RESEARCH PAPER

Evaluation of Anticoagulant Properties of Polysaccharide Extracted from *Sargassum* Species

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ABSTRACT

Sargassum is a marine brown algae with numerous species. Lifestyle changes, urbanisation, and ageing have increased the rate of cardiovascular diseases such as myocardial infarction, stroke, deep-vein thrombosis, and pulmonary embolism. Anticoagulants serve to prevent blood clots and reduce the risk of thrombotic diseases. Heparins and their low-molecular-weight derivatives are the most commonly used medications for treating thromboembolic diseases from more than 50 years. However, they can produce complications such as haemorrhage, hyperkalemia, and thrombocytopenia. As a result, it is necessary to seek alternative sources of natural anticoagulants to treat thromboembolic disorders. Prothrombin Time (PT) and Activated Thromboplastin Time (APTT) assays were conducted. The APTT experiment showed that crude sulfated polysaccharides from *Sargassum species* decrease intrinsic blood coagulation and prolong anticoagulation time. The vast resources of natural and cultivated marine algae can provide bioactive substances with a wide range of novel bioactivities, which are increasingly being explored in the health industry. *Sargassum spp.* had the highest anticoagulant activity in terms of APTT (192 seconds) at 100µg/mL concentration. The crude sulphated polysaccharides (CSP) showed a prolonged clotting time, indicating that polysaccharides have anticoagulant activity.

Keywords: Polysaccharide, Prothrombin, Anticoagulant, *Sargassum* species, Heparin

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INTRODUCTION

In the marine ecosystem, marine algae have the potential to become an excellent source of bioactive compounds such as phenols, carotenoids, vitamins, polysaccharides, proteins, and minerals. Nowadays, there are more than 200,000 eukaryotic marine species validated, among which algae contribute nearly 44,000 described species, among algae, macroalgae (also called seaweed) constitute a new source of compounds, as they have been used traditionally for nutritional or medicinal purposes. Approximately 70% of the Earth's surface is covered by marine water, and thus, the marine world is home to a huge diversity of species. Several organisms have been proposed as sources of known beneficial compounds and other new molecules with biological potential. *Sargassum species* are found throughout tropical and subtropical regions, attached to substrates by their holdfasts. The species is characterised by its short stipe, which branches into many leaf-like laterals and spherical vesicles for flotation. An interesting fact about *Sargassum species* is that communities in some regions, such as Japan and China, have noticed the important role of this species in pharmacology and the food industry. *Sargassum species* are exposed to harsh climatic conditions, resulting in the production of several secondary metabolites and bioactive compounds. FTIR refers to the fact that most molecules absorb light in the infrared region of the electromagnetic spectrum. Because of the variety of functional groups, side chains, and cross-links involved, FTIR is especially useful for identifying organic molecular groups and compounds due to the range of functional groups, side chains, and cross-links involved with distinctive vibration frequencies in the infrared region.

The changes in lifestyle, urbanisation, and ageing have increased the rate of cardiovascular diseases such as myocardial infarction, stroke, deep-vein thrombosis, and pulmonary embolism (Boisson *et al*., 2000). Fucose-containing sulphated polysaccharides from marine brown algae exhibit a unique structure and anticoagulant properties. The anticoagulant activity of sulphated polysaccharides is dependent on the monosaccharide composition, molecular weight, linking pattern, degree of sulphation, and position of the sulfate group. Heparin is widely used to prevent venous thrombosis and treat other coagulation disorders. To overcome the obvious potential side effect of bleeding, researchers studied methods of lowering heparin's anticoagulant activities while increasing its antithrombotic activities, such as chemical modification and fractionation of native heparin into smaller molecular forms. To treat ischemic heart disease and stroke, antiplatelet agents and, in certain circumstances, anticoagulants are used.

Heparin (HP) was the first clinically used anticoagulant, and after more than 50 years since its discovery, it remains the most widely used. Even

though their use is limited to parenteral administration (mainly because of their highly negative charge and large molecular weight), and the new orally active anticoagulants lack the multiple activities of heparin, which is thought to be involved beyond the coagulation cascade, the search for orally active and multitarget small molecules as new antithrombotic drugs is an attractive approach to overcome the limitations of current drugs used in therapy. Although over the last few decades, polysaccharides from algae have been identified as the most therapeutically explored metabolites and suppliers of new antithrombotic agents, in fact, the less explored small molecules have proven to also be an excellent starting point for the development of new and orally effective drugs.

MATERIALS AND METHODS

Collection of Seaweed

Sargassum species was collected from the coastal region of Shrivardhan situated in Raigad District, Maharashtra, India. *Sargassum species* with diverse morphologies were packed in fresh polythene bags, stored in an icebox, and transported to the laboratory. Furthermore, the material was thoroughly rinsed with tap water to remove the salt from the sample surfaces, and the water was drained from the *Sargassum species* and spread on blotting paper to remove any excess water. The dried samples were washed again with sterile distilled water to remove any leftover salt from the surface. The samples were shade-dried for 5 days, then oven-dried at 50°C for 1 hour, and finally crushed in an electric mixer for 2 hours. Finally, the powdered seaweed sample was collected and stored in the refrigerator at 4°C for future use.

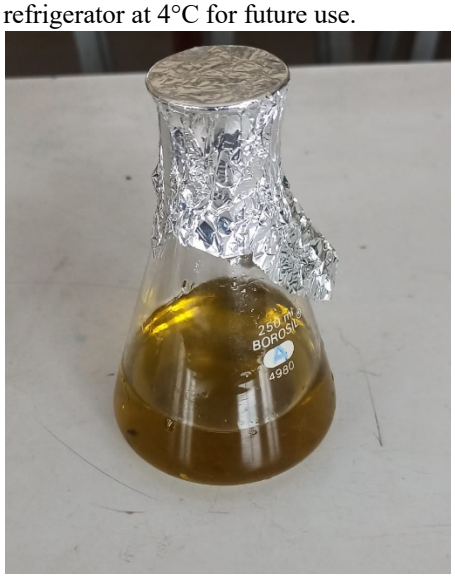

Figure No. 1 *Sargassum species* extract and dried *Sargassum*

Polysaccharide Extraction

The method described by Wassie *et al*. (2021) with slight modifications 15 g of extracted seaweed powder was dissolved in 450 ml of 0.5% EDTA-Na (w/v) and heated at 70°C with agitation for 3 hours. The mixture was centrifuged at 5000 g for 20 minutes at 4°C. The supernatant was neutralised with 0.1 M NaOH and adjusted to pH 7. The extracts were centrifuged and treated with $CaCl_2$. Sulphated polysaccharides were precipitated by adding 80% (v/v) absolute ethanol, then incubated at 4 °C overnight.

Hot Water Extraction

Polysaccharides were extracted from *Sargassum species* using the hot water extraction method as described by Lee (Lee *et al.,* 2001) 15 grams of dry, dehydrated seaweed powder were dissolved in 450 millilitres of distilled water at a 1:30 (w/v) ratio. The mixture was stirred and heated at 70°C overnight for 20 minutes at 4 ºC; the isolated solution was centrifuged at 5000g. To precipitate the alginate, the supernatant was incubated in 1% $CaCl_2$ (w/v) overnight at 4 ºC. After centrifuging the mixture at 5000 g for 10 minutes at 4 ºC, the residual alginate was removed by adding 20% (v/v) absolute ethanol. Sulphated polysaccharide was precipitated by adding 80% (v/v) absolute ethanol and incubating at 4 ºC for an overnight after centrifugation. The Sulphated polysaccharide precipitate was collected through centrifugation at 8000 *g* for 10 minutes at 4 ºC. The sulphated polysaccharide was then oven-dried until a constant mass was obtained. After the drying process, fucoidan was placed in a desiccator for about 4 hours and finally stored in a cool, dry place.

Characterisation of biochemical composition

Fourier transform infrared spectroscopy (FTIR)

One milligram of dried sulphated polysaccharides was combined with KBr salts. The KBr pellets containing sulphated polysaccharides were prepared

using the pressed disc method; FT-IR spectra (spectral range 4000–600 cm-1, resolution 2 cm-1), measured with a SHIMADZU IR Affinity-1 spectrophotometer (Sanjeewa *et al*., 2017)

Anticoagulant activity

The anticoagulant activity of each material was evaluated using the standard coagulation assays APTT and PT, with heparin as a reference.

APTT Assay (activated partial thromboplastin time):

Activated partial thromboplastin time (APTT) was measured using the standard kit on human blood samples obtained from healthy donors and drawn into syringes, adding sodium citrate as an anticoagulant. After mixing 100 µl of plasma with 10µl of different concentrations of sulphated polysaccharides, 100 µl of prewarmed APTT assay reagent was added and incubated for 3 minutes at 37°C. Finally, 100µl of calcium chloride was added, and the time taken for clot formation was recorded. All assays were carried out in triplicate.(Boisson e*t al* .2000)

PT Assay (Prothrombin time)

The standard PT assay Kit was purchased from Diagnostic Enterprises. The prothrombin time (PT) assay was carried out using 100µl of normal human plasma with 10µl of *Sargassum Spp*. extract. After 3 minutes of incubation, PT reagent (200µl) was added to induce clotting, and the time taken for clot formation was recorded.

RESULTS AND DISCUSSION:

Polysaccharides had absorption bands at about 1033 cm-1 and near 1250 to 1260 cm-1, which were caused by symmetric C-O vibration associated with a C-OSO3 group and asymmetric S=O stretching vibration, respectively (Zhang et al. 2010). In addition, polysaccharides from all species displayed absorbance bands at 3383 to 3420 cm-1 and near 2925 cm-1 due to the vibration of O-H and C-H, in that order. The bands near 1620 cm-1 formed due to the vibration of COO- groups (Silva *et al.* 2005). These findings implied that all *Sargassum species* extracts contained sulphated polysaccharides All of the *Sargassum species* extracts under study exhibited absorption bands at approximately 800 and 1740 cm-1, indicating the presence of polysaccharides.The characterisation was based on the corresponding literature and the polysaccharide peak as a reference. However, additional substances with functional groups, such as phenol and proteins, were present, particularly in the raw extracts. Absorption bands at approximately 900 and 1740 cm-1 indicate the presence of polysaccharides.

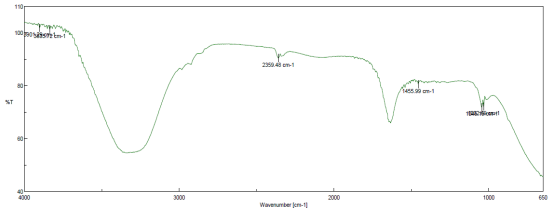

Figure No.2 FTIR of *Sargassum species*

Anticoagulant activity

The anticoagulant activity of sulphated polysaccharides was determined using the APTT test.

Blood coagulation is the result of enzymatic activities that transform inactive proenzymes into active enzymes, which then convert further proenzymes into active forms. In the last phase, the conversion of fibrinogen to fibrin causes the polymerisation of cross-linked fibrin, resulting in clot formation. APTT is a sensitive assay for evaluating the inhibitory characteristics of sulphated polysaccharides on blood coagulation factors VIIIa, IXa, XIa, and Xa, which cause delayed blood coagulation. The anticoagulant activity of each material was evaluated using the standard coagulation assays APTT and PT, with heparin as a reference.

The crude sulphated polysaccharides (CSP) showed a prolonged clotting time, indicating that polysaccharides have anticoagulant activity. *Sargassum species* had the highest anticoagulant activity in terms of APTT (192 Seconds) and Standard Heparin (249 Seconds) at 100µg/mL concentration. The APTT assay showed that crude sulfated polysaccharides from *Sargassum species* inhibit the intrinsic coagulation of blood and increase the anticoagulation time. The prolongation of APTT may be attributed to the interference by anticoagulant agents in the intrinsic coagulation pathway. The inhibition in prothrombin time by polysaccharide is not significant; the standard value for PT ranged from 15 to 17 seconds, and the PT values shown by crude polysaccharide extracted from *Sargassum species* were in the range of 14 to 16 seconds. The prolongation of coagulation time may not be through the extrinsic coagulation pathway.

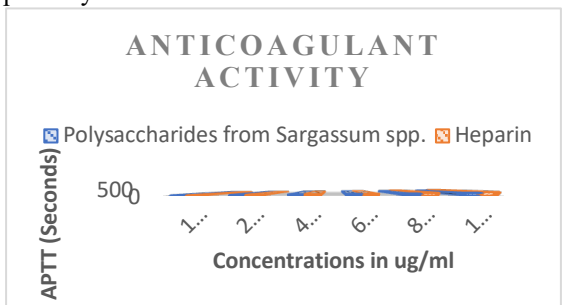

Figure No.3 Anticoagulant activity of *Sargassum species*

Table: APTT and PT assay

Sr. No.	Conc. (µg/ml)	APTT (Seconds)	PT (Seconds)	Heparin (Seconds)	PT (Seconds)
1	10	31	14	35	15
2	20	49	14	62	15
3	40	82	15	91	16
4	60	116	15	122	15
5	80	137	16	195	16
6	100	192	16	249	17

CONCLUSION:

The results suggest that *Sargassum species* possess significant anticoagulant activity and may be a good therapeutic agent for managing and treating cardiovascular diseases. This opens up new opportunities for the seaweed industry globally.

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