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Anticoagulant activity of sulfated polysaccharide isolated from fermented brown seaweed *Sargassum fulvellum*

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Introduction

Sulfated polysaccharides are one of the important bioactive compounds present in marine algae as well as great variety of other organisms like sea urchins. In marine algae, they present as sulfated fucose (fucoidans) and sulfated galactans. Sulfated polysaccharides from marine algae have been demonstrated to have many biological activities such as anticoagulant, antioxidant, antiviral and antihypetention (Painter, 1983).Different extraction techniques have been used to isolate sulfated polysaccharide from seaweeds such as ethanol, hot and cold-water extraction, enzymatic digestion, proteolytic digestion, acid hydrolysis or combination of above treatments.These methods have several limitations such as toxicity effects, high cost, and complex procedure. The purpose of this study was to isolate and characterize sulfated polysaccharide as an anticoagulant from the edible brown seaweed *Sargassum fulvellum* by means of the simple fermentation process and chromatography technique.

Materials and methods

Freeze dried S. fulvellum was dispensed in the glass bottles containing 300 ml of water with 45 g sugar and incubated at 25 ℃ for 10 weeks in order to process fermentation.Total dry matter yield, concentration of sulfated polysaccharide denoted as (SP) and coagulation time (APTT) of the crude seaweed extract (SWE) were measured in every two weeks intervals. The eight-week fermented crude SWE exhibited highest clotting time in the APTT assay. Therefore, this crude SWE was purified by a combination of ion exchange chromatography on DEAE-cellulose and Sepharose 4B gel permeation chromatography.Sulfate, polysaccharide concentration and pH of the purified ASP were measured. APPT assay was carried out for concentration series of purified anticoagulant sulfated polysaccharide (ASP) and

heparin concentrations as standard reference to compare the specific coagulation performance of ASP. The purity and the average molecular mass of the isolated ASP were determined by 0.5% agarose and 6% polyacrylamide (PAGE) gel electrophoresis respectively.

Results and summery

The highest prolongation of APTT (202 s) was observed from crude SWE after the 8th week of fermentation.Therefore, optimum fermentation period was considered as 8 week for this experiment since at that time the highest stable sulfated polysaccharide available for the purification of anticoagulant.The isolated anticoagulant sulfated polysaccharide (ASP) showed single spot on agarose gel electrophoresis, which confirmed the purification status of sulfated polysaccharide. PAGE analysis determined the molecular mass of the purified ASP between 8-20 kDa. Polysaccharide and sulfate concentrations of the purified ASP were 180 and 29.70 µg/ ml respectively with the 1.32% (w/w) polysaccharide recovery from crude polysaccharide applied to DEAE. Purified ASP showed 3.86-pH value and considered as acidic polysaccharide. Moreover, comparison of ASP with heparin showed same relative clotting factor (27.47) at the concentrations of 180 and 60 µg/ml respectively. Therefore, our potent ASP has to be considered as weaker anticoagulant than heparin. Results of the APTT, PT, and TT clotting assays showed that ASP was able to inhibit both intrinsic and extrinsic pathways of blood coagulation cascade. Finally, this study established a feasible and simple experimental protocol to isolate anticoagulant from fermented seaweeds.

References

Painter T.J (1983) Algal polysaccharides. The polysaccharide. Vol 2. Academic Press, New York.pp195-285.