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Purification of novel polysaccharide having anticoagulant properties from fermented red seaweed *Lomentaria catenata*

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Introduction

The necessity of natural sources of anticoagulant compounds has been arisenfrom long time as various side effects were detected from the synthetic drugs. Researches have drawn their attention to isolate bioactive compounds from last 60 years and as a result, several red, brown and green algae are reported as rich sources of anticoagulant, antiproliferative, antiviral and antitumer compounds. Further more these active compounds have been isolated as carbohydrate polymers having sulfate motifs especially fucoidans and sulfated galactans present in brown algae and red algae respectively (Pereira *et al.* 1999).

We isolated anticoagulant activity from *Lomentaria catenata* red seaweed, which is not recognized as possessing anticoagulant activity.

Materials and methods

Instead of performing expensive extraction methods like acid, base and enzymatic hydrolysis, we underwent natural fermentation to extract active anticoagulant compound from seaweed. The freeze dried seaweed was subjected to natural fermentation with non-specific microorganisms for 10 weeks and anticoagulant activity was monitored by activated partial thromboplastin time (APTT) test, prothrombin time (PT) test and thromboplastin time (TT) test. When the fermented algal mixture approached to its maximum anticoagulant activity, the soluble polysaccharides were extracted by ethanol precipitation and freeze dried. The active compound was purified by ion exchange chromatography followed by the gel filtration chromatography. Purity and the molecular mass of the purified polysaccharide were determined by agarose gel electrophoresis and polyacrylamide gel electrophoresis respectively.

Results and summery

After the 4th week, the fermentation mixture of *L. catenata* reaches to its maximum anticoagulant activity >1000 sec, 21.6 sec and >1000 sec for APTT, PT and TT assays respectively. The blood coagulation system can be divided in to two pathways as intrinsic (APTT and TT assays) and extrinsic (PT and TT assays) where a series of factors influenced on (Joachim *et al.*, 2002). The results reveal that the isolated polysaccharide influences both pathways of the blood coagulation system. The molecular weight of the purified carbohydrate polymer is around 500 kDa and the predominant monosaccharide is galactose (28.46%).

Reference

Joachim A., Ali S., Daugschies A. (2002). Fasciola hepatica alters coagulation parameters in sheep plasma in vivo and in vitro. *Parasitology Research.* 89: 53 - 58

Pereira M. S., Barbara M., and Paulo A.S. (1999). Structure and anticoagulant activity of sulfated fucans. *American society for Biochemistry and Molecular Biology.* 274. 7656 - 7667.