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SCREENING OF MARINE GREEN ALGAE *ULVA SPECIES* FOR ANTICOAGULANAT ACTIVITY

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ABSTRACT

The marine green algae *Ulva* sp were collected from Muttam, Kanyakumari District, Tamil Nadu. The algal extract was prepared from the dried, depingmented samples using sterile distilled water and concentrated by precipitation with Acetone. The prepared algal extract was used for anticoagulant assay against human blood plasma and the results of clotting test were expressed as a clotting time ratio, the prepared algal extract was found to be 2.3 PT (Prothrombin time) time ratio. In chemical analysis, algal extract contains 24.10% total carbohydrate and 4.82% proteins. The FTIR analysis 23 functional ions/groups were found between 400-4000 nm and showed the presence of sulphated ions.

Keywords: Green algae, anticoagulant activity, *Ulva* sp., sulphated polysaccharides

INTRODUCTION

Heparin is the most widely known and therapeutically used glycosoaminoglycan and has powerful blood anticoagulant activity. It can be used from an inner anticoagulant surface on various experimental and medical devices such as test tubes and renal dialysis machines. Heparin acts as an anticoagulant, preventing the formation of clots and extension of existing clots within the blood (Bjork and Lindahl 1982). Although heparin is used as a primary anticoagulant drug, it has disadvantages such as production difficulties, hemorrhagic-like side effects (Shanmugam and Mody 2000), an osteoporosis effect with long-term use, and lack of oral bioavailability (Albuquerque *et al*. 2004). Because of the above limitations of heparin, there is a need for antithrombosis research for discovering effective, safer, and easier-to-use anticoagulant agents for short-term treatment of arterial and venous thromboembolic disorders and for long-term prevention of recurrences (Srivastava and Kulshreshtha 1989).

The discovery of alternative anticoagulant molecules must be an important task for scientists. It has been done research in anticoagulant activity of polysaccharides and glycosaminoglycans of diverse sources such as: ascidians (Pavão *et al.*, 1998), sea cucumbers (Vieira *et al*., 1991), echinoderms (Mourão *et al*., 1996), tunicates (Cavalcante *et al*., 2000). These molecules have different degree of sulfate in their structure and therefore are capable of substitute heparin (Farias *et al*., 2000). In 1913, scientists investigated blood anticoagulant properties of marine brown algae (Killing, 1913).

In recent years, polysaccharides of plant origin have emerged as an important class of bioactive natural products, and their blood anticoagulant, antimutagenic, antiviral, hypoglycemic, and anti-inflammatory properties (Srivastava and Kulshreshtha 1989) have been reported. Marine algae are a rich source of sulfated polysaccharides with novel structures, and these compounds have anticoagulant properties. Various anticoagulant-active polysaccharides, especially from red and brown algae, have been isolated and characterized (McLellan&Jurd, 1992). They contain a variety of sulfated galactans and sulfatedfucans, which are among the

most abundant non mammalian sulfated polysaccharides found in nature (Harada *et al.*, 1998). In this present study, an algal extracts from the marine green algae *Ulva* species (it's consumed by local inhabitants as a marine vegetable) was screened for anticoagulant activity.

MATERIALS AND METHODS

Sample collection and processing

Marine green algae *Ulva sp*) was collected from sea shore of Muttom, Kanyakumari district, Tamil Nadu. A matured, healthy sample was collected in a sterile polythene bag and immediately it was transferred into laboratory for further processing.

Salt, sand and epiphytes present on the surface of sample were removed by cleaning the sample with tap water. After cleaning, it was dried at room temperature and made fine powder using electric mixer grinder. Then the powdered sample was depigmented by using methanol.

Preparation of algal extracts

One gram of the ground algal powder was mixed with 50 ml of water and placed in shaking incubator for 12 hours at 70°C. The mixtures were centrifuged at 3500rpm for 20 min at 4°C and filtered with Whatman filter paper. Finally, the supernatant was preliminarily subjected for anticoagulant assay.

The extract was concentrated under reduced pressure. The concentrated samples were precipitated with acetone (1:4 v/v) dehydrated with acetone and dried at 40^0C. The dried product was dissolved in minimum distilled water, and lyophilized to obtain crude sulphated polysaccharide product. The Yield of crude sulphated polysaccharide was calculated by dry weight basis (Shanmugam *et al*., 2001).

Blood coagulation assay

Preparation of plasma: Human blood was collected from healthy volunteers and normal human plasma was prepared as follows: blood was anticoagulant using 3.8% tri sodium citrate solution and it was centrifuged immediately at 3000rpm for 15 minutes. The Plasma was separated and pooled. The pooled plasma was stored at 4^0C and it was used for further process.

Preparation of algal sulphated polysaccharide: The Algal sulphated polysaccharide sample was prepared in normal saline solution. 0.85 g of sodium

chloride was mixed in 100 ml of distilled water. From this solution 1 ml was used for Algal sulphated polysaccharide preparation (Yasantha Athukorala *et al*. 2001).

Anticoagulant activity: Anticoagulant activity of algal sulphated polysaccharide was tested using prothrombin time test with 750ug/ ml concentration. All clotting and control test were performed in duplicate, and average of the two duplicate was recorded. Standard blood anticoagulant heparin140.3 units/mg was used for comparative study. The results of the clotting test were expressed as a clotting time ratio. The ratio was obtained by dividing the clotting time achieved with algal sulphated polysaccharide included in the system by the time achieved under similar conditions with normal saline solution (Shanmugam *et al*., 2001).

Chemical analysis

The carbohydrates concentration was measured by phenol-sulphuric acid method (Krishnaveni *et al*., 1984) and total protein content was estimated using the protocol of Lowry *et al*., (1951). Then the sample was subjected to Fourier Transform Infrared Spectrophotometer (FTIR) analysis. The spectrum (400-4000 nm) was recorded using Attenuated Total Reflectance (ATR) technique beach measurement (Williams and Fleming, 1989).

RESULT AND DISCUSSION

In recent years, investigation of anticoagulant, thrombolytic, and antithrombic reagents from various sources has become a high priority in biomedical research because of the many disorders of blood clotting and fibrinolysis (Matsubara *et al*. 2000). In the present study, anticoagulant activity of the green algae *Ulva* species was screened by prothrombin test (Shanmugam *et al*., 2001).

Blood coagulation assay

All clotting and control test were performed in duplicate, and average of the two duplicate was recorded and heparin for comparative study also recorded. The results were mentioned in table 1.

Clotting time ratio = clotting time achieved with algal sulphated polysaccharide / clotting time achieved normal saline solution = 46/20 = **2.3**

Table: 1 Prothrombin time test

Test sample	Concentration of samples (ul)	Concentration of plasma (ul)	No. of test	Prothrombin time (min)	Average	PT time ratio
Control	-	200	1	8	8	-
			2	8		
Algal SPS	10	190	1	47	46	2.3
			2	45		
Heparin	10	190	1	>120	>120	>11
			2	>120		
Normal saline	10	190	1	20	20	-
			2	20		

The results of the clotting test were expressed as a clotting time ratio. The ratio was obtained by dividing the clotting time achieved with algal sulphated polysaccharide included in the system by the time achieved under similar conditions with normal saline solution

Chemical analyses

The sulfated polysaccharides extracted from *Ulva sp.* have high carbohydrate contents (24.10%) than total protein (4.82%). The sulfated polysaccharides mainly are composed of rhamnose (Wenjun Mao *et al.*, 2006). In FTIR analysis, there are 23 functional ions/groups were detected between 400-4000 nm spectra (Fig. 1).

The two peaks seem to be obvious basic knowledge in the sulphate group detection, the absorption bands can be observed at 3434.37 cm^{-1} (ascribed to O-H stretching), 2925.15 cm^{-1} (C-H stretching), 1632.80 and 1099.46 cm^{-1} (symmetrical and asymmetrical stretching of S-O in SO_3 heparin groups, respectively), 1100-1000 cm^{-1} (Si-O-Si stretching) and at 850 cm^{-1} (=C-H bending). However, observations of the type described can be invaluable in determining the presence of specific functional groups when used in combination with the infrared spectrum.

Similarly, the use of negative spectral curves, viz. the absence of a characteristic group frequency, is equally important. For example, if extracted polysaccharide compound have honey like viscosity and color. In spectra it have a strong peak in between 1200 and 1000 cm-1, if the compound is more sharp in its curve it maybe a sulphate compound or benzaldehyde. If an aldehydic carbonyl group is absent, then the compound probably contains a sulphate group. In context with heparin compound detection the values are the absorptions at 1023.27 and 1124.54 cm^{-1} are considered as a fingerprint of heparin. Therefore, the presence of the absorptions recognized to sulfur-containing groups in the spectrum denotes the preservation of the heparin structure.

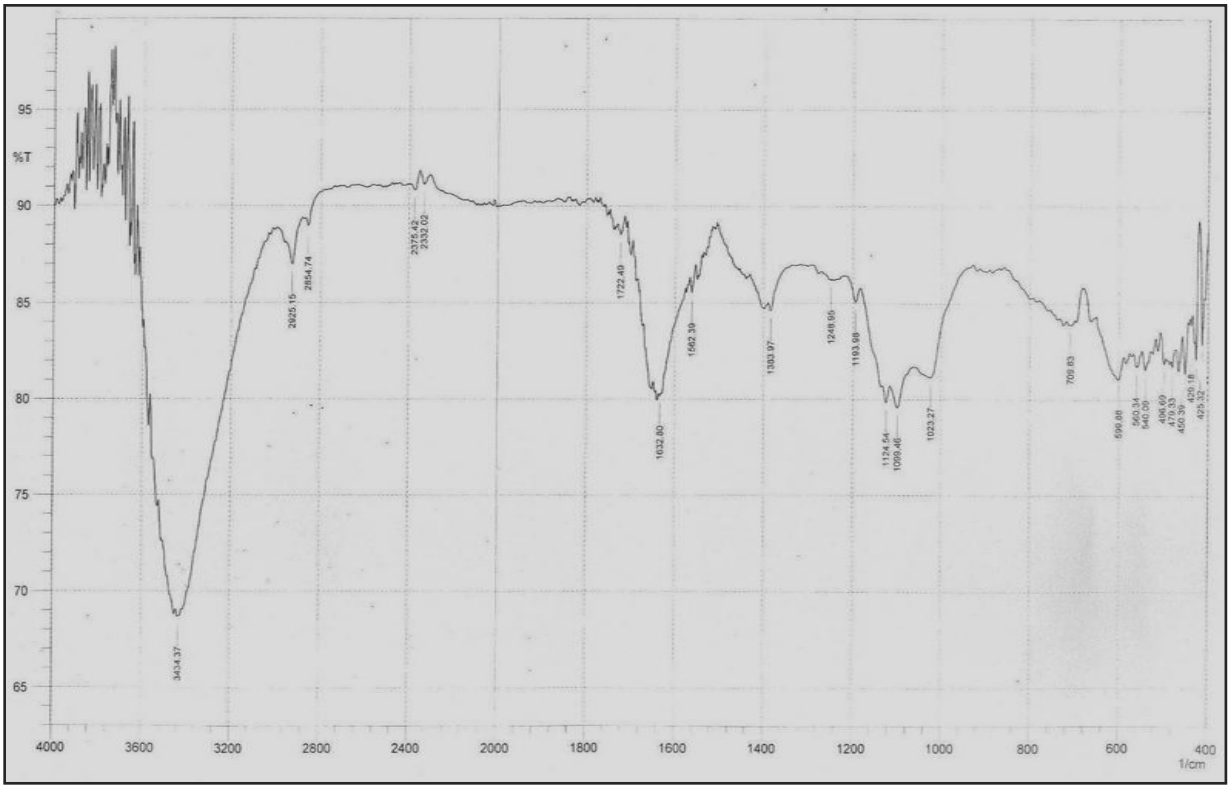

Fig: 1 FTIR chromatogram of Algal extract

Spectrum is of a film deposited from a heparin/acetone mixture. Owing to the presence of OH groups in the monomer and therefore probably also in the film, a band at around 3434 cm^{-1} is expected, but is in medium peak. Two peaks, at 2925.15, 2854.74, confirm the presence of CH groups. The band centered around

1632.80cm^{-1} is indicating the presence of C = O groups. These interpretations are reliable with the features of infrared spectra of films produced. Bands characteristic of heparin (at 1250 and 1020 cm^{-1}) are very little amount, possibly representing poor preservation of the heparin in the deposited material.

Most of green algae exert their anticoagulant activity through polysaccharide, or proteoglycan. In many cases, arabinose and its sulphate derivatives elicit anticoagulant activity of green algae. The anticoagulant activity of most green seaweeds are due to sulphated arabinan, however, sulphated arabinogalactan also have been reported as possible anticoagulant compound in green algae. Therefore, arabinan and sulphate groups of the isolated compounds play a crucial role in prolonging anticoagulant activity of these algal species. As a conclusion, this study prevailed use of Algal biomass for the preparation of medicinally valuable products.

REFERENCES

Albuquerque, I. R. L., K. C. S. Queiroz, L. G. Alves, E. A. Santos, E. L. Leite, and H. A. O. Rocha (2004) Heterofucans from Dictyota menstrualis have anticoagulant activity. Brazillian Journal of Medical and Biological Research, 37:167–171.

Bjork I, Lindahl U (1982) "Mechanism of the anticoagulant action of heparin". *Mol. Cell. Biochem.* 48 (3): 161–18.

Cavalcante, M.C.M., Allodi, S., Valente, A.-P., Straus, A.H., Takahashi, H.K., Mour~ao, P.A.S., Pav~ao, M.S.G (2000) Occurrence of heparin in the invertebrate Styela plicata (Tunicata) is restricted to cell layers facing the outside environment. J. Biol. Chem. 275: 36189–36196.

Farias, W. R. L., A. P. Valente, M. S. Pereira & P. A. S. Mourao (2000) Structure and anticoagulant activity of sulfated galactans. Journal Biological Chemistry 275: 29299-29307.

Killing, H., (1913) Zur biochemie der meersalgen. Z. Physiol. Chem. 83:171–197.

Krishnaveni, S. Theymoli Balasubramanian and Sadasivam, S (1984) Food Chem., 15:229.

Lowry, O.H., Farr, A.L., Randall, R.J. & Rosebrough, N.J. (1951) Protein measurement with the Folin Reagent. J Biol Chem., 193: 265-275.

Matsubara, K., Y. Matsuura, K. Hori, and K. Miyazawa (2000) An anticoagulant proteoglycan from the marine green alga, Codium pugniformis. Journal of Applied Phycology 12:9–14.

McLellan, D. S. and K. M. Jurd. (1992) Anticoagulants from marine algae. Blood Coagulation and Fibrinolysis 3:69–77.

Mourão, P. A. S., M. S. Pereira, M. S. G. Pavão, B. Mulloy, D. M. Tollefsen, M. C. Mowinckel and U. Abilgaard (1996) Structure and anticoagulant activity of a fucosylated chondroitin sulfate from echinoderm. Journal of Biological Chemistry, 271: 23973-23984.

Pavão, M. S. G., K. R. M. Aiello, C. C. Werneck, L. C. F. Silva, A. P. Valente, B. Mulloy, N. S. Colwell, D. M. Tollefsen & P. A. S. Mourão. (1998) Highly sulfated dermatan sulfates from ascidians. Journal of Biological Chemistry 273: 27848-27857.

Shanmugam M., K.H. Mody, R.M. Oza and B.K. Ramavat (2001) Blood coagulant activity of a green marine algae Codium dawarkense in reaction to its growth stage. Indian Journal of Marine sciences. 30: 49-52.

Shanmugam M, Mody KH, Siddhanta AK (2001) Blood anticoagulant sulphated polysaccharides of the marine green algae *Codium dwarkense* (Boergs.) and C. tomentosum (Huds.) Stackh. Indian J Exp Biol. 39(4):365-70.

Shanmugam, S., Mody, K.H., (2000) Heparonid active sulphated polysaccharides from marine algae as potential blood coagulant agents. Curr. Sci. 79: 1672–1683.

Srivastava, R. and D. K. Kulshreshtha. (1989) Bioactive polysaccharides from plants. Phytochemistry. 28:2877–2883.

Vieira, R. P., B. Mulloy and P. A. S. Mourão (1991) Structure of a fucose-branched chondroitin sulfate from sea cucumber. Journal of Biological Chemistry 266: 13530-13536.

Wenjun Mao, Xiaoxue Zang, Yi Li2 & Huijuan Zhang (2006) Sulfated polysaccharides from marine green algae *Ulva conglobata* and their anticoagulant activity Journal of Applied Phycology, 18: 9–14.

Williams, D.H. and I. Fleming, (1989) Spectroscopic Methods in Organic

Chemistry. McGraw-Hill Book Co., New York.

Yasantha Athukorala A, Ki-Wan Lee A, Se-Kwon Kim B, You-Jin Jeon A (2006) Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. Bioresource technology (Online-www.sciencedirect.com).

Harada N, Maeda M (1998) Chemical structure of antithrombinactive rhamnan sulfate from Monostrom nitidum. Biosci. Biotech. Biochem. 62: 1647–1652.