



Research Paper

## **EX VIVO ANTICOAGULANT ACTIVITY OF THE POLYSACCHARIDE ISOLATED FROM *ULVA FASCIATA***

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Hot water soluble polysaccharide was extracted from *Ulva fasciata*. The isolated polysaccharide was purified using gel filtration chromatography on Sephacryl S-400. The chemical composition of the polysaccharide revealed the presence of sulphate. The anticoagulant activity of the purified polysaccharide was studied by prolongation of APTT *Ex Vivo* using rat model. The sulphated polysaccharide prolonged the APTT in a dose dependent manner and can be developed as a promising anticoagulant agent.

**Keywords:** *Ulva fasciata*, sulphated polysaccharide, anticoagulant activity

### **INTRODUCTION**

The cosmopolitan genus *Ulva*, commonly known as the “sea lettuce”, is represented by species distributed in all oceans and estuaries of the world (Guiry and Guiry, 2008). *Ulva* is rich in cell-wall polysaccharides, including cellulose and water-soluble polysaccharides that contain sulphate groups. The main type of water-soluble polysaccharide is ulvan, the main component of which is a disaccharide formed by  $\beta$ -D-glucuronic acid (1, 4)-L-rhamnose 3 sulphate (Lahaye, 1998; Paradossi *et al.*, 1999).

Anticoagulants have been used widely for blood treatment during dialysis and surgery, as medication of disseminated intravascular

coagulation and thrombosis in various diseases, and for blood testing *in vitro* (Tamada, 2004). Heparin preparations are widely used for the treatment and prevention of arterial and venous thrombosis (Fareed *et al.*, 2000). However, this glycosaminoglycan has several limitations due to collateral effects and limited source of material (Mouřao, 2004). So an alternative natural source for anticoagulant is needed. Various anti-coagulant-active polysaccharides, especially from marine red and brown algae, have been isolated and characterized. They contain a variety of sulphated galactans and sulphated fucans and exhibit high anticoagulant activity. However, there are fewer reports of anticoagulant-active

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polysaccharides from marine green algae than those from brown and red algae. Polysaccharides from marine green algae show potent anticoagulant activity, and represent potential source to be explored (Jurd *et al.*, 1995). The present study was designed to isolate and purify polysaccharide from *Ulva fasciata* and to check the anticoagulant activity *ex vivo*.

## MATERIALS AND METHODS

### Plant Material and Reagents

Seaweed specimens were collected from the intertidal and subtidal habitat of Kollam prefecture (Thirumullavaram, Kerala, India) located on the southwest coast. The algal material was identified as *Ulva fasciata*. The seaweeds were washed thoroughly with tap water, dried in air and powdered. The powdered sample was defatted and depigmented by sequential extraction with petroleum ether, chloroform and acetone as solvents in a Soxhlet apparatus. All solvents and chemicals used for the study were of analytical grade and purchased from SRL chemical.

### Extraction and Purification

Powdered algal sample (40 g) was stirred with 800 ml of distilled water for 3 h. The temperature of the extraction was adjusted as 90 °C. Separation of the residue from the aqueous extract was performed by centrifugation at 8000 ×g for 15 min. The pellet was re-extracted in a similar way. The supernatants were combined and dialysed extensively against water. The polysaccharides were precipitated with twice the volume of 95% alcohol. The precipitate was collected by centrifugation at 10000 ×g for 20 min. The collected precipitate was washed with absolute alcohol and lyophilised. The precipitate obtained by the extraction procedure was re-dissolved in water and the protein content was

removed by Sevag method (Sevag, 1934). The aqueous phase was recovered and dialysed against distilled water. The polysaccharides were recovered by precipitation with absolute ethanol. The collected precipitate was washed and lyophilised. Partially purified polysaccharide was further purified by gel filtration chromatography on Sephacryl S-400. The fractions were collected and analysed by Phenol-sulphuric acid method (Dubois *et al.*, 1956). The purified fractions were pooled and polysaccharides were reprecipitated with absolute ethanol and the fraction with high carbohydrate content (UPF 1) was lyophilised and used for further experiments.

### Composition Analysis

Moisture and ash contents of the polysaccharide sample were determined by drying at 120 °C for 2 h and igniting at 550 °C for 6 h. Total sugar content was determined by phenol sulphuric acid method (Dubois *et al.*, 1956) and sulphate content was estimated by turbidimetric method (Dogson, 1961).

### Anticoagulant Action of Polysaccharide Fraction *Ex Vivo*

Male Sprague-Dawley rats weighing 200–220 g were given polysaccharide at three different concentrations (20, 50 and 100 µg/kg b.wt.). PBS was used as a control and administered through intravenous route. After 60 min animals were anesthetized by intramuscular injection of 100 mg/kg b.wt. of ketamine and 16 mg/kg body weight of xylazine. The caudal caval vein was exposed by midline incision and 1.8 mL of blood was collected into a plastic syringe containing 0.2 mL of 100 mM citrate buffer, pH 4.5. The sample is immediately agitated and then subjected to centrifugation in a plastic tube at 1500xg for 10 min, after which plasma was collected and

transferred to a clean plastic tube. Coagulation tests for APTT were performed.

## STATISTICS

All results were expressed as Mean  $\pm$  SD. One way ANOVA followed by Dunnett test was performed using statistical software INSTAT. A value of  $P < 0.05$  was considered significant.

## RESULTS AND DISCUSSION

### Polysaccharide Extraction and Purification

The polysaccharide was extracted with hot water and the yield was found to be 23 % of algal dry weight. Partially purified polysaccharide was further purified by gel filtration chromatography with hot water as the mobile phase (Shonima et al., 2012).

### Chemical Composition of the Polysaccharide

The chemical composition of the polysaccharide was analysed and was given in Table 1.

Composition	Weight %
Moisture	17.3
Ash	20.40
Total carbohydrates	48.60
Sulphate	17.50

### Ex Vivo Anticoagulant Action

*Ex vivo* anticoagulant action was studied and the results are given in Table 2. The polysaccharide in various concentrations were administered in rats and after 5 min the blood was drawn and APTT was determined. Prolongation of APTT was observed in a concentration dependent manner.

Groups	Dose ( $\mu\text{g/mL}$ )	APTT (s)
Vehicle (normal saline)		17.8 $\pm$ .11
PSU	25	22.3 $\pm$ .20**
	50	29.7 $\pm$ .14**
	100	38.3 $\pm$ 19**
Heparin	50	43 $\pm$ 1.4**

Note: Values are mean  $\pm$  SD for six animals. Statistical significance were analysed by One way ANOVA with Dunnett test. The significance compared to control values is denoted by asterisks where  $p < 0.05$  versus control.

## CONCLUSION

The polysaccharide isolated from *Ulva fasciata* was found to be a sulphated polysaccharide. The *ex vivo* anticoagulant activity assay showed that the polysaccharide exhibited potent anticoagulant activity in a dose dependent manner and so it can be developed as a promising anticoagulant for various thrombotic diseases.

## ACKNOWLEDGMENT

The authors would like to thank Dr. M.V.N Panicker for seaweed identification. We express gratitude to Dr. Joby George and Dr. Raveendran (Veterinary doctors, District Veterinary Hospital, Kodimatha, Kottayam) for helping us with the animal experiments. The financial support of Mahatma Gandhi University, Kottayam, Kerala, India is gratefully acknowledged.

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