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EVALUATION OF SULFATED POLYSACCHARIDES AS AN ANTICOAGULANT ACTIVITY – A REVIEW

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ABSTRACT

Marine algae are rich in sulfated polysaccharides (SPs) such as carrageenans in red algae, fucoidans in brown algae and ulvans in green algae. These SPs exhibit many health beneficial nutraceutical effects such as antioxidant, anti-allergic, anti-human immunodeficiency virus, anticancer and anticoagulant activities, Therefore, marine algae derived SPs have great potential to be further developed as medicinal food products or nutraceuticals in the food industry. Researchers have found and isolated various sulfated polysaccharides from brown algae, green algae, and red algae, which were reported to have anticoagulant properties. This contribution presents an overview of nutraceutical effects and potential health benefits of SPs derived from marine algae.

Keywords: Anticoagulant, algae, sulfated polysaccharides, carrageenans

INTRODUCTION

At most the cardiovascular disease like heart disease and stroke like thrombosis are main death and WHO predict 3.6 million people will suffer from this disease during 2030. In area of this cardiovascular disease the heparin as sulphated polysaccharide used for anticoagulant therapy in hematology and transfusion medicine for more than 50 years. However, it has several disadvantages such as production difficulties, chemical in homogenicity, variability in physiological activities, and bleeding **[1, 2]** hence it is necessary to find safe, natural, and easy to use drug instead of heparin.

The anticoagulant activity from sulphated polysaccharide which is derived from marine algae possess similar structure

compared to heparin. Therefore, the sulphated polysaccharide is used for alternative anticoagulant therapy in case of heparin. The sulphated polysaccharides (SPS) from marine algae was demonstrated to have many biological activities such as anticoagulant **[3]**, antioxidant **[4]**, antitumor **[5]**, anti-aging **[6]** and antiviral activities **[7]**. The Chargaff *et al* (1936) a scientist first reported the similar structure of sulphated polysaccharide from marine algae with that of heparin as anticoagulant nature.

Marine algae are rich source of sulphated polysaccharide as abundant in nature with that of animal source. In recent research the scientist are much more concentrated in isolation of sulphated polysaccharide from marine sources for in nutraceutical/ functional food, cosmetic/ cosmeceutical and pharmaceutical applications **[8, 9]** SPs include a complex group of macromolecules with a wide range of important biological activities such as antioxidant **[10, 11]** anticoagulant **[12, 13]**, anticancer **[14, 15]**, antiviral **[16]** anti-allergy and anti-inflammation **[17]**.

Nowdays there is much importance are given to the drugs which is come from the nature sources namely marine sources and also to find new biological variety of marine species. The sources that available nowadays are used as source therapy for thousands of years. The sulphated polysaccharide is classified into three groups namely red algae (rhodophyta) green algae (chlorophyta) and brown algae (phaeophyta) based upon this classification the sulphated groups are present in rich amount in this marine algaes.

Several of this marine algae species shows the pharmacological activities for existing disease like cancer, AIDS, diabetes, arthritis etc **[18]**. The isolation of seaweeds resources possess major metabolites like polysaccharide, lipids, proteins, carotenoids, vitamins and etc. **[19]**. The sulfated fructose and sulfated galactans are other form of anticoagulant marine algae compound present in the sulphated polysaccharide **[20]**.

Some algal species trigger anticoagulant activity through protein or glycoprotein-like compounds **[21]** which binds to the serine protease, an inhibitor of antithrombin, producing a complex which accelerates the proteolysis of the enzyme responsible for coagulation. The preparation of heparin and sulfated glycosaminoglycans from natural sources is expensive and therefore an alternative is desirable **[22]** Anticoagulant and antithrombotic activities are the low molecular weight heparin and unfractionated heparin has most studied properties of sulphated polysaccharide where they are currently used as anticoagulant and also for prevention of deep vein thrombosis disorder **[23]**.

However, the side effects of heparin are

reported like thrombocytopenia, hemorrhagic effect and inability to bind or stop of thrombin bound to fibrin **[24, 25, 26]**. Moreover, heparin is extracted from animal sources are purified and its recovery is poor. The sulphated polysaccharide purified are much better purified and the recovery is good and also alternative source of anticoagulant **[27]**.

Collection of algae, Extraction and Purification of sulfated polysaccharides

In march 2004 on north coast of brazil the marine species *H. floresia* (Clemente) *C. Agardh* was collected. Initially, the sulfated polysaccharides were extracted from the red seaweed *H.floresia* by mechanical stirring for 24h at room temperature in water at 1.5% (w v-1). The residue was removed by centrifugation (5.000 × g for 15 min. at 4oC). The supernatant was precipitated with absolute Et OH (1:3; v v-1), centrifuged, re-dissolved in distilled water, dialyzed against water, freeze dried and denominated Hf1. The residue was re-extracted at 800c for 4 hrs and the centrifugation is done on the same condition and once more hot extraction are repeated with second extract residue, the precipitated supernatants were with Et OH (1:3; v v-1) AND hf2, Hf3 are denominated for second and third extractions respectively in hot distilled water 600c the weight of 0.02g of each of Hf1, Hf2, Hf3 fraction was dissolved and centrifuged. The Hf1, Hf2, Hf3 soluble fraction are originating as clear supernatant liquid and then it was freeze dried for further uses. These fractions were used in the subsequent studies. The algae were oven dried at 45˚C for 48h. The dry seaweed (120g) was milled and stirred for 30min with 900mL of n-hexane. The supernatant was concentrated in vacuo and the treatment was repeated four times. The treated seaweed was dried at room temperature for 48h and soaked into 2000mL of a solution containing 96% ethanol and 36% of formaldehyde in a 4:1v/v ratio. After 72h the seaweed was decanted and air dried.

One hundred grams of the dried alga were stirred with 3% CaCl2 solution at 850C for 4h, and the mixture was cooled and centrifuged at 3000 × g for 20min. The solid was recovered and the extraction process was repeated three more times. The supernatants were dialyzed using Spectra/Pormembrane (MWCO3500) (Spectrum Laboratories, Rancho Domínguez, CA,USA) against tap water, followed by distilled water, concentrated in vacuo and freeze-dried (Christ Alpha 1–2 Freeze Dryer, OsterodeamHarz, Germany). The resulting solid was dissolved in 75mL of distilled water, stirred for 2h with 1MHCl (50mL) and centrifuged. The supernatant was neutralized with1MNaOH solution, dialyzed against distilled water, concentrated and freeze-dried **[28]**.

In this method 800ml of distilled water with 40g of powdered algal sample was stirred for 3 hrs. 900c was adjusted for the extraction for separation of aqueous extract residue for the separation and centrifugation at 8000 × g for 15 mins. Re-extract the pellets with the same way. Dialyzed the supernatants with combined extensively against water. Precipitate the polysaccharide with the doubled the amount of 95% alcohol. The centrifuged precipitate was collected and centrifuge at 10000×g for 20mins. Then the precipitate is collected washed with absolute alcohol and lyophilized.

The extraction precipitate obtained was redissolved in water and remove the protein content Sevag method **[29]**. The water phase dialysed before it recovers against distilled water and same as for polysaccharide recovered by ethanol. The precipitate which is collected was washed and lyophilized, the polysaccharide which is partially purified further purified through gel filtration chromatography on sephacryl S-400. The obtained polysaccharide was collected and analysed by phenol-sulphuric acid method [30].The polysaccharides fraction purified and pooled and reprecipitated with absolute with ethanol. the high carbohydrate content (UPF1) was lyophilized and used for further experiments.

Evaluation of anticoagulant activity

Methods used to determine the anticoagulant activity are usually commensurate with the historical phase of hemostatic investigation. Thus, early studies have used whole blood clotting times and USP/BP assays the recent studies were used clot end point APTT, PT and TT. Direct antiXa, direct anti-thrombin activities, potentiation of ATIII and HC-II inhibition of thrombin were also measured using chromogenic substrates for a precise mechanism of action. Potency was expressed by (a) time delayed (second) (b) clotting ratio (c) heparin units/mg and (d) relative anti-thrombin activity of heparin.

Anticoagulant Methods

Anticoagulant activity

The coagulation factor of blood coagulation in the injury vessel wall were stopped the blood flow, when there is abnormal condition exposure to non-endothelial surfaces at vascular injury sites occurs. The blood coagulation was stopped by endogenous and exogenous anticoagulant interferes with coagulation factors for the anticoagulant activity **[31]**. Heparin has been the most commonly used antithrombotic/anticoagulant drug.

Heparin has side effect such as thrombocytopenia, hemorrhagic, ineffectiveness in congenital or acquired anti thrombin deficiencies and not able to inhibit thrombin bound to fibrin **[32]**. Due several side effects of heparin the sulphated

polysaccharides are derived from the marine sources which has less side effects when compared to commercial heparin. Therefore, various anticoagulant SPs from marine algae have been isolated and characterized. Two types of SPs are identified with high anticoagulant activity including sulfated fucoidans from marine brown algae **[33]** and carrageenan from marine red algae **[34]**.

There are some reported about sulphated polysaccharide from green algae when compared to brown and red algae **[35]** Jurd *et al*. **[36]** in species like *codium fragile* (Chlorophyceae) contain xylo arabino glactans and in species *codium cylindricum* it contain sulfated galactan which is responsible for anticoagulant activity **[37]** In addition, Maeda *et al* **[38]** reported the green algae *Monostroma nitidum* have six fold higher activity than heparin and in comparison with brown algae exhibit more anticoagulant activity than green and red algae extracts.

The anticoagulant activity wasdetermined by APTT, PT for the above species and PT Since a few studies reported the prolongation of PT by marine SPs, it suggests that marine SPs interfered a little or may not inhibit the extrinsic pathway of coagulation. The relationship between structure and anticoagulant activity of some SPs has been reported **[39]**. The biological active proteins are depending upon the sulfated groups in sulphated polysaccharide which increase the specific or non-specific binding sites. In sulfated galactans the anticoagulant activity is due to presence of nature of sugar residue, the sulfation position and sulfate contents in SPS **[40]**.

Moreover, the O-sulfated 3-linked with galactans have increased the inhibition of thrombin and factor Xa by antithrombin and/or heparin cofactor II in the intrinsic pathway of blood coagulation **[41]**. Furthermore, the carrageenans which has high molecular weight and with high sulfate contents show higher anticoagulant activity than low molecular weight and sulfate contents **[42]**. The currently used LMW Heparin and unfractionated Heparin are derived from the sulphated polysaccharide. The sulphated polysaccharide derived from marine sources are less potent or same contents of the anticoagulants of heparin. Collectively, these evidence shows that sulphated polysaccharides from marine algae are promising potential to use as anticoagulant or medicinal purpose for pharmaceutical purpose.

Anticoagulant Action of Polysaccharide Fraction from *Ulva fasciata* was determined by with three different concentration namely 20, 50, 100g/kg b.wt of male Sprague-dawley rats are weighed at 200-220g, the PBS used as control the route of administration is i.v. the ketamine is given as dose of 100mg/kg b.wt after

60mins to anesthetized in intramuscular route and 16mg/kg of xylazine. The caudal vein was exposed by midline incision and 1.8ml blood was collected with citrate buffer of PH 4.5. The samples were immediately shake and transferred to centrifugation in a plastic tube at 1500×g for 10mins, after which plasma was collected and transferred to a clean plastic tube. Coagulation tests for APTT were performed **[43]**. The sulphated polysaccharide isolated from various species had a particular biological action and has sulfated radicals, these compounds were analyzed with different assays.

It was estimated that the Caulerpa polysaccharides contains galactose and have specific heparin cofactor-II dependent thrombin inhibition **[44]**. This may be an important tool to design novel anticoagulants **[45]**. A sulphated polysaccharide derived from *B.occidentalis* (Rhodophyta) by Faris *et al*., **[46]** the anticoagulant exhibit by this methods are 150.00 IU mg-1 and unfractionated heparin as 193.00 IU mg-1 with one third of 2-0 sulfated units with anticoagulant activity. The fraction FII normal coagulation time was increased up to polysaccharide concentration of 100 mcg ml-1 and the APTT time with high polysaccharide concentration of 250mcg ml-1 with normal APTT time. The Extracted from *S. wightii, T. conoides, P. tetrastromatica, S.tenerrimum and T. ornata* were in the range of 14 to 16 s. The prolongation of coagulation time may not be through extrinsic coagulation pathway **[47]**. The amount of crude heparin and heparin like glycosaminoglycans was estimated as 46g/kg wet weight. The 1080IU/KG and 28 IU/KG from *chaetomorpha antennia* crude sample were yield for the activity of heparin like glycosaminoglycans in the experimental samples by metachromatic dye method **[48]**. The in vitro anticoagulant activity of SP fractions from red algae *Halymenia pseudofloresia* using citrated rabbit plasma and observed marked changes in activated partial thromboplastin time **[49]**. The fractions obtained in the first (464.20, 211.60, 103.50, and 101.70 IU/mg) were more active compared to those from the third extraction (137.10, 96.50, and 89.20 IU/mg). Its actions were considered superior to the existing heparin standard (100 IU/mg) and SPs from the same genus species *Halymenia* sp. **[50]**. The *Ulva fasciata, Dilophus fasciola* exhibited remarkably high anticoagulant activities after 10 and 20 minutes. While, low anticoagulant activity was recorded for *Laurencia papillosa* and *Gracilaria. cylindrica*.

The anticoagulation mechanism of algae may be attributed to direct inhibition of thrombin and potentiating of antithrombin III. Prolonged activated partial

thromboplastin time (APTT), suggesting inhibition of intrinsic factors and increased intrinsic pathway-dependent clotting times. Generally, the anticoagulant activities of the extracts were less than heparin which was used as standard anticoagulant. This anticoagulation activity may be due to the presence of uronic acids **[51]**. Anticoagulant activity of the algal ethanol extract was determined according to the method of Hassan *et al*. Blood was collected from NRC rates into 8% sodium citrate solution in the proportion of 1:19 volumes of blood.

The mixture was immediately agitated by gentle inversion, centrifuged at 5000 rpm at 4°C and the separated canary yellow plasma was pooled. Algal ethanol extract (0.8 ml), and standard heparin sodium solution (0.5 U.S.P. units / 0.8 ml) were used as positive control, or 0.8 ml saline solution as negative control was placed in glass tubes. Then, 1 ml plasma and 0.2 ml of 1% calcium chloride solution were added to each tube. Tubes were stopper immediately and the time was recorded, and inverting three times in such a way mixed the contents the inner surface wet on the entire tube. The time required for clotting was determined.

Assay by batteries assessment of anticoagulant of sulphated polysaccharide from seaweeds have been studied. The APTT, TT, PT, antithrombin to anticoagulant factor Xa activities was doned and compared with heparin **[52]**. The *in vitro* and invivo anticoagulant activity was investigated from sulphated polysaccharide brown seaweeds *Ecklonia cava.* It extended the coagulation time in Wistar rats in a dose- and time-dependent manner **[53]**. By APTT Test the SP anticoagulant activity are evaluated from *D.cervicornis* Species. It possesses SP Prolong the coagulation time only 1.4 folder lesser than Clexane species an LMW Heparin.

In PT Test shows the extrinsic coagulation pathway of *Caulerpa cupresoides* has aggression. The water soluble sulfated arabino galactans from *Codium fragile* and *Codium vermila* prevent anticoagulation but induce platelet aggregation. It was showed that the high sulfated content in SP shows the higher anticoagulant activity. In this regard, *C. vermilara* proved to be superior with a higher degree of sulfation and arabinose content **[54]**. The green algae *Monostroma latissimum* sp with hot water extract of sulfated rhaman polysaccharide shows anticoagulant activity.

The anticoagulant activity as evaluated by assays of the APTT and TT promises that it can be a potential source of anticoagulant **[55, 56]** isolated sulfated two rhamnose containing SP from marine green algae *M.nitidum* shows it anticoagulant activity. The results showed that both the SP

possesses high anticoagulant activities, it potent the thrombin blocked mediator through heparin cofactor II. They also hastened thrombin and coagulation factor Xa inhibition by potentiating antithrombin III **[57]** extracted sulfated heterofucans from *C. cervicornis* which prolonged APTT. Four sulfated polysaccharides doubled APTT with only 0.1 mg/ml of plasma, only 1.25-fold less than Clexane. By proteolytic digestion the heterofucans extracted from brown seaweed *D.menstrualis* shows sequential acetone precipitation as per the Albuquerque et al (2004). The anticoagulant activities of these heterofucans were determined by APTT test. 20g/ml a fucan fraction shows significant anticoagulant about 4.88-fold lesser than Clexane (4.1 g/ml) **[58]** isolated a highly sulfated (21.76 %), 100–500 kDa molecular weight galactan anticoagulant from red algae *Lomentaria catenata* with microbial-fermented freeze-dried.

It demonstrated that the anticoagulant compound showed better efficacy than heparin and prolonged activity toward APTT and PT assays **[59]** studied that the SP from the brown seaweed *L. saccharina* shows promising activity on thrombosis. **[60]** studied the anticoagulation efficacy of sulfated b-D-mannan extracted from green seaweed *C. vermilara* and reported that higher sulfate content leads to more pronounced effect. Fucoidan has been proposed as a potential substitute of the anticoagulant heparin, with added merits. Unlike mammalian mucosa-derived heparin, fucoidan is extracted from plants, so less likely to contain infectious agents, such as viruses or prions **[61]**. The current findings promise a host of possible candidates for natural anticoagulant preparation.

CONCLUSION

In animal sources have more potent anticoagulant activity and the molecular weight is high as approximately 40000 Daltons the main side effects are bleeding in the inside tissues because of high molecular weight. So now in need of low molecular weight anticoagulant it would be possible by only through the seaweeds the anticoagulant are less potent when compare to animal sources and the side effect is very less as the molecular weight is less than the animal sources.ie approximately 20000 Daltons and it also produce by enzymatic hydrolysis which have still reduce in molecular i.e. low molecular weight heparin or sulphated polysaccharide at less than 10000 Daltons.

As per the sulphated polysaccharide which is present in different seaweeds possess anticoagulant. The anticoagulant various according to their yield obtained, when the sulfated content was high the anticoagulant is also high. The marine contain only less species of sulphated polysaccharide and it

should be finding out the species with high sulfated groups and then with the local people or by the government it should be cultivated and also it gives opportunities to local people to improve their financial status and to society to produce with cheap amount of anticoagulant for the purpose of patient who in need of anticoagulant therapy.

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