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Review Article

Marine Sourced Glycosaminoglycans 'GAGs'

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Abstract: Globally, there is an uprising interest for availability of tangible, novel, added value, sustainable commercial exploitable therapeutic compounds from naturally derived sources. The paper will highlight bioactive compounds of therapeutic potential from marine sources such as polysaccharides, especially glycosaminoglycans. It is now well documented, and this effort combined with a green technological approach in sustainable natural biodiversity will allow the development of a new generation of therapeutics. Outsourcing for a biocompatible, non-mutational bioactive compound such as GAG's in a worldwide research and industrial agenda. Hence, the thorough understanding on how the biosynthesis, structure and function of complex glycosaminoglycans will reveal the polysaccharides from the marine environment could provide a valid alternative to traditional polysaccharides such as glycosaminoglycans.

Keywords: Marine, Sulfated Polysaccharides, GAG, Crown-of-Thorns (COT).

1. Introduction

The oceans cover more than 70% of the world surface and between 36 known living phyla, 34 are taxonomied within the marine environments with more than 300000 odd known species of fauna and flora[1,2,3]. *Per se*, the biodiversity of the marine environment and its associated biological and chemical biodiversity constitute a tangible practically unlimited bioresource of new bioactive substances or in outsourcing of biocompounds in the field of the development and optimization of novel sustainable commercially exploitable bioactive marine based product[4,5].

Marine organisms have been known from time immemorial to possess curative powers. But until recently, their bioactive compounds, nutraceutical properties, and green technology optimized commercial potential remained undiscovered. The marine world represents a largely untapped reservoir of bioactive ingredients that can be applied in numerous aspects of food processing, storage, and fortification. Thus, the marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural or chemical features not scientifically evidence located within terrestrial natural products. These marine-derived functional ingredients are such as certain polysaccharides (sulfated or total), polyphenols, polyunsaturated fatty acids and carotenoids, which have been reported to have a definitive global role as nutraceutical in improving human health and nutrition[6].

Plants and terrestrial microorganisms (especially those from soil samples) are traditionally in the focus of the search for new drug candidates from nature (also called "bioprospecting"). Due to the repeated re-isolation of already known compounds, the emphasis on the search for new drug-like chemical entities is nowadays shifting to less investigated organisms from unique habitats such as the sea[7,8]. So far, seven drugs derived from marine natural products are already registered either in the EU or in the US and many more are under clinical or preclinically investigated.

The concept of nutraceutical has been derived by coining the terms "nutrition" and "pharmaceutical". In this context, active substances with pharmaceutical properties are delivered to the humans through food-based approaches to prevent or treat certain disease conditions. Since the natural sources are recognized as

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safe for human consumption, the active substances produced in the diverse group of marine organisms have a wider role in the nutraceutical industry. These marine-derived active ingredients include certain polysaccharides, polyphenols, bioactive peptides, polyunsaturated fatty acids, and carotenoids which are known to have anticancer, anti-inflammatory, antioxidant, anti-obesity, hypocholesterolemic, antimicrobial, prebiotic, and probiotic activity enabling them to be applied as nutraceutical.

Marine organisms produce a rich variety of sulphated glycosaminoglycans with characteristic variations of sugar composition and sulphation patterns determined by the species of origin. The distinct molecular structures and biophysical properties of marine GAGs reflect the evolutionary adaptation and diversification of sulphated polysaccharides to complex and changing habitats. Marine GAGs are derived from organisms that have significant regenerative capacity. They may have interesting effects on the activities of many growth factors, morphogens etc. (for example FGF, HGF/SF, VEGF, GDNF) that are activated by Heparan Sulphate co-receptors.

Marine eco-environment or biosphere represents tremendous flora and fauna biodiversity therapeutics exploitable tangibilities sources and original polysaccharides worth further in-depth optimization and investigation. However, these marine sources are very much species specific. However, the abundance and stable morphological characterization are an adjusted much sort of, as such this marine environment can provide a validated alternative to traditional or folk medicine.

Taxonomized, elucidated design, optimized marine outsourced compounds can potentially be exploited for naturally derived drug discovery and for the delivery of better sustainable new marine-derived outsourced product for pharmaceutical and therapeutics application and efficacies.

Among the various sources for the development of new drugs, compounds from living organisms, so-called natural products, are of particular significance[9]. Nowadays, approximately one third of today's best selling drugs are either natural products or have been developed based on lead structures provided by nature[10].

Some literature have reviewed that over 14,000 different natural products from marine organisms have been described[11], hundreds of patents describing new bioactive marine natural products have been filed[12]. Stated that various marine natural products are currently in clinical trials mostly in the areas of cancer, pain or inflammatory diseases.

2. The present understanding about GAG

Glycosaminoglycans (GAGs) which are sometimes known as mucopolysaccharides[13] are large, complex carbohydrate molecules that interact with a wide range of proteins involved in physiological and pathological processes[14,15]. There are two types of GAGs, sulfated GAGs and nonsulfated GAGs. GAGs can be sulfated (chondroitin sulfate, dermatan sulfate, heparin/heparan sulfate, keratin sulfate) or (hyaluronic acid).

GAGs such as heparin, heparan sulfate (HS), and dermatan sulfate (DS) serve as key biological response modifiers by acting as:

(1) Stabilizers, cofactors, and co-receptors for growth factors, cytokines, and chemokines;
(2) Regulators of enzyme activity;
(3) Signaling molecules in response to cellular damage, such as wounding, infection, and tumorigenesis; and
(4) Targets for bacterial, viral, and parasitic virulence factors for attachment, invasion, and immune system evasion[16].

Glycosaminoglycans are found in not only vertebrates but also many invertebrates, implying a conserved function in the animal kingdoms[17]. The presence of GAGs in various vertebrates, as well as invertebrates, has been well documented and increasing interest has been shown by different sectors such as research, biochemical industries, biopharmaceutical, nutraceutical and biomedical[18]. Glycosaminoglycans are the major component of the extracellular matrix molecules in animal tissues, play an important role in various physiological events[17].

Glycosaminoglycans (GAGs) present in all animals: some of them such as heparin and dermatan sulfate are extracted from mammalian mucosa for therapeutical uses[19]. Table 1 below shows the therapeutic properties and potential modulated from marine sources which are from GAG, mucopolysaccharides and proteoglycan.

Table 1. Therapeutic properties/potential modulated from some voucher specimen for marine sources (GAG / Mucopolysaccharides / Proteoglycan).

Species	Tissues	Biological Properties / Potential	References
Sea-cucumber (*S. Hermanni*)	Visceral	Anti-inflammatory	Siti Fathiah *et al*., 2011
Fish (sharks, skate, codfish, salmon, and trout)	Cartilages, ligament	Antitumor, Anti-pathogenic	International Patent Application, US 20070010430.
Starfish (Crown-of-Thorns)	Integument	Antithrombotic, Anti-inflammatory	Nur Afiqah *et al*., 2012
Sponges (Porifera)	Body wall	Anti-pathogenic, Anticoagulant	Zierer *et al*., 2000
Mussels (*Perna canaliculus*)	Integument	Anti-inflammatory	International Patent Application, US nr. AU 2002242861

3. Biomedical interests in GAG

The exploration of the marine potential of what concerns the isolation of compounds and its further use and application in the biomedical field is still in its infancy. The number of naturally derived products continues to expand steadily in terms of number of compounds investigated, which has been closely followed by the increase of intellectual property, namely by the number of patents filled[20,21,22,23,24,25,26,27,28,29,30]. Still, to a large extent, the marine environment is regarded as a largely untapped source of chemical diversity[31]. In fact, the harvesting of marine potential is not limited to drug discovery alone. Although the main emphasis has been given to pharmaceuticals, other potential applications for marine-derived materials have been additionally explored. Among the wide variability of marine origin molecules, algae sulphated polysaccharides are of proven economical importance, demonstrated by their wide application in food industry and medicine and because they found no equivalent in terrestrial organisms[18].

4. Insight Biochemistry of GAGs

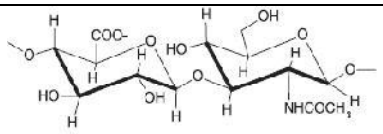
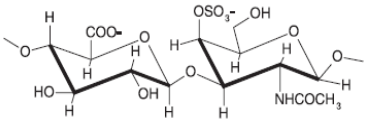
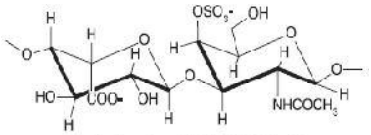
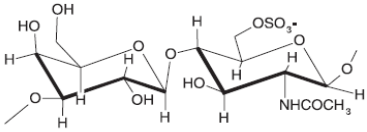
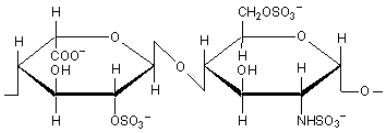
From 1930 to 1960, great strides have been made in analyzing the chemistry of these polysaccharides preparations (also known as "mucopolysaccharides"), yielding the structure of hyaluronan, dermatan sulfate (DS), keratan sulfate (KS), different isomeric forms of chondroitin sulfate (CS), heparin and heparan sulfate (HS). Together, these polysaccharides came to be known as glycosaminoglycans (sometimes abbreviated as GAGs) to indicate the presence of amino sugars and other sugars in a polymeric form[32]. Table 2 shows repeating disaccharide units of various glycosaminoglycans (GAGs).

Glycosaminoglycans are linear polysaccharides, whose disaccharide building blocks consist of an amino sugar (*N*-acetylglucosamine, glucosamine that is variously N-substituted, or *N*-acetylgalactosamine) and a uronic acid (glucuronic acid or iduronic acid) or galactose. Sulfated glycosaminoglycans can be covalently bound to a protein to form proteoglycans[19]. Proteoglycans consist of a core protein and one or more covalently attached glycosaminoglycan chains (Fig. 1).

Proteoglycans consist of a protein core (*brown*) and one or more covalently attached glycosaminoglycan chains ([*blue*] HS; [*yellow*] CS/DS). Membrane proteoglycans either span the plasma membrane (type I membrane proteins) or are linked by a GPI anchor. ECM proteoglycans are usually secreted, but some proteoglycans can be proteolytically cleaved and shed from the cell surface[32].

Glycosaminoglycans consist of repeating disaccharide units composed of an N-acetylated or N-sulfated hexosamine and either a uronic acid (glucuronic acid or iduronic acid) or galactose. Hyaluronan lacks sulfate groups, but the rest of the glycosaminoglycans contain sulfates at various positions. DS is distinguished from CS by the presence of iduronic acid. Keratan sulfates lack uronic acids and instead consist of sulfated galactose and *N*-acetylglucosamine residues[32].

Table 2. Repeating disaccharide units of various GAGs.

GAGs	Disaccharide units
Hyaluronan (HA)	 D-GlcA-β(1→4)-D-GlcNAc-α(1→4)
Chondroitin sulfate (CS)	 D-GlcA-β(1→3)-D-GalNAc4S-β(1→4)
Dermatan sulfate (DS)	 L-IdoA-α(1→3)-D-GalNAc4S-β(1→4)
Keratan sulfate (KS)	 D-Gal-β(1→4)-D-GalNAc6S-β(1→3)
Heparan sulfate (HS) or Heparin	iduronate-2-sulfate *N*-sulfo-glucosamine-6-sulfate  α-*N*-sulpho-6-sulphoglucosaminyl-(l→4)-iduronate 2-sulphate

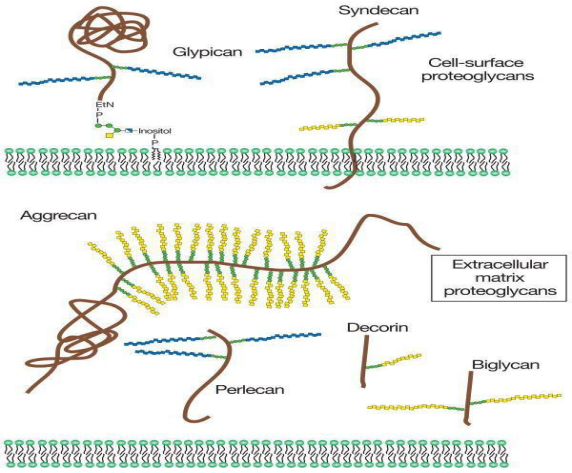


Fig. 1. Proteoglycans core protein and covalently attached glycosaminoglycan chains.

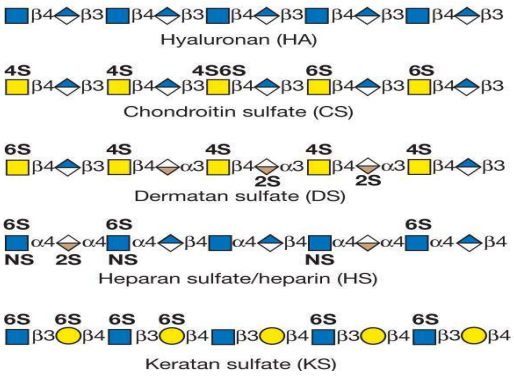


Fig. 2. Depicts characteristic features of the major types of glycosaminoglycans found in vertebrates.

5. Bioactive complexes of GAG from marine species

GAGs are biopolymers industrially extracted from various animal organs. There are patent applications, which disclose obtaining methods of some GAGs derivatives of heparinoids from bacteria *Escherichia coli*[33] or heparin and heparinoids from fish (carp, mackerel, cod, herring, anchovy, shark and salmon) and other marine organisms with anticoagulant and antithrombotic activity exclusively[34,35,36]. Another patent application discloses a pharmaceutical composition with anti-inflammatory properties, which contain sulfated hexosamines and a protein extract from *Perna canaliculus*[37]. Other inventors isolate GAGs from cartilaginous fish that present antitumor effect[38]. However, it seems that these products do not capitalize the whole therapeutic potential of the GAGs concerned.

6. Dilemma of inland source GAGs

There is an immense diversity of marine plants and animals from which an estimated 14,000 pharmacologically active compounds have been isolated[39]. GAG has never been reported in plants. Reports also revealed structural diversity in vertebrate GAGs. Among the sulfated glycosaminoglycans, heparan sulfate, a ubiquitous cell surface component of mammals and other vertebrates, is the one that exhibits the highest structural variability according to the tissue and species of origin[40]. Table 3 shows a comparison of why GAG harvesting from inland or marine invertebrates sources is better considered[41].

7. GAGs from marine sources

Among the various sources for the development of new drugs, compounds from living organisms, so-called natural products, are of particular significance[40]. The oceans cover more than 70% of the earth's surface and are an indispensable source of protein for human nutrition. With regard to drug discovery and development, however, the oceans started to attract interest from pharmaceutical companies and research institutions only approximately 50 years ago with the discovery of the sponge-derived nucleosides spongothymidine and spongouridine[42].

Table 3. Comparison table to illustrate why GAG harvesting from inland or marine invertebrates sources is considered better.

Vertebrates / Inland	Marine Invertebrates
1. Heterogenous structure	1. Homogenous structure
2. Diverse sulfation pattern	2. Sulfated total-, N-, and O-sulfated Glycosaminoglycans
3. Mutational defects in most genes – biosynthetically derived enzyme which causes severe consequences.	3. Stable expansion of sulfated structures: Pharmacologically active compounds are associated to a hetero undescribed compound (reaction of waste to benefit opportunities)
4. Risk for the presence of infections.	4. No alteration in structure (morphologically undefended)
5. Can reliable of availability (cost, volume) restrict to certain use.	5. Originally species specific

The presence of sulfated GAG in a diverse range of marine phyla-like sponges (Porifera)[43] and several classes of fishes (Actinopterygii and others), particularly in commercially relevant species like sharks, skate, codfish, salmon, and trout is being discussed. It is because, marine-derived GAGs are being extensively studied because of their pharmaceutical activities like anti-pathogenic, antitumor and anticoagulant and as new biomaterials with application in different areas such as biomedical, bioengineered biomaterials applications, tissue engineering and regenerative medicine research[18].

Results from some recent studies also suggest that echinoderms are a potential source of glycosaminoglycans (also known as mucopolysaccharides). These are especially effective in improving skin appearance, healing wounds and are also important for the healthy functioning of joints[44].

According to[45], better yield of sulfated GAGs has been successfully extracted from locally harvested Crown-of-thorns (*A. planci*) and in that quantitative studies conducted, it seems to suggest that a sustainable volume for production technology is achievable. The high content of GAGs from the wasted COT biomass can thus be a better tangible source of collagenous threads as compared with other outsourced Echinodermata mass. It is thus, evidently based proven that the locally harvested Crown of Thorns (COT) which is also a phylum echinoderm are of the potential tangible of GAGs source.

Literature have shown that body's coelomic fluid from COT's contained the highest amount of total sulfated GAG, followed by body's integument, arm's internal tissue, arm's integument, body's internal tissue and the lowest amount was extracted from arm's coelomic fluid[45].

On the other hand, literature have been documented that sea cucumbers or 'GAMAT' can help cure certain ailments and diseases[46]. Sea cucumber and their products have long been purported as a source of traditional medicines due to their various important nutritional and medicinal values[47]. In addition, the coelomic fluid of certain sea cucumbers has been reported to contain high bioactive substances that suggest orchestrating an important role in wound healing[48].

However, according to[49], the integument body wall of *S. hermanni* and *S. vastus* has the richest source of sulfated GAGs followed by the visceral internal organs and coelomic fluid. The data are congruent with nutrient analyses that showed the integument body wall and the visceral internal organs (especially intestines) part of sea cucumbers to have a much higher nutrient value of protein and lower in fat[50].

Thus, based on the previous study conducted both by[45,49] it was objectively proved that (homogenous, non-adulterated, therapeutic) GAG can be derived from COT and sea cucumber biomass. Table 5 and 6 below, revealed that COT possessed more total sulfated GAGs content per milligram as compared with the sea cucumber. Although, GAGs have been isolated in other Echinoderms, especially the sea cucumber[51] however, there is poor scientific evidence and research's to elucidate the presence of GAG in COT biomass especially from the local coastal region. Hence, GAG derived from COT biomass is still a scientific lacuna especially in determining its prowess as an alternative source, especially (from marine sources) for wound healing dynamics. Apart from that, it is also scientifically noted that the COT body region contained the highest amount of total sulfated GAG as compared to its arm, even though the internal tissue from the body is of low amount[45]. Table 4 shows the differences between marine species and its potential for commercialization.

8. The Future for Marine outsource GAG

The oceans are the source of a large group of structurally unique natural products that are mainly accumulated in invertebrates such as sponges, tunicates, bryozoans, and molluscs[52]. Marine polysaccharides present an enormous variety of structures. They are still underexploited and therefore they should be considered as an extraordinary source of chemical diversity for drug discovery[53]. Marine species offer a great diversity of polysaccharides showing interesting biological properties mimicking those described for the mammalian GAGs. Table 7 below summarizes the technical microscopical features related to "efficiency studies" observed in respect to GAG adulteration and its potential for the future used.

Table 4. Differences in taxonomy, local geochemical signature and therapeutic value between marine species.

	Starfish	Sea cucumber	Sandfish	Golden Sandfish	Small Fish
Reference	Nur Afiqah *et al.*,2011	Kariya *et al.*,1990	Natacha Agudo, 2006	Daniel Azari Beni Giraspy & Grisilda Ivy	Natalia Rosoiu *et al.*, 2008
Family	Acanthasteridae	Stichopodidae	Holothuriidae	Holothuriidae	-
Genus	Acanthaster	Apostichopus	Holothuria	Holothuria	-
Species	*A. planci*, Linnaeus, 1758	*A. japonicus*	*H. scabra*, Jaeger	*H. lessoni*, Conand	*Engraulis encrasicolus ponticus*
Therapeutic value	Positive bioactive compound from anatomical parts	Sulfated Gag from body	Food Delicacy	Food Delicacy	Positive biological active compound
Temp.	-	15-21ºC	27-30ºC	25-27ºC	-
Salinity	-	27-35 ppt	28-36 ppt	37-38 ppt	-
Dissolved oxygen	-	> 5mg/L	5-6 mg/L	>5.5mg/L	-
pH	-	8.1-8.3	6-9	8.2	-

Table 5. Percentage (%) division of O- and N-sulfated GAGs from Sea Cucumber [49].

Sea cucumber species	Anatomical parts	O-sulfated GAGs (%)	N-sulfated GAGs (%)
S. hermanni	Integument body wall	55.04 ± 2.55	44.96 ± 2.6
	Visceral internal organs	44.94 ± 12.37	55.06 ± 12.4
	Coelomic fluid	62.71 ± 12.8	37.29 ± 12.8
S. vastus	Integument body wall	70.79 ± 7.45	29.21 ± 7.5
	Visceral internal organs	69.99 ± 11.68	30.01 ± 11.7
	Coelomic fluid	82.31 ± 6.7	17.69 ± 6.7

Table 6. Percentage (%) division of N- and O-sulfated Glycosaminoglycans (GAGs) from COT[45].

Species	Anatomical parts	O-sulfated GAGs (%)	N-sulfated GAGs (%)
Acanthaster planci **(Body)**	Integument body wall	17.06 ± 0.50	82.94 ± 0.50
	Internal tissue	20.00 ± 0.30	80.00 ± 0.30
	Coelomic fluid	24.99 ± 2.25	75.01 ± 2.25
Acanthaster planci **(Arms)**	Integument body wall	18.05 ± 0.75	81.95 ± 0.75
	Internal tissue	16.50 ± 0.14	83.50 ± 0.14
	Coelomic fluid	13.14 ± 0.80	86.86 ± 0.80

Table 7. Technical microscopical features in relation to "efficiency studies" observed to GAG adulteration.

Novelty	Process	Microscopical features
Engineered cell nuclear line model	1) Tissue organization	1) Collagen restoration binding 2) Keratin deposition 3) Mutagenesis or vascularization 4) Polymorphonuclear infiltration 5) Fibroblast proliferation
	2) pH fluid (intracellular / extracellular) neutralization	1) Reconstruction of extracellular matrix / basement membrane
Tissue therapy	3) -cell-matrix interaction -osteogenesis -monolayer cell proliferation	1) Migration of endothelial cells

9. Justification why local geochemical signatures such as sea cucumbers' and COT are now being pursued and harvested for therapeutic efficacies

To date, researchers have isolated approximately 7000 marine natural products, 25 percent of which are from algae, 33 percent from sponges, 18 percent from coelenterates (sea whips, sea fans and soft corals), and 24 percent from representatives of other invertebrate phyla such as ascidians (also called tunicates), opisthobranch molluscs (nudibranchs, sea hares etc.), echinoderms (starfish, sea cucumbers etc.) and bryozoans (moss animals). A simplistic analysis of these data reveals that as the search for "Drugs from the Sea" progresses at the rate of a 10 percent increase in new compounds per year, researchers are concentrating their efforts on slow-moving or sessile invertebrate phyla that have soft bodies, and the lack of spines or a shell, *i.e.* animals that require a chemical defense mechanism [54,5].

A very different kind of substances has been obtained from marine organisms among other reasons because they are living in a very exigent, competitive, and aggressive geochemical signatures surrounding, very different in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules. As such, in an associated perspective, these compounds seem to be very useful and promising for biomedical research to clarify many normal and pathological mechanisms of action in the human body as well as in the design of very specific and potent new pharmaceuticals for a wide variety of diseases. In tandem with this, and with sustainable green technological approaches improvement of aquaculture, the potential of these marine microorganisms to be exploited for the obtainment of lead molecules is clearly immense as feasibility to cultivate in laboratory conditions also improves and the industrial biotechnological techniques to optimize and manipulate its variables from the initial sources of bioactive natural products excel.

10. Conclusion

This writes up hope to highlight and reveals that there is an acute need for developing new therapeutic bioproducts based on the optimized study that may allow improving the efficiency and therapies means of sulfated GAG. Both marine invertebrates (Crown-of-thorns and Sea Cucumber) have shown positive effects as biocompound reactant. Encouraging results in animal studies and clinical trials will support the clinical relevance of these glycosaminoglycans based drugs and the use of glycosaminoglycans as therapeutic targets.

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