Application of glycosaminoglycan polysaccharides extracted from aquatic animals in medical science

Ahmadi M.[*1]; Rostamzad H.[2]

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Abstract

Aquatic animals, due to their unique environment, are known as producers of natural products with specific structure and diverse biological activities. Aquatic wastes are rich in bioactive compounds, which are mostly wasted with limited use and low commercial value, and even cause resource wastage and environmental pollution. One of these compounds is sulfated glycosaminoglycans (GAGs**).** Glycosaminoglycan polysaccharides are extracted from various parts (whole fish, head, flesh, fins and tail, gut and bone) fish, shrimp, molluscs and algae by two enzymatic and chemical methods. GAGs are negatively charged linear polysaccharides composed of repetitive disaccharide units of hexosamine (glucosamine or galactosamine), uronic acid (glucuronic or iduronic acid), or galactose. The most common GAGs are chondroitin sulfate (CS), dermatan sulfate (DS), hyaluronic acid (HA), keratan sulfate (KS) heparin (HE), and heparan sulfate (HS**)**. GAGs have biological functions in medical science such as immunomodulators, antioxidants, antivirals, anti-inflammatories, neuroprotectors, antiproliferative, and anticoagulants. Heparin is a highly sulfated glycosaminoglycan that has natural anticoagulant properties. Heparin isolated from some molluscs has shown the same structural characteristics and anticoagulant activity as heparin isolated from mammals. Also, glycosaminoglycans extracted from fish swim bladder have shown good anticoagulation activity**.** GAGs can be extracted from fish cartilage. Although cartilage is a low-value material, it is rich in chondroitin sulfate, which has been reported as one of the GAGs for the treatment of osteoarthritis. Due to the various side effects of sulfated glycoaminoglycan extracted from pig intestine or cow lung, there is a need for alternative natural compounds, and aquatic animals are natural sources available for extracting these compounds. Therefore, the use of non-edible aquatic by-products can be useful as one of the added value sources for GAGs extraction. Recent research focuses on the use of GAGs in tissue engineering constructs for the regeneration of damaged tissue such as implants, nerve regeneration, bone repair and damaged cartilage repair.

Keyword: Fish, Glycosaminoglycan, polysaccharide, Heparin, Anticoagulant

1- Aquatics Fish Processing Research Center, Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education & Extension Organization (AREEO), Bandar Anzali, Iran.

2- Department of Fisheries, Faculty of Natural Resources, University of Guilan, Someasera, Guilan, Iran

[*]Corresponding author's Email: Shillat2017@gmail.com

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Introduction

The aquatic processing industry produces a lot of waste, which as a raw material is a source of bioactive compounds for extraction. One of these compounds is glycosaminoglycans (GAGs), which can be extracted from the intestines, skin, scales, swim bladder of fish and shrimp waste (Heloisa *et al*, 2021). Glycosaminoglycans (GAGs) are linear polysaccharides containing repeating disaccharide units with a high negative charge that are mostly present on cell surfaces or in the extracellular matrix. Based on the disaccharide composition, GAGs are classified into four families, including heparan sulfate (HS), chondroitin sulfate (CS), hyaluronan (HA), and keratan sulfate (KS). The biological activities of GAGs depend on their ability to interact with a variety of proteins including growth factors, cytokines, enzymes, serine protease inhibitors and extracellular matrix proteins (Pan *et al*., 2018). GAGs have anticoagulant, antithrombotic, neuroregenerative, antitumor, antiviral or anti-inflammatory activities. The main glycosaminoglycans are chondroitin sulfate, dermatan sulfate, hyaluronic acid, heparan sulfate, and heparin. Active glycosaminoglycan heparin has natural anticoagulant properties. It is used as medicine due to its high anticoagulant activity. The production of commercial heparin relies on the intestines and lungs of cows and pigs as raw materials. Considering the emergence of mad cow disease, the existence of religious-cultural beliefs and the absence of non-animal sources of heparin, it motivates to identify alternative heparin-like or heparin compounds from marine sources (Hemmati *et al*., 2014). Chondroitin sulfate has anti-inflammatory properties with anti-cancer effects. It plays a role in creating the strength and flexibility of cartilage, glucosamine helps to inhibit inflammation and the growth of cartilage cells. Another important compound is hyaluronic acid (HA), a natural polysaccharide composed of glucuronic acid and N-acetyl-D-glucosamine units. HA is known for its excellent water holding capacity, making it an effective humectant. With age, the level of HA in the body decreases, which leads to a decrease in moisture retention in the skin, resulting in dryness and wrinkles. Oral consumption of HA can help increase moisture both on the surface of the skin and inside it. In addition to individual benefits, the combination of gelatin, hyaluronic acid, and chondroitin has the potential to be used in wound healing applications(Sureandiran *et al*., 2024). Therefore, the use of non-edible aquatic by-products can be useful as one of the added value sources for GAGs extraction.

Chondroitin sulphate

One of the most predominant glycosaminoglycans (GAGs) is chondroitin sulfate (CS), which is present in the extracellular matrix of tissues (Wang *et al*., 2020). Fish and fish waste (fins, scales, skeleton, bone, and cartilage) are good sources of CS, because they are inexpensive and available. The first step in the production of pure CS from fish samples is the chemical or enzymatic extraction of crude GAGs. Studies show that enzyme-based extraction is more effective than chemical extraction. Different types of enzymes, solvents and detergents can be used to break the structure and remove CS from proteoglycans (Nogueira *et al*., 2019). Papain is a frequently used enzyme that has been evaluated in several types of tissue samples for its ability to release GAG (Nakano *et al*., 2012). Chemical hydrolysis of the tissue is usually performed first to ensure complete degradation of the proteoglycan core, followed by removal of proteins to recover specific GAGs from the resulting extracts, which is the most commonly used method. Each CS disaccharide unit contains only one sulfate group, which is a critical determinant of its pharmacological and pharmacokinetic activity. CS, due to its anti-inflammatory properties, is mainly used to treat osteoarthritis. Its applications in other fields of medicine, including antiviral, anti-infective, tissue regeneration and tissue engineering, have been well evaluated. In recent years, studies show that CS can be used as a biomarker for cancer diagnosis and treatment (Urbi *et al*., 2022).

Heparin

Heparin, a sulfated glycosaminoglycan found in mammalian and vertebrate tissues, is widely used in medicine due to its anticoagulant, antithrombotic, and antilipid activities. Studies have shown that heparin-like compounds are present in some invertebrates. A substance called mactin, with anticoagulant activity and structural similarities to mammalian heparins, has been isolated from the molluscs Cyprinia islandica and Mactrus pussula (Carl *et al*., 1999). Heparin is the most widely used GAG due to its ability to anticoagulate blood. As such, heparin is widely used in the treatment of myocardial infarction, unstable angina, venous thrombosis, and pulmonary embolism. Heparin also serves as a starting material for the preparation of low molecular weight heparins (LMWHs). Studies show that heparin extracted from the skin of commercially caught edible fish exhibits potent anticoagulant activity (Krichen *et al*., 2018).

Hyaluronic acid (HA)

Hyaluronan, also known as sodium hyaluronate, is a naturally occurring polysaccharide that plays an important role in biological processes, including healing, tissue repair, and joint lubrication (Dicker *et al.,* 2014). HA is non-sulfated, which sets it apart from most other GAGs. This lack of sulfate groups means that HA does not have a strong negative charge, making it more hydrophilic and less likely to be a problem for growers. Hyaluronan is a type of hyaluronic acid that can be extracted directly from animal sources such as fish cartilage, fish eyes, sharks (skin and cartilage), bivalve mollusks, and oysters. The key difference between its marine source and its mammalian source is its molecular size, which is smaller than that of mammalian-derived hyaluronan, allowing it to penetrate the

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skin and improve hydration. Studies also show that it has antioxidant properties and may be beneficial for overall skin health. Hyaluronic acid (HA) is highly biocompatible, and gut bacteria can break down hyaluronan, allowing it to be naturally excreted from the body (Abdallah *et al*., 2020). The biodegradability of hyaluronan makes it an ideal material for temporary medical devices, such as drug delivery vehicles or tissue engineering scaffolds .And it is often used as a carrier for other wound healing agents and in cosmetic formulations because it increases the absorption of other active ingredients into the body (Abdel-Rahman *et al*., 2023).

Fucoidan

Fucoidan as one of the most important sulfated polysaccharides found in the extracellular matrix of brown algae and it has numerous applications in medical sciences and pharmacy. Fucoidan compounds have been extracted from invertebrates such as sea urchins, etc. In addition to anticoagulant activity, fucoidan also has other biological activities such as antiviral, anticancer, antitumor, anti-inflammatory, and antioxidant activities. Fucoidan is able to prevent oxidative damage. Therefore, it is the most important factor in cancer prevention and can increase the performance of drugs under treatment according to the type of cancer cells or help prevent the disease (Radkhah *et al*., 2019). Fucoidan polysaccharide reduces tumor volume and weight by participating in chemical pathways. Therefore, it can be important for use as a dietary supplement by cancer patients along with other chemotherapy drugs (Wu *et al*., 2016). It also protects the body against toxicity associated with chemotherapy agents and radiation (Atashrazm *et al*., 2015).

Sources of glycosaminoglycans

1. Fish
2. Shrimp
3. Molluscs
4. Algae

Glycosaminoglycans extraction procedures

7-1. Enzymatic Extraction

Exogenous enzymes like papain and pronase can be used to liberate whole glycosaminoglycan (GAG) from the tissues.

Parameters affecting enzymatic extraction

1. Enzyme Concentration
2. Digestion Time
3. Temperature
4. pH

Chemical extraction

GAGs are highly negatively charged in nature and are precipitated using positively charged chemicals (Raghuraman, 2013).

Conclusion and recommendations

Marine resources are a rich and valuable source of sulfated glycosaminoglycans (sGAGs) with potential therapeutic applications and anticoagulant properties. Anticoagulants as a drug

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have many uses, because major fatal diseases such as stroke, heart disease and cancer can be treated with anticoagulant drugs. With the increasing growth of the fish processing industry, the need to reduce pollutants and increase the yield of raw materials, fish processing waste can be used to extract anticoagulant drugs. New GAG compounds from other marine organisms with potent anticoagulant activities are still the focus of research for the development of new anticoagulants.

References

Abdallah, M.M., Fernández, N., Matias, A.A. and Bronze, M.D.R., 2020. Hyaluronic acid and Chondroitin sulfate from marine and terrestrial sources: Extraction and purification methods. *Carbohydr. Polym.*, 243, 116441. DOI:10.1016/j.carbpol.2020.116441

Abdel-Rahman, R.M. and Abdel-Mohsen, A.M., 2023. Marine biomaterials: Hyaluronan. *Mar. Drugs*, 21, 426. http://doi.org/10.3390/md21080426

Atashrazm, F., Lowenthal, R.M., Woods, G.M., Holloway, A.F. and Dickinson, J.L. 2015. Anti-tumor activity of fucoidan in acute promyelocytic leukemia and its synergy with arsenic trioxide and ATRA in vitro and in vivo. *Experimental Hematology*, 43, 51-106. DOI:10.1016/j.exphem.2015.06.076

Dicker, K.T., Gurski, L.A., Pradhan-Bhatt, S., Witt, R.L., Farach-Carson, M.C., Jia, X., 2014. Hyaluronan: A simple polysaccharide with diverse biological functions. *Acta Biomater.*,10, 1558–1570. DOI:10.1016/j.actbio.2013.12.019

Nakano, T., Pietrasik, Z., Ozimek, L. and Betti, M., 2012. Extraction, isolation and analysis of chondroitin sulfate from broiler chicken biomass. *Process Biochem*, 47, 1909–1918. DOI:10.1016/j.procbio.2012.06.018

Nogueira, A.V., Rossi, G.R., Iacomini, M., Sassaki, G.L., Trindade, E.S. and Cipriani, T.R., 2012. Viscera of fishes as raw material for extraction of glycosaminoglycans of pharmacological interest. *Int. J. Biol. Macromol*, 121, 239–248. DOI:10.1016/j.ijbiomac.2018.09.156.

Radkhah, A. and Sadeghi Nejad Masouleh, A., 2019. A review on the anticoagulant and anticancer properties of fucoidan polysaccharide extracted from brown algae. *Journal of Aquaculture and Exploitation*, 8, 4, 79-92. DOI:10.22069/japu.2020.16463.1491

Raghuraman, H., 2013. Extraction of sulfated glycosaminoglycans from mackerel and herring fish waste. *Submitted in partial fulfilment of the requirements for the degree of Master of Applied Science*, 156 P. http://hdl.handle.net/10222/36256

Urbi, Z., Azmi, N.S., Ming, L.C. and Hossain, M.S., 2022. A Concise Review of Extraction and Characterization of Chondroitin Sulphate from Fish and Fish Wastes for Pharmacological Application. *Curr. Issues Mol. Biol.*, 44, 3905–3922. https://doi.org/10.3390/cimb44090268

Wang, W., Shi, L., Qin, Y. and Li, F., 2020. Research and application of chondroitin sulfate/ dermatan sulfate-degrading enzymes. *Front. Cell Dev. Biol.*, 8, 560442. DOI:10.3389/fcell.2020.560442

Wu, L., Sun, J., Su, X., Yu, Q., Yu, Q., and Zhang, P., 2016. A review about the development of fucoidan in antitumor activity: Progress and challenges. *Carbohydrate Polymers*, 154, 96-111. DOI:10.1016/j.carbpol.2016.08.005

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