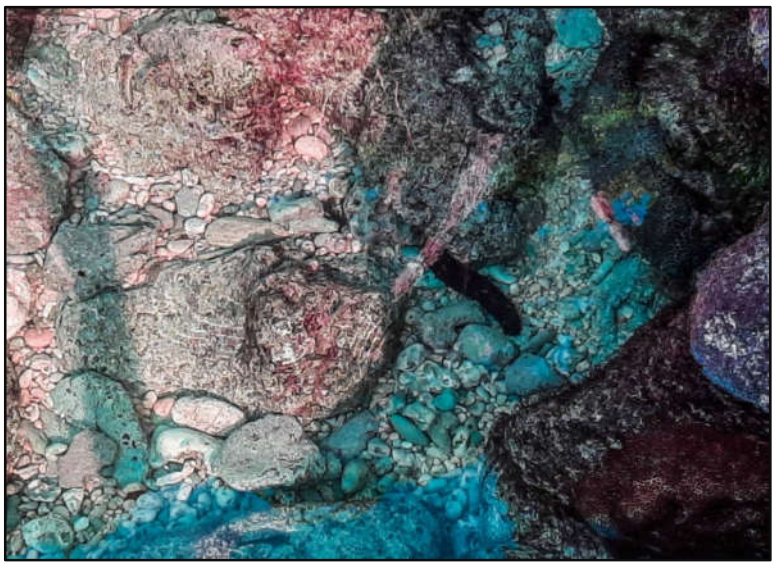

Fig. 3: Sea Cucumber in the Neil Island at Andaman & Nicobar Islands[4*]

OVERVIEW:

Most sea cucumbers have a soft and cylindrical body more or less long, rounded and occasionally fat, without solid appendages. Their shape ranges from almost spherical for "sea apples" (genus:*Pseudocolochirus*) to serpent-like for Apodida or the classic sausage-shape, while others resemble caterpillars. The mouth is surrounded by

tentacles, which can be pulled back inside the animal. [2*]

Anatomy

Sea cucumbers are typically 10 to 30 cm in length, although the smallest known species are just 3 mm long, and the largest can reach 3 meters. The body ranges from almost spherical to worm-like, and lacks the arms found in many other echinoderms, such as starfish.[4*]

Body plan

The body of a holothurian is roughly cylindrical. It is radially symmetrical along its longitudinal axis, and has weak bilateral symmetry transversely with a dorsal and a ventral surface.[4*]

Digestive system

A pharynx lies behind the mouth and is surrounded by a ring of ten calcareous plates. [4*]

Nervous system

Sea cucumbers have no true brain. A ring of neural tissue surrounds the oral cavity, and sends nerves to the tentacles and the pharynx.[3*, 4*]

Respiratory system

Sea cucumbers extract oxygen from water in a pair of "respiratory trees" that branch in the cloaca just inside the anus, so that they "breathe" by drawing water in through the anus and then expelling it. [4*]

Circulatory systems

Like all echinoderms, sea cucumbers possess both a water vascular system that provides hydraulic pressure to the tentacles and tube feet, allowing them to move, and a haemal system. The latter is more complex than that in other echinoderms, and consists of well-developed vessels as well as open sinuses. [1*, 3*]

Locomotive organs

Sea cucumbers possess penta-radial symmetry, with their bodies divided into five nearly identical parts around a central axis& secondarily evolved a degree of bilateral symmetry. For example, because one side of the body is typically pressed against the substratum, and the other is not, there is usually some difference between the two surfaces (except for Apodida).Like sea urchins, most sea cucumbers have five strip-like ambulacral areas running along the length of the body from the mouth to the anus. The three on the lower surface have numerous tube feet, often with suckers that allow the animal to crawl along; they are called trivium. The two on the upper surface have under-developed or vestigial tube feet, and some species lack tube feet altogether; this face is called bivium. [4*]

Endoskeleton

Sea cucumbers have an endoskeleton just below the skin, calcified structures that are usually reduced to isolated microscopic ossicles (or sclerietes) joined by connective tissue. In some species these can sometimes be enlarged to flattened plates, forming an armour. In pelagic species such as *Pelagothuria natatrix* (Order Elasipodida, family Pelagothuriidae), the skeleton is absent and there is no calcareous ring.[1*]An internal skeleton composed of plates of calcium carbonate in most sea cucumbers, however, these have become reduced to microscopic ossicles embedded beneath the skin. A few genera, such as *Sphaerothuria*, retain relatively large plates, giving them a scaly armour. [1, 4]

Pharmacology & Chemistry of Metabolites of Sea Cucumber

In recent years, attention has been devoted to developing bioactive agents from natural food sources to produce pharmaceutical grade anti-inflammatory supplements. Sea cucumbers are nutrient-rich, invertebrate deep-sea dwellers that have been used for centuries as an anti-inflammatory and anti-disease food source and for treating ailments in Korea, Japan, Indonesia, and China. [5, 7]

- **Chemical composition of various sea cucumbers**

Functional ingredients from sea cucumbers have become an increasingly interesting way to develop new foods as well as biomedicine products. Sea cucumbers are a source of high value-added compounds with health benefit effects to be used as functional ingredients. Bioactive peptides, vitamins, minerals, fatty acids, saponins, carotenoids, collagens, gelatins, chondroitin sulfates, amino acids, fatty acids and other bioactive compounds are example of such sea cucumber derived functional ingredients that can be added at different stages of the food and biomedisine production process.[4] The sea cucumber species (Stichopus hermanni, Thelenota ananas, Thelenota anax, Holothuria fuscogilva, Holothuria leucospilota, Holothuria atra, Holothuria scabra and Actinopyga mauritiana) described in this article has been selected considering edible species, medicinal effects, and low toxicity. The aforementioned selected varieties are some of high-value sea cucumbers in Asia. Assuming that any new functional ingredient obtained from sea cucumbers could be used for further development of new products in food and pharmaceuticals industries. Another important factor is their nutritional value and potential as new sources of functional ingredients. [6, 12]

- **S. hermanni (local name in Indonesia: gamat emas, gamat kacang, taikongkong):**

Sea cucumber S. hermanni (curryfish, golden sea cucumbers) belongs to the genus Stichopus; these species were formerly known as Stichopus variegatus. In Indonesia and Malaysia, sea cucumber S. hermanni (Fig. 4) has long been used for the preparation of traditional medicinal products like gamat water and gamat oil. These species are gaining much recognition among consumers, medical and biomedical researchers due to their potential health benefits. In Asian region communities, S. hermanni have been exploited for medicinal purposes; however such applications needs to be proven on a scientific basis using some clinical models.[1*, 6]

Fig. 4. *Stichopus hermanni* from Lembeh strait, Indonesia.[6]

S. hermanni contains high amount of protein (47.00% ± 0.36%) and low percentage of lipid (0.80% ± 0.02%). This sea cucumber contain significant amount of sulfated glycosamino-glycan. Glycosamino-glycan are long,[20] unbranched polysacchari - des composed of repeating disaccharide units

consisting of alternating uronic acids (D-glucuronic acid or L-iduronic acid) and amino sugars (D-galactosamine or D-glucosamine) Glycosaminoglycan are divided into non-sulfated and sulfated glycosaminoglycan. Sulfated glycosaminoglycan extracted from *S. hermanni* possess various chemico-biological functions. Compared to other parts such as internal organs and coelomic fluid; integument body wall of *S. hermanni* contain highest glycosamino-glycan, both sulfated and non-sulfated. Further, sulfated glycosamioglican[16] from integument has been demonstrated to accelerate wound healing process in rats. More than 60% of wound heal area in rats was observed after daily treatment with sulfated glycosaminoglycan (20 µL of 1 µg/mL) for 12 days. The healing activity of sulfated glycosaminoglycan was mediated through acceleration of wound contraction in wound healing phase I. In addition, 40% of *Stichopus hermanii* extract were able increase the number of lymphocytes during the healing process of traumatic ulcer on Wistar rat's oral mucous. Most recently, Arundina et al. (2016) extracted *S. hermanni* from Kalimantan, Indonesia and demonstrated their growth stimulating effects in mesenchymal stem cells. Mesenchymal stem cells are self-renewing cells that have the capacity to differentiate into adipocytes, chondrocytes, myocytes, and osteoblast. Following treatment with *S. hermanni* extract and osteogenic induction medium for 4 weeks, mesenchymal stem cells were differentiated into osteoblast. Collectively, it can be assumed that sea cucumber *S. hermanni* is able to accelerate wound healing process. Further, these sea cucumber species *c*an be used to prepare lotion or a topical ointment for wound healing management. [6]

Sea Cucumber as Neuroprotective:

Neuroprotection may defined as mechanisms and strategies used in order to protect neuronal cells against injury, apoptosis, dysfunction and or degeneration in the central nervous system (CNS). In the CNS, there are two classes of cells, including neuron, and glia (microglia, astrocytes and the related Schwann cells and oligodendrocytes). Astrocytes plays an important structures that provide housekeeping functions necessary to maintain neuronal function, actively shape synaptic function, and act as neural precursors in adult neurogenic regions. In addition, astrocytes also preserve the host integrity following injury. Recently, Patar et al. (2012) prepared water extract of *S. hermanni* from Malaysia and showed their growth promoting effect to promote proliferation of spinal astrocytes. In pathological cases like spinal cord injury, proliferating reactive astrocytes are proven essential for early regeneration process, provide neuroprotective effects and preserve motor function after acute injury. Further, it was demonstrated by GC-MS results that 37% of the total *S. hermanni* water extracts were comprised of amino acids (37%) followed by hydrocarbon (21%), ester compounds (16%), the orther remaining compounds consisted of phenols, alcohol groups and unidentified compounds. The 2-carbamoyl-3-methylquinoaxaline was found to be the most abundant compounds in *S. hermanni* extracts.Interestingly, quinoxaline d erivatives has been reported to involved in reducing neurological deficits and glia loss after spinal cord injury. These, quinoxaline may contribute to the neuroprotective effects of *S. hermanni*. It is an alternative source to synthetic ingredients that can contribute in wound healing and neuroprotection.

Until now, wound healing as well as neuroprotective activities of *S. hermanni* have been observed in vitro. Therefore, further research studies are needed in order to investigate *S. hermanni* biological activities in vivo as well as human subject.[1*, 3*, 4*, 7]

***T. ananas* (local Indonesian name: teripang nanas) and *T. anax* (local Indonesian name: teripang babi, teripang donga, teripang duyung)**

T. ananas and *T. anax* are two sea cucumber species belong to the Stichopodidae family which found in tropical waters. *T. ananas* (Fig.3) are known as pineapple sea cucumber or prickly redfish. These species is considered as commercial sea cucumber species and one of the most popular edible sea cucumber species consumed in China and Southeast Asian countries. Due to intense commercially exploitation population, these sea cucumber species declined by 80–90% in at least 50% of its range and listed as endangered species by the *International Union for Conservation of Nature*. Medicinal value of *T. ananas* including antioxidant, antiinflammatory, antitumor, antiproliferative, anticoagulant and antiviral effects have been established.[4*, 1]

Fig. 5. *Thelenota ananas* from Kupang, Indonesia.[4]

Wu et al. (2010) have isolated novel fucosylated chondroitin sulfate (Fig. 3) from the body wall of the sea cucumber *T. ananas*, which consisted of *N*-acetylgalactosamine (GalNAc), glucuronic acid (GlcUA), and ester sulfate with about 1:1:3.7, respectively. Fucosylated chondroitin sulfate is a water-soluble depolymerized glycosaminoglycan isolated from echinoderm sea cucumber. Physicochemical of the fucose branch are differ according to the sea cucumber species. Anticoagulant activity of the fucosylated chondroitin sulfate from *T. ananas* as measured by the activated partial thromboplastin time assay varies in proportion to the molecular weight follows a logarithmic-like function. The molar ratio for types of fucose branch found in *T. ananas* is 25:22:53 for 3-monosulfate, 4-monosulfate and 2,4-disulfate, respectively. Fucose content and composition correlate with anticoagulant activities of fucosylated chondroitin sulfate; in addition to the composition. More recently, it was demonstrated that anticoagulant activity of fucosylated chondroitin sulfate from *T. ananas* was mediated by inhibition of intrinsic

tenase. However, fucosylated chondroitin sulfate from sea cucumber *T. ananas* also activated factor XII which further lead to hypotension when injected intravenously in rats. Interestingly, the activation of factor XII could be diminished by the low molecular weight fucosylated chondroitin sulfate; suggesting that molecular weight also plays an important role in anticoagulant effect of fucosylated chondroitin sulfate. Not only anticoagulant activity, low molecular weight fragment of fucosylated chondroitin sulfate from sea cucumber *T. ananas* which prepared by free radical depolymerization has been demonstrated to inhibit virus HIV replication. Fucosylated chondroitin sulfate was effective in blocking laboratory strain HIV-1IIIB entry and replication, and inhibiting infection by clinic isolate HIV-1_{KM018} and HIV-$_{1TC-2}$. Fucosylated chondroitin sulfate might possess potential to be further developed as a novel HIV-1 entry inhibitor for treatment of HIV/AIDS patients, particularly for those infected by T-20-resistant variants. However, further study to elucidate fucosylated chondroitin sulfate structure and activity relationship will be required in the near future.[4*, 1, 4]

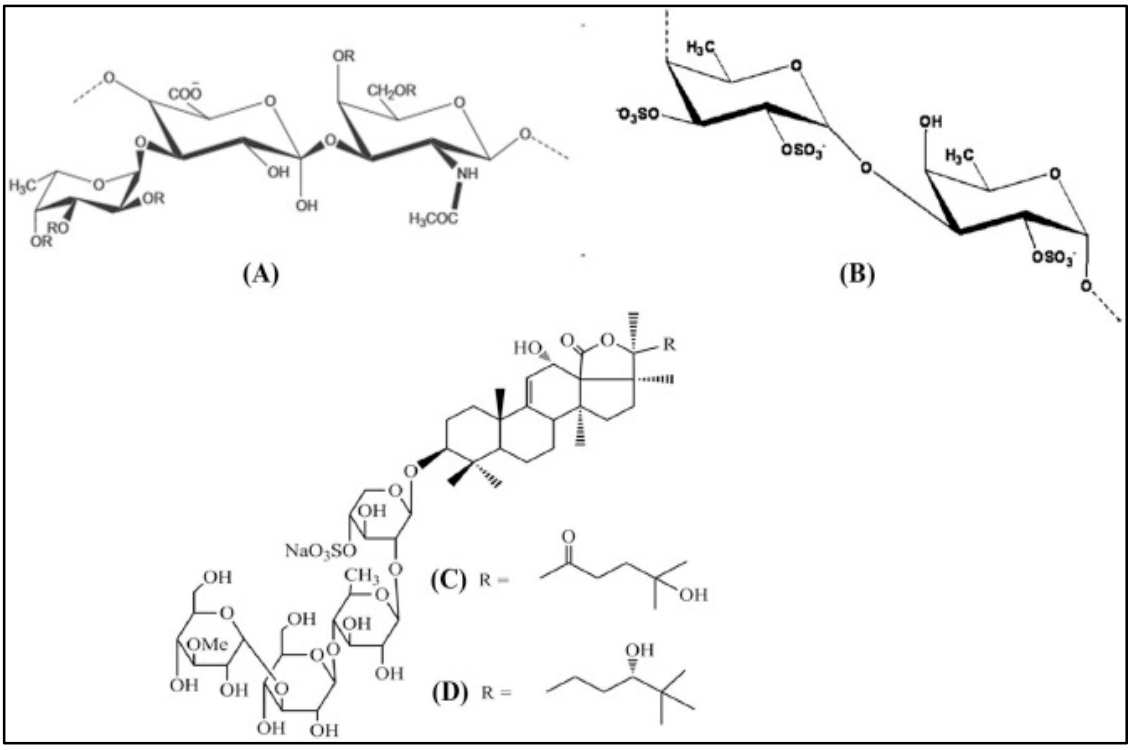

Fig. 6. Functional materials-derived from sea cucumbers.

Fucosylated chondroitin sulfate (A); Fucoidan (B); Holothurin A_3 (C); and Holothurin A_4 (D).[5, 13]

Fucoidan (4) is sulfated polysaccharide found in brown algae and sea cucumbers. In sea cucumbers, it was first isolated from *Ludwigothurea grisea*. Recently, low molecular weight fucoidan which composed of a novel tetrafucose repeating units has been isolated from sea cucumber *T. ananas* by enzymatic degradation. Fucoidan from *T. ananas* was proven to possess a significant superoxide radical scavenging activity with an IC_{50} value of 17.46 ± 0.14 µg/mL. [9] The radical scavenging effect of fucoidan on superoxide radicals improved along with the increasing sulfate

content. However, additional 2-O-sulphation in a specific residue increase the radical scavenging effect; suggesting that antioxidant activity of fucoidan derived from *T. ananas* depends on the sulfation pattern not simply on sulfate content. [11, 14]

Triterpene glycosides or also referred as saponins are substances consisting of a sugar moiety attached to a triterpene or steroid aglycone. These substances are widely distributed in plants, marine invertebrates and are characteristic secondary metabolites of echinoderms, octocorals, and sponges'two triterpene glycosides (stichoposide C and stichoposide D) have been isolated from the *T. ananas* and *T. anax.* The structural differences between stichoposide C and stichoposide D are sugar residue; where stichoposide C has quinovose, and stichoposide D has glucose as the second monosaccharide unit. Stichoposide C showed potent anticancer activity in leukemia cells (HL-60) and mouse subcutaneous tumor cells (CT-26) by inducing apoptosis through the activation of both intrinsic and extrinxic pathway. [5, 7, 11, 17]

Fig. 7. *Holothuria fuscopunctata.* [1*]

Sea cucumbers consist of vitamins, minerals, cerebrocides, peptides, and lectins, and also contain unique molecules, such as sulfated polysaccharides, 12-methyltetradecanoic acid (12-MTA), philinopside E, triterpene glycoside compounds, glycosaminoglycan, and chondroitin sulfates. In sufficient quantities, these unique compounds are known to possess anti-microbial, anti-oxidant, anti-angiogenic, anti-inflammatory, immunomodulatory, and anti-tumoral properties. As supplements, these sea cucumber extracts have been shown to suppress inflammation and increase innate immune responses. In this review, we will discuss the anti-inflammatory, anti-tumorigenic, and immune properties of bioactive agents extracted from sea cucumbers.[13]

Bioactive components of sea cucumber extracts and their biological effects on various human cancer cells and cancer animal models.

- **Anti-Inflammatory Function of Sea Cucumber Extracts:**

Inflammation reflects a sequence of events that occurs in response to injury or foreign body entry; this process is highly coordinated and involves various cell types. A normal inflammatory response is characterized by the infiltration of leukocytes and release of other activated inflammatory mediators at the injury/infection site, and

eventually will be resolved or regulated with the release of anti-inflammatory mediators [6]. This action is needed to restrict the ongoing inflammation and stop its development into chronic inflammation. Persistent, long-lasting inflammation may lead to inflammatory disease and, eventually, cancer [2, 3]. Although several anti-inflammatory agents, like non-steroidal anti-inflammatory drugs (NSAIDs), are available, their use is restricted in dosage or intervals and special precautions are advised due to their gastrointestinal toxicity. Therefore, there is a need for the development of natural anti-inflammatory agents that have the potential to self-limit or resolve inflammatory events, without progressing into chronic inflammation.[6, 7]

Sea cucumber extracts are reported to affect soft tissue repair. Different sea cucumber extracts display different functions, with varied effects when tested in vitro or in vivo. Compared with the high dose (10 mg/kg), low-dose Stichopus sp1 extract (1 mg/kg) was shown to promote healing properties in rabbits with fractures; these findings indicate that doses are important, and these compounds may not produce dose-dependent effects. The major fatty acids in sea cucumber extracts, EPA and DHA, contribute to tissue/wound healing. EPA and DHA are known n3 fatty acids, which inhibit prostaglandin synthesis by suppressing COX-2 and 5-LOX expression under inflammatory conditions, and also act as anti-thrombotics, which accelerate the healing process. These tissue repair properties might play a role during tumor development, when the unique fatty acid content may help to inhibit cancerous transformation of epithelial cells.[7]

Holothuria tubulosa, Leptogorgia ceratophyta, Coscinasterias tenuispina, and *Phallusia fumigata* extracts were shown to be effective in down regulating pro-inflammatory marker cyclooxygenase (COX) activity in inflamed mouse tissues. As it is well documented that COX-2 is involved in carcinogenesis, ***sea cucumber extracts should be further analysed for their anti-cancer properties.***[5, 7]

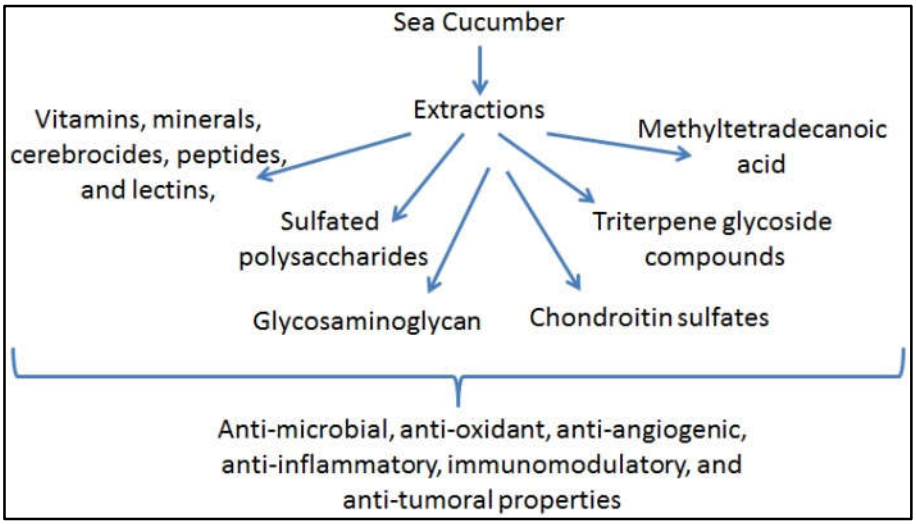

Fig.8:Bioactive components of sea cucumber extracts and their biological effects on various human cancer cells and cancer animal models.[7, 9]

- **Biologically Active Compounds with Anticoagulant and Antithrombotic Activity**

The first studies estimating anticoagulant activity of biologically active compounds from sea cucumbers were carried out and published in the

1980s when large amounts of sulfated polysaccharides rich in fucose were found in the body wall of the sea cucumber *Holothuria grisea* (*Ludwigothurea grisea*). It contained several fractions of this polysaccharide varying in molecular weight and chemical structure. The high molecular weight fraction contained the most fucose and small amounts of galactose and aminosugars. The polysaccharide fraction with lower molecular weight generally consisted of sulfated fucans. The fraction with the lowest molecular weight making up the largest portion of the polysaccharide contents of the sea cucumber was composed of sulfated polysaccharides with approximately equimolar quantities of glucuronic acid, *N*-acetylgalactosamine, and fucose. Also, it had higher sulfate content then the two higher molecular weight fractions mentioned above.[15]

Nowadays, at least three types of polysaccharides are known to be in the body wall of sea cucumbers. They are sulfated fucans, fucosylated chondroitin sulfates and neutral glycans. The first two of them wereidentified in all species that were investigated whereas neutral α-glucan was isolated only from the sea cucumber *Holothuria edulis*.[11]

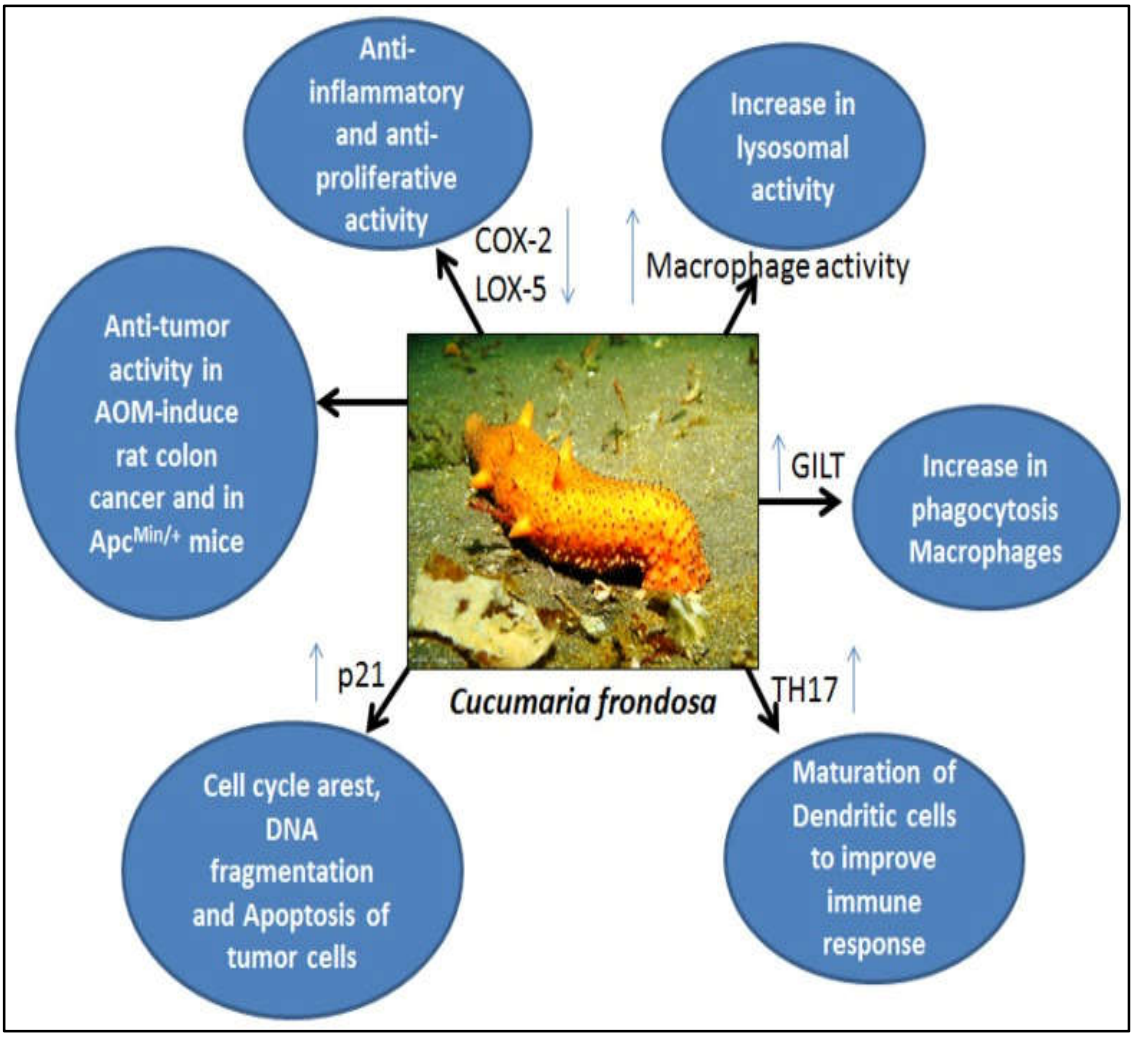

Fig. 9:Sea cucumber and its various effects on molecular targets and over all response upon treatment in vitro and in vivo cancer models.[7]

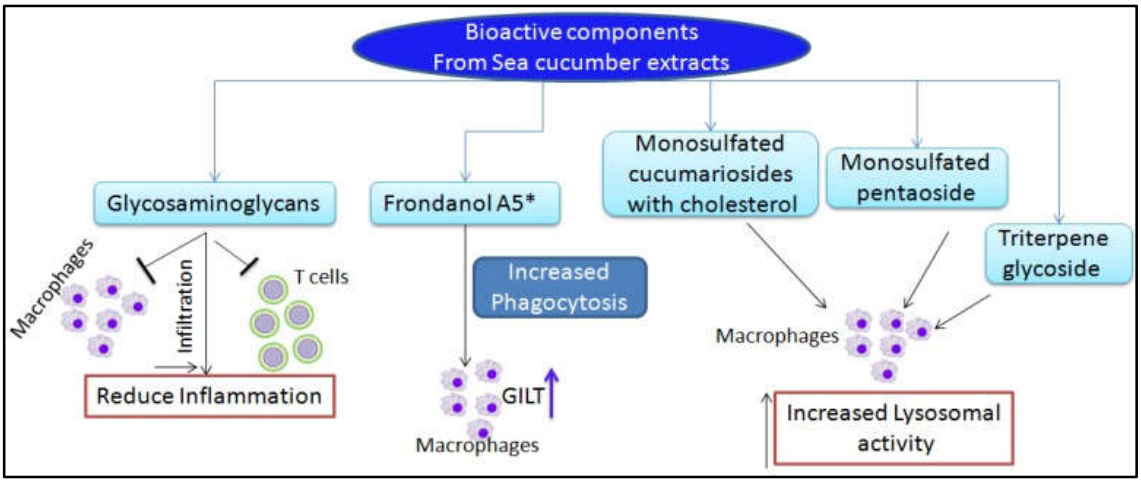

Fig. 10: Bioactive compounds isolated from sea cucumbers show immunomodulatory effects and improved immune responses by modulating innate immune cells. * Frondanol A5 consists of monosulfated triterpenoid glycoside Frondoside A, disulfated glycoside Frondoside B, trisulfated glycoside Frondoside C, eicosapentaenoic acid, 12-methyltetradecanoic acid, and fucosylated chondroitin sulfate, as well as canthaxanthin/astaxanthin in small quantities.[13]

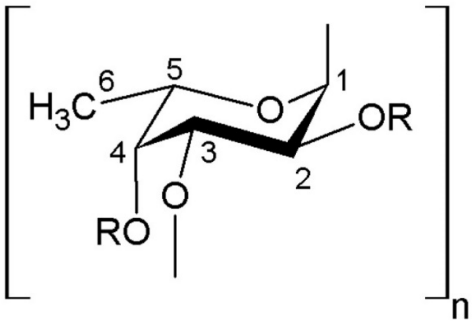

Fig. 11:The basic backbone structure of 1-3-linked fucose residues in many sulfated fucans from marine organisms. *R* may be hydrogen, sulfate, or a galactose or fucose side chain.[11, 14]

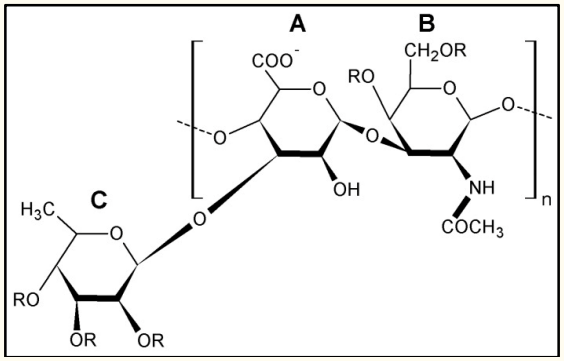

Fig. 12:Preponderant structure of the fucosylated chondroitin sulfate. The backbone is made up by repeating disaccharide units of alternating β-D-glucuronic acid (A) and *N*-acetyl-β-D-glucosamine (B). The β-D-glucuronic acid residues bear 2, 4-disulfated fucose branches (C) at the 3-*O*-position. R may be hydrogen or sulfate.[11, 13, 14]

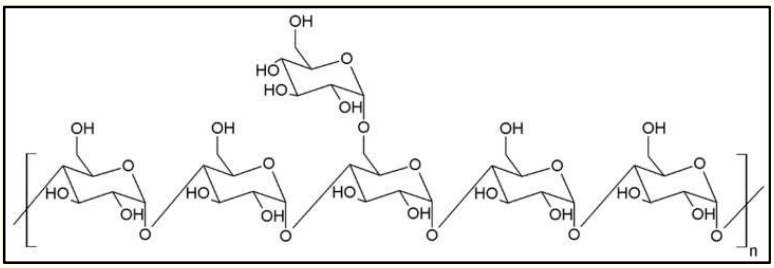

Fig.13:Neutral glucan from sea cucumber *Holothuria edulis*. The major component of polysaccharide is an α-(1→4)-D-glucan branched with a single α-D-glucose at C-6 every five residues on average.[11]

All these polysaccharides consist of similar structural features of repeating units of oligosaccharide, whose residues have specific patterns of sulfation. All echinoderms contain linear polymer sulfated fucans consisting of regular tandem repeats such as di-, tri- or tetrasaccharide repeating units with defined glycosidic linkages and distinctive sulfation patterns at O-2 and O-4 [15]. Each holothurian species contains polysaccharides with specific sulfation patterns and positioning of the glycosidic linkage.[8]

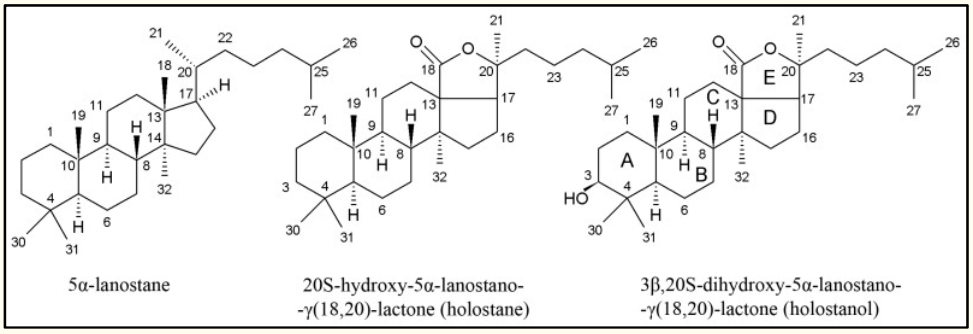

Fig. 14:Structures of lanostane, holostane and holostanol. A, B, C, D, and E: rings of the pentacyclic triterpene.[5]

The sea cucumber *Isostichopus badionotus* was found to have the repeating tetrasaccharide unit [→3Fuc (2S, 4S) α1→3Fuc (2S) α1→3Fuc (2S) α1→3Fucα1→] n[9]. *Apostichopus japonicus* (*Stichopus japonicas*) was shown to have two different types of sulfated fucan polymers. One of them consists of (1→3)-linked linear fucosyl residues that are substituted at C-4 with fucosyl residues and has 3.41 mmol fucose/g and 2.35 mmol sulfate/g. Using gel permeation chromatography, its molecular weight was found to be 9 kDa. The second one is generally a chain of unbranched (1→3)-linked fucosyl residues. It has 3.90 mmol fucose/g and 3.07 mmol sulfate/g contents, and its molecular weight is 32 kDa. Both have unbranched (1→3)-linked fucosyl residues. [5]

Biologically Active Compounds with Anticancer Activity

- **Triterpene Glycosides (Saponins)**

The first study suggesting anticancer properties of biologically active compounds found in sea cucumbers was the article authored by Nigelli in 1952 [2*, 2] demonstrating that an injection of a glycoside fraction from sea cucumber *Actinopyga agassizi* consisting of a number of triterpene

glycosides (holothurins) into a sarcoma-180 node inhibited tumor growth in mice. Later, it was shown that healthy mice injected with Krebs-2 ascitic tumor cells and treated with holothurin prevented induction of the tumor growth. Holothurin was also shown to inhibit growth of epidermal carcinoma cells.[19]

Triterpene glycosides also called triterpene saponins are secondary metabolites typically produced by all sea cucumbers. Nowadays, the number of triterpene glycosides isolated from sea cucumbers exceeds 300. From a chemical point of view, these glycosides are amphiphilic compounds that have aglycone (lipophilic) and glycone (hydrophilic) moietes. The majority of the triterpene glycosides contain the so-called holostane type aglycone comprising of lanostane-3β-ol with a γ (18, 20)-lactone in the E-ring of the pentacyclic triterpene [3β, 20S-dihydroxy-5α-lanostano-γ (18, 20)-lactone]. Some glycosides contain a non-holostane type aglycone which do not have γ (18, 20)-lactone in the tetracyclic triterpene. Generally, the triterpene glycosides consist of two to six monosaccharide units such as D-xylose, D-quinovose, D-glucose, 3-*O*-methyl-D-glucose, 3-*O*-methyl-D-xylose and rarely 3-*O*-methyl-D-quinovose, 3-*O*-methyl-D-glucuronic acid and 6-*O*-acetyl-D-glucose covalently linked to C-3 of the aglycone. The sugar units of the glycone part are generally arranged in linear or branched chains. In addition, these chains may carry from one to three sulfate groups. About sixty percent of the sea cucumber triterpene glycosides have sulfate groups linked to the monosaccharide units of the carbohydrate chain. The chemical structures of 341 glycosides isolated from more than 50 species of holothurians are provided in the review article of M.A.M. Mondol et al. [10]

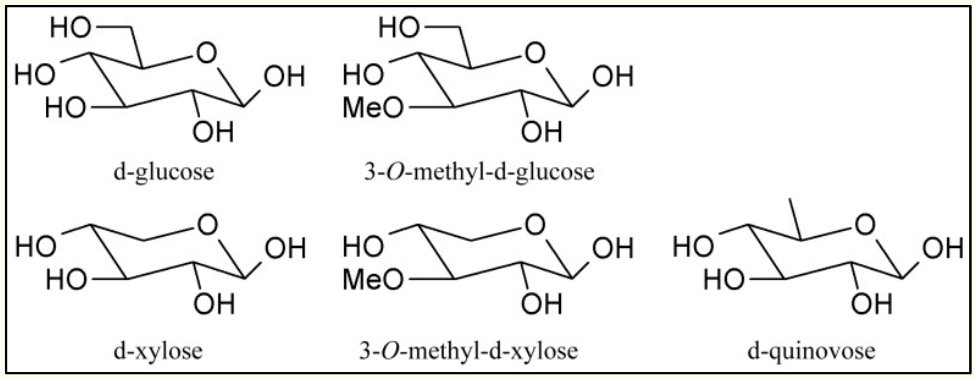

Fig. 15:Common sugar units present in sea cucumber triterpene glycosides.[5, 11]

- **Cell cycle arrest**

Mammalian cells progress through several cell cycle phases (G1, S, G2, and metaphase) during cellular division. Cell cycle checkpoints, notable features of cell cycle progression, provide essential surveillance to prevent cells from entering the next phase before the previous phase has been completed. Thus, halting the cell cycle can lead to prevention of cancer cell growth and division and is one of the major strategies for cancer therapy.[7, 8]

Cucumarioside A2–2 demonstrated anticancer effects through its ability to cause the arrest of the cell cycle during the DNA synthesis (S) phase and was shown to induce programmed death in Ehrlich carcinoma mouse tumor cells. [17]In another study, Echinoside A and Ds-echinoside A, triterpenoid glycosides isolated from *Peasonothuria graeffei*, caused the arrest of the cell cycle during the G0/G1 phases in hepatocellular liver carcinoma cells (HepG2). A reverse transcriptase-polymerase chain

reaction assay showed that both triterpenoid glycosides increased expression of cell-cycle-related genes, including p16, p21, and c-myc, and decreased expression of cyclin D1. Frondanol A5, an isopropyl alcohol/water extract of *Cucumaria frondosa*, inhibited growth during the S and G2/M phases and led to increased levels of p21WAF1/CIP and decreased levels of Cdc25c. A polar fraction of Frondanol A5 was effective at inducing G2/M cell cycle arrest, and also potently decreased expression of cyclin A, cyclin B, and Cdc25c in S2013 and AsPC-1 cells.[7, 8, 9]

- **Reduction of tumor growth**

Tumour growth inhibition by Frondoside A was initially demonstrated in a xenograft model using AsPC-1 pancreatic cancer cells. Frondoside A was also shown to inhibit tumour growth and reduce tumor volume by 87% in an athymic mouse model using MDA-MB-231 breast cancer cells.[5]

Philinopsides A and E, novel sulfated triterpenoid glycosides derived from *Pentacta quadrangulari*, were also shown to reduced tumor growth. Philinopside A reduced tumor growth in the sarcoma 180 mouse model, whereas philinopside E inhibited tumor growth in both sarcoma 180 and hepatoma 22 mouse models.[7, 8, 17]

- **Frondoside A**

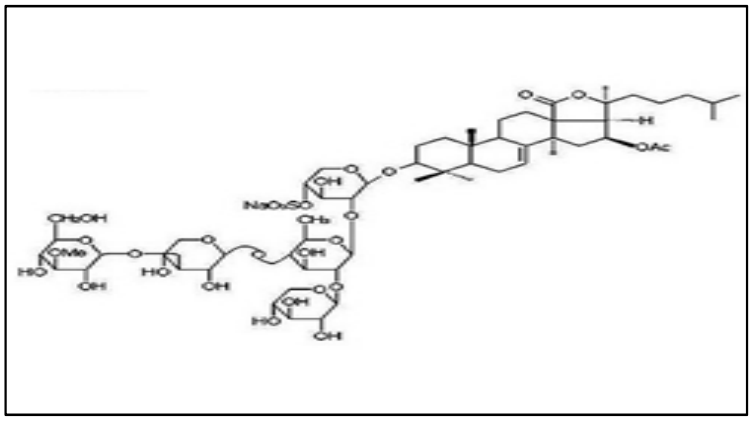

Fig 16: Frondoside A is a triterpenoid saponin isolated from the sea cucumber *Cucumaria frondosa* with potential anticancer properties.

Frondoside A is of interest as a potential anticancer agent. It has potent cytotoxicity against THP-1 and HeLa tumor cell lines with IC50 values of 4.5 ug/mL and 2.1 ug/mL, respectively. It has been shown to inhibit proliferation and induces apoptosis of human pancreatic cancer xenografts. It has also inhibited the migration and invasion of breast cancer cell line MDA-MB-231 in vitro. Additionally, Frondoside A strongly decreased the growth of MDA-MB-231 tumor xenografts in athymic mice without any toxic side effects. Another study has shown that Frondoside A has potent anti-metastatic activity. The glycoside inhibited tumor metastasis in a murine model of metastatic breast cancer, and its mechanism of action is partially attributed to its ability to antagonize prostaglandin E receptors EP4 and EP2. All these studies support Frondoside A as a promising novel therapeutic cancer agent.[3]

A study by Ma et al. found that Frondoside A had potent anti-metastatic activity in a syngeneic murine model of metastatic breast cancer. Intraperitoneal administration of Frondoside A in mice implanted with mammary tumors in mammary glands, inhibited tumor metastasis to the lungs. Along with the anti-metastatic activity described in vivo, Frondoside A also inhibited

migration of tumor cells in vitro through inhibition of prostaglandin E receptors, EP2, and EP4. [17]

Other studies have also reported that Frondoside A has the promising anti-metastatic potential for breast cancer therapy. Park et al. investigated the anti-metastatic effects of Frondoside A in MBA-MB-231 human breast cancer cells, and found that Frondoside A inhibited TPA-induced activation of AP-1 and NF-κB and decreased TPA-induced activation of ERK1/2, PI3K/Akt, and p38 MAPK signals caused a reduction in expression of MMP-9. In addition, Frondoside A decreased invasion of MDA-MB-231 tumour cells in a Matrigel invasion assay. [2*, 3*, 3, 7, 11]

Frondoside A was shown to inhibit angiogenesis as indicated by reduced CD31 staining, used to measure microvessel density, in LNM35 lung cancer xenografts and was shown to block basal and bFGF-induced angiogenesis using the CAM assay. Frondoside A treatment demonstrated significant inhibition of metastasis in a lung cancer xenograft model and an in vitro Matrigel invasion assay, without toxic side effects.[9]

- **Cerebrosides**

Cerebrosides, neutral glycosphingolipids, are composed of two different structural units: hexose (glucose for glucocerebrosides or galactose for galactocerebrosides) and ceramide constituted of a sphingoid base, also called a long-chain base (LCB), and an amide-linked fatty acid (FA). Ceramide formation occurs through a β-glycoside bond between the hydroxide radical at C-1 of ceramide and hexose. The most common sphingoid base of mammalian sphingolipids is sphingosine (4-sphingenine, d18:1) usually attached to a branched 2-OH or non-hydroxyl alkyl within 16–24 carbon atoms. Sphinganine (dihydrosphingosine, d18:0), phytosphingosine (4-hydroxysphinganine, t18:0) and some others are often found in small amounts. Sphingoid bases from plants include d18:1, d18:2, and 4-hydroxy-8-sphingenine (t18:1).[13]

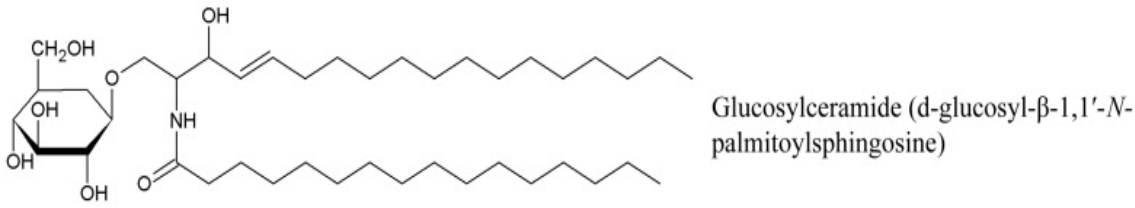

Fig.17:Representative structure of glucosylceramide (d-glucosyl-β-1, 1′-N-palmitoylsphingosine).[21]

Sphingoid bases in marine invertebrates have been reported to be significantly different from those found in plants and animals. Sphingolipids of marine invertebrates have a unique triene type of sphingoid bases with a conjugated diene The fatty acid moieties of the sea cucumber cerebrosides are usually saturated, monounsaturated and α-hydroxyl fatty acids with about twenty fatty acids total. In most analysed holothurians the glycosyl group is a glucose unit whereas galactose is found only in the sea cucumber *Bohadschia argus*. It is obvious that modification in chain length and degree of saturation and/or hydroxylation of LCBs and FAs leads to extensive variation of the cerebroside structure. [15]

Investigation of the antitumor potential of cerebrosides showed that such long-chain bases as d17:1, 4, 8-sphingadienine (d18:2), d18:3 and d19:3 inhibit proliferation of human colonic cancer cells DLD-1, WiDr and Caco-2 in a dose-

dependent manner due to induction of apoptosis (Table 1)

Table 1: Cytotoxicity of sea cucumber cerebrosides against cancer cells.

Compounds	Source	Type of Tumor Cell Lines	Pharmacological Effect, Anticancer Mechanism	Reference
Sphingoid bases	*Stichopus horrens*	Human colon cancer cells Caco-2, DLD-1 and WiDr	Reduction of cell viability, induction of apoptosis, increasing caspase-3 activity	[5]
Glucocerebrosides, sphingoid bases	*Cucumaria frondosa*	Caco-2	Inhibition of cell proliferation	[7]
Glucocerebrosides	*Acaudina molpadioides*	Murine sarcoma cells S180	Induction of apoptosis	[11]
Sphingoid bases	Species not specified	Human hepatoma cells Hep-G2	Reduction of cell viability, induction of apoptosis, upregulation of death receptor-5, apoptosis inducer protein Bax, growth arrest and DNA-damage-inducible protein DNA-damage-inducible gene 45 and peroxisome proliferator-activated receptor-γ, downregulation of protein kinase p-AKT, increasing of caspase-3 and caspase-8 activities	[8]

Similar molecular compounds belonging to glucocerebrosides were extracted from *Cucumaria frondosa*. Their structural characteristics were as follows: the fatty acid part was usually saturated, monounsaturated and α-hydroxyl fatty acids, sphingoid base consisted of dihydroxy and trihydroxy chains and glucose was attached as monosaccharide moiety. Cerebrosides isolated from sea cucumber *Acaudina molpadioides* were composed of glucose, amide-linked fatty acid consisting of fatty acids and the sphingoid base. This type of cerebrosides in in vitro experiments inhibited proliferation of the sarcoma S180 cells by inducing apoptosis. [7, 9, 13, 19]

- **Fucosylated Chondroitin Sulfates**

Native FuCS was isolated from sea cucumber *Cucumaria frondosa* and used to prepare low-molecular-weight FuCS. The latter was given to male C57BL/6 mice with implanted Lewis lung carcinoma and led to inhibition of tumor growth and intensity of metastasis in a dose-dependent manner. The low-molecular-weight FuCS increased p53/p21 expression and apoptosis due to enhanced activity of caspase-3 resulting in cell cycle arrest in

Lewis lung carcinoma cells. Its mechanism of antiproliferative influence is related to suppressed expression of vascular endothelial growth factor, increased expression of tissue inhibitor of metalloproteinase-1 and down-regulation of MMPs as well as significantly reduced activity of the extracellular signal-regulated protein kinase 1/2/p38 mitogen-activated protein kinase/NF-κB pathway which plays the leading role in expression of MMPs. These results suggest that low-molecular-weight FuCS may be considered a promising anti-tumor drug candidate.[11]

FuCS was shown, in animal studies with mice, to reduce pulmonary metastasis of B16F10 melanoma cells because it inhibits P-selectin-mediated adhesion of the tumor cells to platelets and tumor cell migration demonstrated by specific in vitro experiments. This key element of glycosaminoglycans antimetastatic influence results in dramatic reduction of P-selectin-mediated adhesion of tumor cells and in down-regulation of protein levels of such integrins as focal adhesion kinase and MMP-2/9 in B16F10 cells [10]. Specific glycosaminoglycan isolated from sea cucumber *Holothuria leucospilota* has the capacity to inhibit thrombin-induced platelet activation and aggregation of platelets, reduce adhesion of the breast cancer cells to platelets and prevent adherence of the platelet-cancer cell complex to fibrinogen, weaken formation of the complexes between platelets and cancer cells and suppress mRNA and protein levels of β1 and β3 integrins, MMP-2 and MMP-9. It can also increase expression of MMP inhibitor and tissue inhibitor of metalloproteinase-1 in MDA-MB-231 cells. Thus, the mechanism of antitumor activity of holothurian glycosaminoglycans is directly dependent on its antiplatelet properties and capacity to inhibit cellular adhesion between tumor and normal cells.[11, 13, 14]

- **Antibacterial and Antifungal Agents:**

According to numerous studies, sea cucumbers contain biologically active compounds with either very low or even no antibacterial properties regarding many common pathogens. For example, a number of experiments focused on exploring ethyl acetate, methanol, or water-methanol extract obtained from the body wall, coelomic fluid or cuvierian organs of sea cucumbers. It was found out that these extracts do not kill or prevent proliferation of *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus* and some other pathogens. Some studies showed moderate antibacterial activity of holothurian compounds when they were applied in very high concentration. Weak bacteriostatic activity against human pathogens was noted in experiments with hydroalcoholic, n-hexane, chloroform, and methanol extracts obtained from different tissues and organs of sea cucumber *Holothuria leucospilota* used in concentrations of 1000 and 2000 µg/mL. Even such high concentrations did not result in bacteriostatic effects. Similarly, ethanol extracts of sea cucumbers *Actinopyga echinites*, *Actinopyga miliaris*, *H. atra* showed slight antimicrobial activity against some bacterial strains such as *E. coli*, *Aeromonas hydrophila*, *Enterococcus* sp., *Pseudomonas aeruginosa, Klebsiella pneumoniae*, *S. aureus, Salmonella typhi* and *Vibrio harveyi*. Extracts of *H. scabra* were tested on many bacterial strains with no signs of bacterial inhibition regardless extract concentration. The absence of antibacterial activity of the *H. scabra* extracts against *S. aureus* and E. coli were confirmed by studies testing compounds obtained from *H. leucospilota* in the Persian Gulf.[18, 19]

In some studies protein hydrolysates from holothurians by enzyme hydrolysis of the fresh sea

cucumbers were explored for antibacterial activity against pathogenic Gram positive and Gram negative strains. The result showed quite low efficacy for pure holothurian peptides used as antibacterial agents in comparison with common synthetic antibacterial drugs. At the same time, protein hydrolysates from sea cucumbers are considered a promising non-toxic and safe alternative natural source to chemical food preservatives.

Despite their low antibacterial activity, holothurian extracts were shown to possess activity regarding fungal pathogens. A crude methanol extract of *Actinopyga lecanora* demonstrated marked antifungal activity against *Candida albicans* (minimum inhibitory concentration, MIC, 62.5 µg/mL), *C. neoformans* (MIC, 125 µg/mL), *Sporothrix schenckii* (MIC, 62.5 µg/mL), *Trychophyton mentagrophytes* (MIC, 125 µg/mL) and *Aspergillus fumigatus* (MIC, 31.2 µg/mL). Methanol extracts of these sea cucumbers were found to contain three active triterpene glycosides, namely, holothurin B, holothurin A and another new compound. Comparative studies have shown that holothurin B exerts more pronounced antifungal effects inhibiting *Trychophyton mentagrophytes* proliferation more than the commonly used specific antifungal drug fluconazole. Furthermore, holothurin B is

characterized by MIC as very close to fluconazole in inhibiting the growth of *Sporothrix schenckii* and *Aspergillus fumigatus*. The other two triterpene glycosides were not found to possess any antifungal properties. Holothurin B may be a lead prototype for development of new safe antifungal agents.[7, 16]

The triterpene glycosides holotoxins B, A and A1 isolated from A. japonicus exerted fungicidal effect against various Candida species including several clinical isolates, although its antifungal activity was lower than that of amphotericin B and miconazole by an average of 17.5–23 times.[7]

- **Antiviral Compounds**

Some recent studies with the compounds isolated from sea cucumbers have shown their antiviral potential. The water extract of *Holothuria* sp. (from the Persian Gulf) exerted significant activity against herpes simplex virus type 1 (HSV-1) in cell culture at half maximal cytotoxic concentration (CC_{50}) 32.57 mg/mL and IC_{50} inhibition of virus adhesion to cells and intracellular replication of 120.2 and 189.9 µg/mL, respectively. [13, 16]

FuCS obtained from sea cucumber *Thelenota ananas* effectively inhibited entry and replication of the laboratory strain HIV-1_{IIIB} (4.26 and 0.73 µg/mL, respectively), prevented infection of clinical isolates HIV-1_{KMO18} and HIV-1_{TC-2} (23.75 and 31.86 µg/mL, respectively), suppressed HIV-1 drug-resistant virus and inhibited HIV-2_{ROD} and HIV-2_{CBL-20} replication (100 µg/mL). It should be emphasized, that this FuCS showed high antiviral activity against T-20-resistant strains with EC_{50} values between 0.76 µg/mL and 1.13 µg/mL. It was clearly shown that FuCS can bind the recombinant HIV-1 gp120 protein, but inhibition of recombinant HIV-1 reverse transcriptase was not observed. These results suggest FuCS may have potential to be further developed as a novel HIV entry inhibitor for treatment of HIV affected patients. [4, 5, 6, 7, 16]

- **Antioxidants:**

Mechanisms of many pathogenic disorders are often linked to the damaging influence on cell membranes, proteins, lipids, DNA and other biomolecules with unpaired electrons referred to as free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS). All individual cells as well as whole organisms possess their own protective systems that scavenge free radicals reducing their damaging effects and repair cellular

damage using compounds known as antioxidants. Oral administration of Holothuria atra extract exerted hepatoprotective and antioxidant effects in rats with 7,12-dimethylbenzanthracene-induced hepatorenal dysfunction, namely, significantly diminished the level of the kidney dysfunction markers, serum creatinine, urea and uric acid, and normalized levels of malondialdehyde, reduced glutathione, glutathione-*S*-transferase, superoxide dismutase, and catalase in the liver tissue.[7] Similarly, Holothuria arenicola extract given to mice with liver disorder induced by bile duct ligation through gastric gavage normalized the antioxidant enzyme, glutathione-S-transferase, and superoxide dismutase and catalase activities. Several other sea cucumbers like *Holothuria arguinensis* from the North-Eastern Atlantic and *Holothuria atra* from the coast of Red Sea also were shown to have high antioxidant potential.[15, 18]

- **Angiotensin-Converting Enzyme Inhibitors:**

Angiotensin converting enzyme (ACE) is a key component of the complicated system for blood pressure regulation. ACE promotes conversion of inactive angiotensin I into the active vasoconstrictor compound angiotensin II via renin-angiotensin and the kinin-kallikrein systems. At the same time ACE contributes to inactivation of a specific vasodilator, bradykinin. Nowadays, ACE is considered the most effective biotarget in the treatment of blood pressure disorders. Besides numerous synthetic compounds there are plenty of natural sources which, can provide more effective and safe novel ACE inhibitors. Bioactive peptides from marine species are among them.[18, 19]

Peptide hydrolysates of proteins obtained from sea cucumber *Actinopyga lecanora* were prepared using several enzymes including alcalase, papain, bromelain, flavourzyme, pepsin, and trypsin. These hydrolysates demonstrated significant ACE inhibiting effects along with antioxidant activity. Alcalase hydrolysates were shown to have highest ACE inhibitory activity (69.8%) after 8 h of hydrolysis. [1, 2, 7]

Sea cucumber *Stichopus horrens* was hydrolyzed using several enzymes. The outcomes showed that alcalase hydrolysate has the highest amount of protein (39.8%) followed by flavourzyme hydrolysate (32.7%). As expected alcalase hydrolysate showed the highest inhibitory activity regarding ACE with IC_{50} value of 0.41 mg/mL. [7]

Therefore, bioactive peptide hydrolysates from various sea cucumbers may be used as ingredients in manufacturing functional foods, supplements and pharmaceuticals reducing the risk of heart disease in hypertensive persons.[15, 19]

- **Immunity Stimulating Agents**

Frondoside A and cucumarioside A2-2 from *C.f. japonica* were used for treatment of peritoneal mouse macrophages in vitro in doses 0.1 and 0.02 µg/mL, respectively, and this resulted in the marked increase of lysosomal activity by 20–30%. The cucumariosides I2, B2 and A5 isolated from sea cucumber *Eupentacta fraudatrix* used in doses 1–5 µg/mL similarly increased lysosomal activity by 15–16%. Immunostimulating activity of these compounds depends on structural peculiarities. Even slight changes of triterpene glycoside structural characteristics resulted in dramatic changes in their effects. Immunomodulatory activity of sea cucumber glycosides depends on both aglycone and carbohydrate chain structures.[13, 14]

CONCLUSION:

Bioactive compounds isolated from the sea cucumber for use as anti-cancer agents has attracted the attention of cancer researchers because of their natural origin and long history as a nutritious food. Sea cucumbers contain many marine-derived

agents that have the potential to inhibit the growth of several different types of human tumor cells as demonstrated in in vitro studies, in vivo murine models, and human studies. Triterpenoids, including triterpene glycosides, are the most abundant group of secondary metabolites found in marine sources including sea cucumbers. This review demonstrates sea cucumber triterpene glycosides are potential drug prototypes for the development of novel pharmaceuticals with antitumor activity. A range of triterpene glycosides, holothurin B, marmoratoside A, impatienside A, and bivittoside D also exert powerful antifungal effects which sometimes are close to modern antifungal pharmaceuticals, and are considered potentially novel antifungal drugs. Regarding antioxidant, immune stimulating and glucose-lowering activity demonstrated by triterpene glycosides from sea cucumbers, our opinion is that presently, there is not sufficient grounds to consider them as potential prototypes for antioxidant, immune stimulating and glucose-lowering pharmaceuticals. Several secondary metabolites derived from sea cucumbers demonstrate anti-cancer properties through multiple mechanisms including cytotoxic activity, induction of apoptosis, cell cycle arrest, reduction of tumor growth, suppression of invasion and metastasis of tumor cells, inhibition of angiogenesis, and decreased drug resistance. Moreover, the vast diversity of active compounds is quite impressive and includes dozens of types of polysaccharides, cerebrosides bases and hundreds of types of tri-terpeniods posing a serious problem for researchers to select the most promising lead molecules. Isolation and purification of the separated components requires many resources, in particular, financial. According to the principles of drug discovery, molecules must have high potential value. Thus, these molecules must be structurally novel and possess pharmacological activity. Information provided in this present review demonstrates the pharmacological activity of components isolated from sea cucumbers.

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CONFLICT OF INTEREST REPORTED: NIL ; SOURCE OF FUNDING: NONE REPORTED