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Anticoagulant Activity of Sulphated Polysaccharides Extracted from *Sargassum polycystum* (Sargassum weed) as an Alternative for Heparin Therapy

Darlyne Luijoyce H. Leonor[1*], Princess B. Bocalan[1], Nomer John O. Brucal[1], Shinette Athrea A. Catapang[1], Therese Veronica V. Javier[1*], Andrei C. Lawas[1], Oliver Shane R. Dumaoal[2]
College of Allied Medical Professions,
Lyceum of the Philippines University,
Capitol Site, Batangas City
[1]Student Researcher
[2] Faculty Researcher
*Correspondence: javiernikki@gmail.com

Abstract

Few studies have explored seaweeds as plentiful supplies of sulphated polysaccharides. Studies revealed that sulphated polysaccharides appeared to have antiviral, anti-inflammatory, antioxidant, and anticoagulant properties. Among these different biological processes, this review mainly focused on the anticoagulant activity of extracted polysaccharides originating from *Sargassum polycystum*. However, isolated polysaccharides from *S. polycystum* are still poorly investigated. Thus, further evaluation would contribute to the discovery of potential alternative heparin therapy. Innovations in this matter would be of significant help in resolving such diseases resulting from abnormalities in blood flow which required the use of anticoagulant and thrombolytic agents..

Keywords: Diseases, Marine; Medicinal; Substitute

Introduction

Natural macromolecules consisting of various subunits present in any life forms are known as sulphated polysaccharides. Decades ago, biological activities exhibited by polysaccharides showed a promising outcome to food and medical fields (Xie et al., 2016). This traditional claim has put to numerous studies and showed that organisms with sulphated polysaccharides have natural anticoagulant, anti-inflammatory and antiviral properties (Wang et al., 2013). In addition, sulphated polysaccharides' anticoagulant property is influenced by their molecular weights. Hence, excellent anticoagulant activities are demonstrated in having low molecular weight. Heparin is an anticoagulant extensively used worldwide. Although their low weight derivatives are causing possible side effects such as inability to produce clot-bound thrombin, interchangeable impacts of anticoagulant, and thrombocytopenia. With this, exploring for supplementary sources of anticoagulant agent are needed (Magalhaes et al., 2011).

On the report of Department of Health, circulatory and ischemic cerebrovascular diseases are few of the leading causes of death in the country. Failure in blood coagulation system activation causes thrombus formation. In line with these diseases, anticoagulant and thrombolytic agents are needed to maintain normal blood flow. Sulphated polysaccharides derived from seaweeds are efficient and can influence various biological activities such as coagulation of blood.

Variety of brown algae usually grows in Southeast Asia, Pacific and Western Atlantic Ocean, and one of which *is Sargassum polycystum.* Farmers used *S. polycystum* as a natural fertilizer and herbicide to supplement the soil and improve growth and productiveness of plants. *S. polycystum* is one of the most edible seaweed to man, however, its rapid production hinders fishermen's work and competes with corals and fishes for nutrients. In line with this, removal and disposal of fully grown *S. polycystum* is needed, and making them worthless and irrelevant (Li et al., 2015).

In brown algae, α-L-fucose consisting of sulphated homopolysaccharides and sulphated heteropolysaccharides is known to be one of the most commonly polysaccharides that can be found, such as fucan and fucoidan, respectively. Studies revealed that fucan and fucoidan compounds present in *Sargassum genus* proves that it consists of glucuronic acid, galactose and mannose compounds with semi-sulphated side chains consisting of galactose, xylose and fucose compounds (Dore et al., 2013).

Algal polysaccharides activity showed significant medical purposes similar with heparin but only few studies supported it. Large amounts of sulphated polysaccharides are present in *S. polycystum* and demonstrate a significant anticoagulant activity. However, in several databases (ScienceDirect, Google Scholar and PubMed) there is still lack of studies on the use of *S. polycystum* for its anticoagulant properties.

The review focuses on the demonstration of *S. polycystum* extract containing sulphated polysaccharide that exhibits anticoagulant effects similar with heparin as therapy. The polysaccharide extract can potentially provide an alternative usage for the alleged seaweed and diminish its environmental impact. Through this review, a seaweed attributed for its known therapeutic value and is widely distributed in the country will be further utilized. If proven to be significantly helpful, this would contribute to the list pf potential alternative anticoagulant. This can be locally available as substitute anticoagulant for commercial heparin with less consequences and can be offer at a low-priced.

ARTICLE SELECTION AND RESEARCH CRITERIA

References for this study were gathered and searched from Science Direct, Google Scholar, and PubMed. Keywords that were included in search strategy to gather informations were anticoagulant, heparin, sulphated polysaccharide, and *S. polycystum*. All articles were screened by checking the abstract, introduction, methods, and conclusion. The studies focused on sulphated polysaccharides that were extracted from seaweeds which show anticoagulant activity. The studies that have been chosen were properly screened by checking reference for pertinent citations. In totality, there were 1,900,358 journals (spanning from 2010-2020) screened and out of these, 50 articles were chosen which are relevant to the study

Epidemiology of thrombosis

Thrombosis is a multifaceted disorder that prevents blood from flowing normally through the circulatory system. Thrombosis is the formation of blood clot or fibrin clots that blocks your blood vessel causing ischemia and necrosis (Wang et al., 2013).

Thrombosis is more likely to happen in conditions with increased platelet count and high levels of fibrinogen seen among in patients with hypertension, diabetes, hypercholesterolemia, and heavy smokers. Obesity is an important modifiable risk factor for

venous thromboembolism (VTE). Clots in the veins are somewhat different to those in arteries and there are several risk factors however, the clots are generally located in two places namely the legs (deep vein thrombosis [DVT]) and the vessels of the lungs (pulmonary embolus [PE]) (Bland et al., 2014).

Venous thrombosis is usually associated to aging, with low rate of about 1 out of 10,000 per year before the fourth decade of life, rapidly rising after 45 years of age, and approaching 5 to 6 per 1,000 annually by the age of 80. Thus, a higher fatality percentage from thrombosis is seen in older persons. It is likely that thrombosis is less diagnosed in certain debilitated elderly patients, so these estimates are probably underestimating. The reasons for an increased thrombosis risk with age are not yet clear but may be related to the increasing presence of other diseases predisposing to thrombosis, to increasing coagulation potential, or some other factors other than age, including exogenous factors such as surgery, mobility, pregnancy, trauma, hospitalization and the puerperium and hormone use, and endogenous factors such as obesity, some types of cancer and inherited and acquired disorders of hypercoagulation (Cushman, 2007).

Diagnosis of thrombosis

Different clinical approaches for the detection of thrombosis are highly available but some are not applicable in remote areas (Beyer & Schellong, 2005). Despite the variety of diagnostic methods, there are no validated techniques existing with much reliability. Therefore, the growing interest in the search of up-to-date diagnostic strategies is being addressed to reduce unnecessary investigations and lowering the overall costs associated with the investigation of patients suspected with thrombosis. Exposure of patients to risks was being prevented as well (Liang et al., 2018).

However, other clinical practices are invasive in nature, thus, causing serious complications and discomfort in the patients (Baumann Kreuziger et al., 2017).

Clinical symptoms of thrombosis are evry so often nonspecific and sometimes confusing. Prompt and accurate finding has a great importance as delayed recognition may progress from fatal disability to death. Given the probability of missed diagnosis or obtaining false positive results, the diagnostic tools must be assessed with correlated test since there is still no standardized system in evaluating suspected cases (van Dam et al., 2020). In current clinical setting, the gold standard remains to be venography although rarely performed nowadays while D-dimer is more widely

employed especially concerning the emergency responses in cases of suspected thrombosis (Kacmaz et al., 2017). In addition to conventional imaging methods as presently the first line tests, the introduction of D-dimer assays, rapid enzyme linked immunosorbent and clinical criteria have been studied for use in screening suspected thrombosis (van Dam et al., 2020).

Venography was formally considered the diagnostic reference standard for thrombosis but due to its invasive nature, the need for radiation, contrast media, and the painful injection of veins, alternative diagnostic imaging has been tested (Beyer & Schellong, 2005). These disadvantages and limitations have made venography replaced by ultrasonography. Currently, ultrasonography is the most validated and accurate non-invasive imaging method but requires highly skilled operators (Bernardi & Camporese, 2018). D-dimers are fibrin degradation products produced when fibrin is degraded by plasmin therefore, concentrations are elevated in patients with venous thrombosis (Bernardi & Camporese, 2018). D-dimer levels can be determined through several means namely: rapid enzyme linked immunosorbent assay (ELISA) and by quantitative and qualitative latex assays. Additionally, these methods yield almost 100% sensivity and specificity (Akman et al., 2004). A normal D-dimer measurement completely ruled out thrombosis. On the other hand, consistently rise of D-dimer does not always indicate thrombosis (Kacmaz et al., 2017). Hence, it still appeared to have no aid in diagnosing thrombosis as single test approach (Kraaijpoel et al., 2017). D-dimer is only a valuable in excluding but not diagnosing thrombosis (Akman et al., 2004).

Anticoagulant

The anticoagulant activity is one of the most studied property of sulphated polysaccharides. Currently, the sulphated polysaccharides used in most therapeutic practices as anticoagulant are unfractionated heparins. Since heparin is of animal origin, it may comprise harmful contaminants for human blood leading to possible side effects like blood loss and diminished platelet count and might comprise one's condition through these compounds. This rises the pressure to look for potential anticoagulant agents alternative to heparin for advanced health care system (Liang et al., 2018). Activated partial thromboplastin time (APTT) was recognized for the quantification of anticoagulant activity (Naqash & Nazeer, 2011). Results were expressed by dividing the accomplished clotting time consumed with anticoagulant compared to the time reached using the control

(Magalhaes et al., 2011). In earlier years, it has been defined that sulphated polysaccharides derived from green algae have an anticoagulant activity (Li et al., 2015).

Anticoagulant tests suggest that sulphated polysaccharides had particularly reactive APTT delaying activity and inhibitory action to intrinsic factor Xase, thus these can equally be reduced with the decline in their molecular weight (Yang et al., 2018).

Numerous studies discovered that declining molecular weight for unfractionated heparin and produced low molecular weight heparins (LMWH) can reduce the anticoagulant activity of clotting assays. Overall charge of a heparin chain also enables strong interactions among heparin, ATIII and Factor IIa, making it important for the anticoagulant action. Thus, heparin treatment options could be limited by manifestation of impurities such as acetate, chloride, water excess and structural heterogeneity (Monakhova et al., 2019).

Saccharides like heparin and fondaparinux are one of the numerous medications that take part in treatment of blood coagulation related conditions. However, continuous monitoring is needed with these anticoagulant therapies in order to prevent hemorrhage and the allergic reactions. It is applied in diverse health conditions like thrombosis (Imran et al., 2020).

Heart attack, deep vein thrombosis and pulmonary emboli are thromboembolic disorders which are some of the main causes of mortality in developing country (Hmidani et al., 2019). Increase in elderly population and increase risk of cardiovascular incidents lead many of the researchers to strive to develop new and better anticoagulants (Imran et al., 2020).

Heparin

In 1918, American physiologist William Henry Howell together with his medical student Emmet Holt published a paper focused on pro-antithrombin and heparin. Howell termed heparin as an anticoagulant extractable from. In 1928, Howell published an article titled “The Purification of Heparin and Its Chemical and Physiological Reactions” and stated that heparin was in fact a sulphur-containing complex carbohydrate and not a phosphatide (Copeland & Six, 2009).

Heparin was originated from the liver cells of dogs. Canine liver isolate was discovered to be a good inhibitor of the coagulation cascade of the human blood. Brinkhous et al. (1939) demonstrated that the heparin requires a plasma cofactor, making it an indirect anticoagulant. In 1968, Abildgaard termed this cofactor antithrombin

III and is now identified as AT. Heparin-AT interaction is the main mediator of anticoagulant activity of heparin (Tahir, 2007).

Heparin, among others, is one of the anticoagulant agents commonly used in the medical laboratory science field and clinical cardiopulmonary bypass. It is a highly sulphated, linear glycosaminoglycan that is produced by mast cells. Its chemical structure is made up of repeating monomeric disaccharides of uronic acid and glucosamine, and its narive structure is in helical form. In 1937, McLean discovered that the heparin is able to bind with antithrombin III which causes accelerated thrombin inhibition thus, it has been practiced as an anticoagulant in the medical field (Ma et al., 2018).

Heparin is the only polysaccharide used for prevention and treatment of VTE worldwide that shows a heterogeneous structure in terms of sulphation pattern and monosaccharide confirmation. Heparin also binds to the coagulation proteases. Recent studies indicate that this binding is weaker and less specific than the binding to antithrombin. Proteases and coagulation cascade interaction (thrombin, serpin inhibitors, heparin cofactor II, antithrombin, and activated factor Xa) is associated to the anti-thrombotic and anticioagulant action of heparin (dos Santos-Fidencio et al., 2019). As medication, it is usually administered in the span of 7-10 days intravenously. Unresolved questions about this treatment have been raised whether it should be administered continuously or intermittently, and whether the dosage control throughout the therapy is useful or not. Fondaparinux, a pentasaccharide chemically made from cellobiose and D-glucose, is the only available alternative to animal-extracted heparin that has the minimum binding site of antithrombin III (Lord et al., 2016).

Disseminated Intravascular Coagulation (DIC) is a condition in which blood clots form throughout the body and blocking small blood vessels. It is a result of pathologic activation of fibrinolysis and coagulation system with depletion of multiple coagulation factors, is a common manifestation of a considerable range of disorders. Patients with DIC have a low or rapidly decreasing platelet inhibitors and increased markers of fibrin formation and degradation, hastened fibrinogen turnover and manifestation of cold-precipitating fibrinogen. One of the most widely used blood anticoagulant for this disease is heparin. It was believed that heparin interferes the process through inhibiting the activation of clotting mechanisms (Klein & Bell, 1974).

Potentiated Antithrombin (AT) inhibits pro-coagulation factors IIa and Xa's action, which then leads to reduced coagulation

in the blood. Heparin's biological activity including its inhibitory ability on enzymatic blood clot formation in vivo and in vitro, can be validated through several methods. The chromogenic method for anti-Xa and anti-IIa assay using commercially available kits is common for this event. A more sophisticated method that is surface plasmin resonance-based was recently developed, in which anticoagulant activity was evaluated by measuring the competitive antithrombin binding of commercial and standard USP heparin (Monakhova et al., 2019).

Accordingly, heparin and AT's interaction are very important step in the anticoagulation process which serves as key step for anticoagulant activity measurement of heparin and because of these improved assays for the low molecular weight (LMW) heparins, biosynthetic heparins, bioengineered heparins, and heparins extracted from the organs of different animals have been developed. With the discovery and development of new heparin-based drugs, innovative methods for the easy, rapid, and accuarte analysis of anticoagulant activity are needed to ensure quality control of these products (Zhao et al., 2017).

Immune heparin-induced thrombocytopenia (HIT) is a result of an antibody formation triggering complex of heparin and platelet factor IV. Cross-linking of heparin complexes and FIIa receptors on platelets enhances platelet aggregation and activation. The clinical sequelae range from thrombocytopenia to arterial thromboembolism and skin lesions. The mortality rate caused by HIT is up to 30%. Switching to a nonheparin anticoagulant is mandatory for patients with strongly suspected or confirmed HIT, Fondaparinux is an antithrombin-dependent, selective factor Xa inhibitor, has been the most successful off-label synthetic, ultra low molecular weight pentasaccharide in the market. However, the possibility of a low frequency of clinically relevant cross-reactivity with fondaparinux does exist thus, it is not currently approved in any jurisdiction for therapy in patients with suspected or confirmed HIT (Schindewolf et al., 2017).

Sulphated polysaccharides

Importance in exploring seaweeds as rich source of sulphated polysaccharides has grown to researchers (Kim & Wijesekara, 2011) yet only scarce knowledge on this matter was established (Deniaud-Bouët et al., 2017).Numerous studies have suggested variety of properties possessed by sulphated polysaccharide. It has also been examined and further discovered

that sulphated modification greatly influenced the sulphated polysaccharides' biological activities (Yuan & Macquarrie, 2015)

Sulphated polysaccharides' properties regarding biological effects are likely dependent on its chemical composition, including monosaccharide, sulphate content, structural characteristics, and molecular weight. Its chemical structure varies regarding several aspects, such as maturity of algae, geographic setting, seaweed specie, harvesting period, and the extraction technique employed (Pre-proofs, 2019).

The major grouping of aquatic algae Chlorophyta (green), Rhodophyta (red), and Phaeophyta (brown) varies with the amount of sulphated polysaccharides present. The core sulphated polysaccharides constituent in aquatic algae includes ulvan (green), carrageenan (red), and fucoidan and laminarans (brown) (Kim & Wijesekara, 2011).

Marine algae are rich in polysaccharides that contain sulphate moieties in their structures. Sulphated polysaccharides and their lower molecular weight oligosaccharides derivatives from seaweeds have been shown to possess a variety of biological activities such as anticoagulant, antiproliferative, anti-cancer, anti-viral, antihyperlipidemic and antihepatotoxic activities (Costa et al., 2010).

Fucoidans are special complex polysaccharides which contains L-fucose and sulphate ester groups, mainly brown seaweed derived. It has been reported to demonstrate physiological and biological functions such as anticoagulant, antithrombotic, anti-inflammatory, ant-viral, and anti-tumor activities (Faggio et al., 2016).

The immediate action of sulphated polysaccharide on thrombin can be related with the capacity of ploymers to bind thrombin suppressing their reactant movement. It has been shown that the polysaccharide extract from marine algae has an ability to slow blood clotting prolonging the PT and APTT (Faggio et al., 2016).

Marine microalgae like seaweeds has its advantage because it is easily growing and harvesting does not depend on the climate or season. Being easily controlled it enables the production of polysaccharides particularly the sulphated polysaccharides. Applications include the following namely: have been found: antiviral agents, antioxidants, anti-inflammatory properties and its role in the immunomodulatory system (Filomena et al., 2013).

Marine organisms play a vital role in human health and nutrition. Seaweeds are one of the important marine living resources

that have a lot of health benefits including anticoagulant, antiviral, etc. Sulphated polysaccharides from marine seaweeds and heparin share similar ionic structure. Sulphated galactan from red seaweeds, sulphated arabinan from green seaweeds, and fucoidan from brown seaweeds have been well demonstrated for their anticoagulant activity (Shobharani et al., 2014).

Sargassum polycystum

Seaweeds are marine macroalgae that can be seen in bodies of water such as lakes, ocean, and river. These can be classified as red and brown, which are uniquely marine and green seaweeds, which are common in freshwater and with different types of species (Nagappan et al., 2017). Antibacterial, anticoagulant, anti-inflammatory, and anti-oxidant are biological activities exhibited extensively by seaweeds owing why it is noticeable to be one of the most predominant objects that can be studied (Li et al., 2015). The prevalent type among seaweeds which can grow up to 20 meters long are the brown seaweeds. The sulphated polysaccharide established in brown algae are manily fucoidan. It is termed due to its multiplex aulphated polysaccharide and it has been discovered to have medical purposes and also a source of food products (Barros Gomes Camara et al., 2011).

In this study, *S. polycystum* is the sample of choice because contains sulphated polysaccharide which has been identified to have a dominant anticoagulant property. Fucoidan which was extracted from the *S. polycystum* is a natural sulphated polysaccharide with heterogeneous formation and biological qualities (Majdoub et al., 2009).

The therapeutic effects of *Sargassum spp.* are rendered to be methodically plausible and may be described in relation of their in vivo and in vitro activities such as antiviral, antibacterial, anti-inflammatory, and anticancer actions. These pharmacological activities can be primarily credited to the most important bioactive metabolites namely: meroterpenoids, phlorotanins, and fucoidans. *Sargassum spp*. seems to possess iodine which is of great value used for the treatment of thyroid disease (e.g. Hashimoto's thyroiditis) and plays a significant role in immunomodulation (Liu et al., 2012).

S. polycystum, commonly known as *lusay-lusay*, is a seaweed originating from the Sargassaceae family. It is easily distinguishable through its brown or dark green color and a holdfast, stipe, and frond. It is branchy, has leafy-like appendages structure

that of a berry (called pneumatocyst, which are gas-filled structures filled with oxygen) that helps the fronds float (Costa et al., 2010).

It is found mostly in shallow reef flats and rocky bottoms. Commonly edible in nature, it has a salty, slightly bitter flavor (Liu et al., 2012). There are some compounds that are unavailable in terrestrial vegetables and also have naturally active compounds like flavonoids, pheophytine, polyphenols, terphenoids, sargaquinoic acids, sargachromenol, sterols and sulphated polysaccharides (Dore et al., 2013).

Sulphated polysaccharides' biological features that were reported up to date are anti-tumor, anticoagulant, anti-oxidant, immunomodulatory, inflammation, antiviral, anti-protozoan, antibacterial, and antilipemic (Dore et al., 2013). Bioactive sulphated polysaccharide extracted from seaweeds are generally classified into three major types namely: Fucan which embodies a family of water soluble, rich in SP sulphated L-fucose which are removed from extracellular matrix of the weeds (Xie et al., 2016)**.** Next is fucoidan, the sulphated alpha L-fucan which demonstrated a wide range of pharmacological activities, and lastly, Carrageenans which belongs to the family of linear SP which are removed from red seaweeds (Li et al., 2015).

It is a medicinal plant that has anti-obesity property (Awang et al., 2014) and contains iodide, which has great therapeutic effect on iodine deficiency induced endemic goiter and can temporarily inhibit hyperthyroidism (Liu et al., 2012).

In other studies, it is written that extracts from *S. polycystum* at 150 mg/body weight were helpful in alleviating tissue in diabetes-related injuries. The 300 mg/body weight doses were constructive for pancreas but may be toxic to the kidney and liver of diabetic rats (Motshakeri et al., 2013).

Table 1 shows the summary of sulphate content from different aquatic seaweeds after various extractions. The results obtained indicates similarity with the conventional method.

Table 1
Sulphate Content of Different Marine Seaweeds

Seaweed/ Algal spp.	Sulphate (%)	Reference
Monostroma angicava (green)	26.6	Li et al. 2017
Codium divaricatum (green)	23.7	Li et al. 2015
Enteromorpha linza (green)	34.4	Wang et al. 2013
Sargassum vulgare (brown)	22.6	Dore et al. 2013
Gracilaria corticata (red)	29.08	Seedevi et al. 2017
Ascophyllum nodosum (brown)	29.33	Yuan & Macquarrie 2015

CONCLUSION

Few studies were conducted to further explore the significant role of sulphated polysaccharides in different biological processes. The information presented in the gel electrophoresis findings showed that the extracted polysaccharides subjected to agarose gel migrates towards heparin standard band. Thus, verifying the heparin-like sulphated polysaccharide content. Based on the results from different journals that were reviewed, it can be concluded that all algal polysaccharides exhibited substantial anticoagulant activity.

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