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**Purification and characterization of *Pleurotus sajor-caju* (Fr.)  
*Sing* gluco-oligosaccharide (*Ps*-GOS) obtained from *Hevea*  $\beta$ -  
1,3-glucanases digestion of insoluble  $\beta$ -Glucans**

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**ABSTRACT**

The objective of this study was to purify and characterize of *Pleurotus sajor-caju* (Fr.) *Sing* gluco-oligosaccharide (*Ps*-GOS) obtained from the digestion of its insoluble  $\beta$ -glucan with  $\beta$ -1, 3- glucanase derived from concentrated *Hevea* latex serum. A short-chains and water soluble *Ps*-GOS was characterized, with LC-MS and IR, and confirmed to be a mixture of  $\beta$ -1, 3/1, 6-glucan. Further purification of *Ps*-GOS with anion -exchange (DEAE-cellulose) and size-exclusion (Bio-Gel P10) chromatography were applied and obtain a various size of purified gluco-oligosaccharide with a molecular weight range from 1.5 to 20 kDa. In addition, our preliminary study was demonstrated that *Ps*-GOS could inhibit tumor growth and metastasis of human tumor, CaSki and B16F1 cell, xenografts in immunodeficient mice. Moreover, the immunohistochemistry study indicated that the level of inflammatory activity after mice were treated with *Ps*-GOS was decreased. Nevertheless, to further understand the anti-inflammatory mechanism, we will investigate its anti-inflammatory activities of purified *Ps*-GOS in lipopolysaccharide- induced RAW264.7 macrophage.

**Keywords:** Anti-inflammation,  $\beta$ -Glucan, *Hevea* glucanase, RAW264.7  
Macrophage.

## INTRODUCTION

Inflammation is the body's immune response against invading pathogens. Macrophages recognize bacteria or other pathogens by binding to LPS or specific components of pathogens that lead to stimulate macrophage activation to produce and secrete proinflammatory cytokines such as tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin 6 (IL-6) and nitric oxide (NO), prostaglandin E2 (PGE2) (Zhang *et al.*, 2011; Szliszka *et al.*, 2011; Lorsbach *et al.*, 1993; Snyder and Brecht, 1992; Posadas *et al.*, 2000)

However, prolonged secretion of high levels of inflammatory cytokines from macrophages is leading to chronic inflammation and then causes various chronic diseases such as cancer. Hence, inhibition of macrophage activation is beneficial to prevent chronic inflammation that leads to many diseases (Aktan, 2004; Guzik *et al.*, 2003; Van der Vliet *et al.*, 2000)

Many studies have been demonstrated that oligo- and polysaccharides had immunomodulatory and various therapeutic activities (Chen and Yan, 2005; Yoon *et al.*, 2007). Therefore, we investigated the anti-inflammatory activities of small size and water-soluble *Pleurotus sajor-caju* (Fr.) Sing glucan oligosaccharide (*Ps*-GOS), that obtained from degradation of insoluble  $\beta$ -1,3-glucan by  $\beta$ -1,3-glucanase from *Hevea* latex enzyme. In addition, our preliminary result from human tumor xenografts in immunodeficient mice indicated that *Ps*-GOS inhibit the growth and metastasis of CaSki cancer and melanoma cell lines B16F1, respectively (data not shown). Together, the result from immunohistochemistry also indicated that the level of inflammatory activity was decreased in mice that treated with *Ps*-GOS. Hence, to understand the inflammatory inhibition mechanism of *Ps*-GOS, the objective of this study was, therefore, to purify and characterize the purified *Ps*-GOS. In addition, its anti-inflammatory activities in lipopolysaccharide-induced RAW264.7 macrophages of the purified *Ps*-GOS will be further investigated.

## MATERIAL AND METHODS

### Materials

Dried grey oyster mushrooms (*Pleurotus sajor-caju* (Fr.) Sing) were purchased from local market. DEAE-Cellulose was purchased from Sigma and Bio-Gel<sup>®</sup> P-10 from Bio-Rad. All other chemicals used were of high-quality analytical grade.

### **Extraction of insoluble $\beta$ -glucan**

Dried grey oyster mushrooms (50 g) were boiled for 2 hrs, followed by centrifugation. Residue was stirred in 1M NaOH at 130 °C for 10 hrs and 2% acetic acid at 100 °C for 8 hrs, respectively. Then, washed residue with ethanol and residue was dried by vacuum oven. The  $\beta$ -glucan assay was conducted according to the method of McCleary and Glennie-Holmes (1985), using the Mushroom and Yeast  $\beta$ -glucan kit (K- YBGL) (Megazyme International Ireland Ltd., Wicklow, Ire-land).

### **$\beta$ -1, 3-glucanase isolation from fresh latex**

The protocol for  $\beta$ -1,3-glucanase preparation was modified following the research of Churngchow *et al.*, 1995. Briefly, the latex was collected into a container chilled in ice, filtered through cheese-cloth and centrifuged. It was separated into 3 parts: a top layer of rubber particles, a clear serum (C-serum) and a pellet or bottom fraction containing the lutoids (Moir, 1959). Later, the bottom fraction was frozen and thawed to obtain soluble proteins consisted in the lutoids (B-serum. Finally, lutoid membranes were separated from B-serum by centrifuging. Approximate five liters of B-serum (5.12 mg/ml of protein) was further concentrated 10-fold by ultrafiltration (Pellicon XL Biomax 10 kDa), operating under 20 psi at room temperature. The concentrate enzyme fraction (19.48 mg/ml of protein), containing the hydrolytic enzymes, especially  $\beta$ -1,3-glucanase, left in the retentate was collected and used as the starting material in the soluble  $\beta$ -glucan preparation.

### **Digestion of insoluble $\beta$ -glucan**

Insoluble  $\beta$ -glucan (2.63±0.99 g) was stirred in *Hevea* glucanase at 45 °C for 3 weeks, followed by centrifugation. The supernatant was filtered through ultrafiltration membrane (molecular weight cutoff 10 kDa) and the polysaccharide smaller than 10 kDa (permeate), was called *Ps*-GOS, was precipitated with cold ethanol and dried by freeze-dryer (0.74±0.001 g).

### **Purification of *Ps*-GOS**

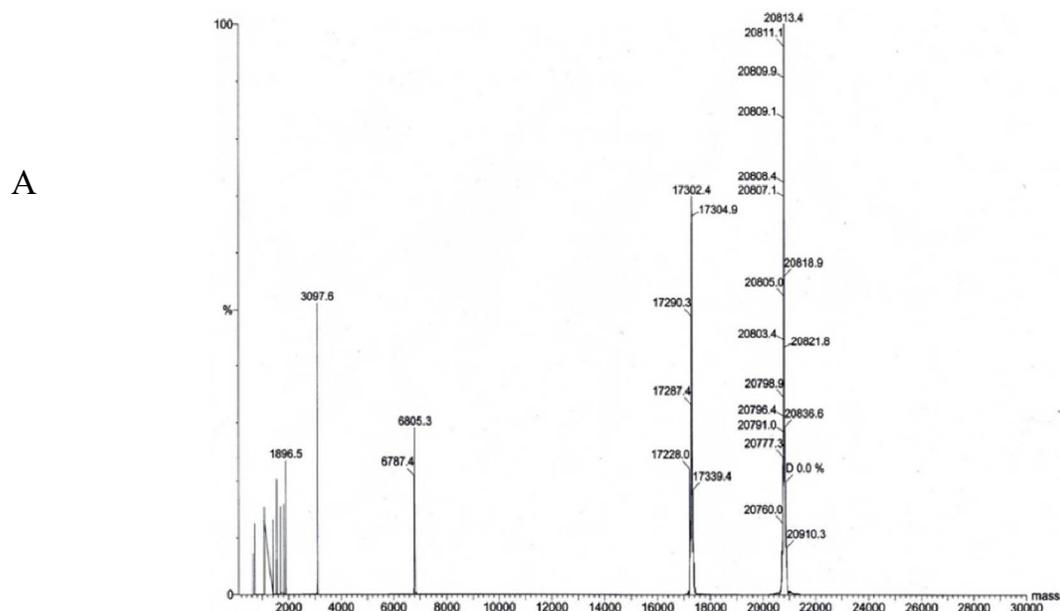
Crude *Ps*-GOS (100 mg) was dissolved in distilled water (1 ml), followed by centrifugation and supernatant was loaded on the DEAE-Cellulose column (3cm×60cm) and eluted with distilled water, then 0.2, 0.5 and 1 M NaCl solution at a flow rate of 0.8 ml/min, 1 ml each fractions. The polysaccharide was detected by the phenol-sulfuric acid method and a UV detector at 490 nm, respectively. The unbound fraction, was called partial purified *Ps*-GOS, was dried by freeze dryer. Partial purified *Ps*-GOS (100

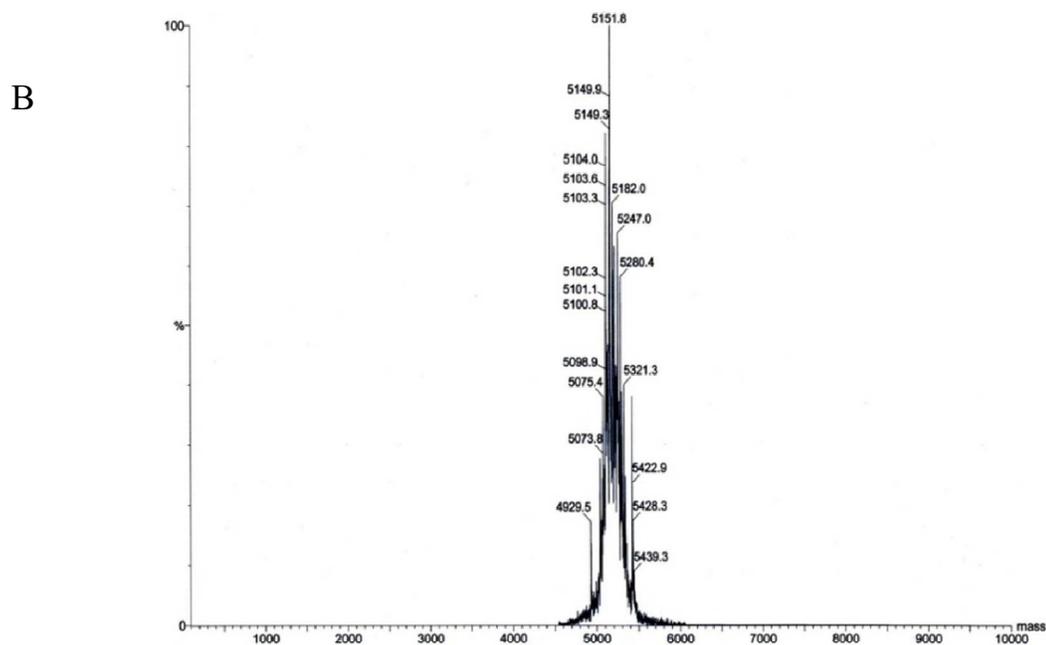
mg) was dissolved in 1 ml of distilled water, followed by centrifugation and supernatant (500µl) was loaded on the Bio-Gel P-10 column (2cm×60cm) and eluted with distilled water at a flow rate of 0.8 ml/min, 1 ml each fractions. The polysaccharide was detected by the phenol-sulfuric acid method and a UV detector 490 nm, respectively. The obtained polysaccharide was dried by freeze dryer. However, anti-inflammatory activity of purified *Ps*-GOS in lipopolysaccharide-induced RAW264.7 macrophage will be further investigated.

## RESULTS

### Small size and water-soluble gluco-oligosaccharide (*Ps*-GOS) was obtained from *Hevea* glucanase digestion.

The result obtained from ESI-MS indicated that the size of insoluble  $\beta$ -glucan was reduced from 20813 Da to 5151 Da (Figure 1A and B) after digestion, a percentage of yield about 35.55. In addition, the water solubility of *Ps*-GOS was increased when compare to the substrate, insoluble  $\beta$ -glucan. *Ps*-GOS was further purified by chromatography techniques.

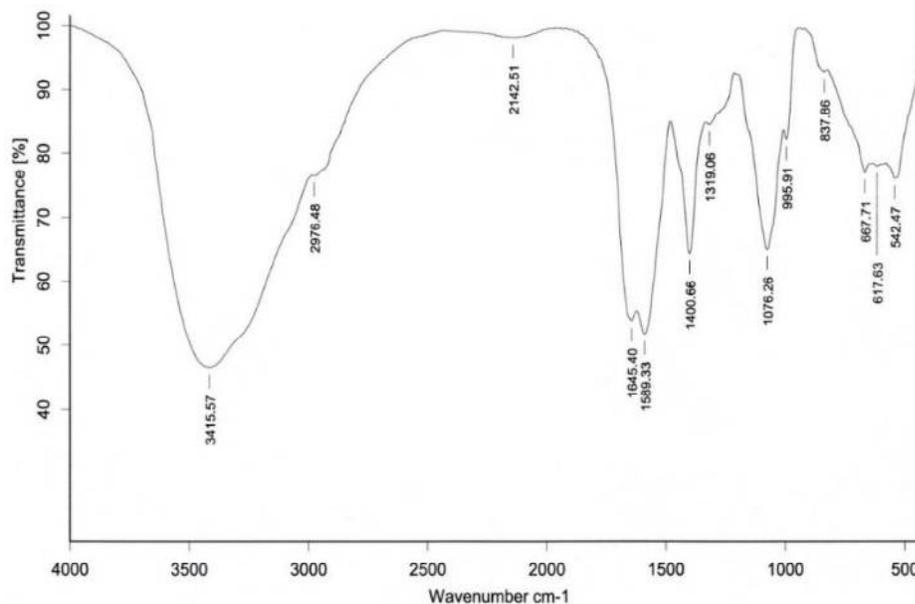




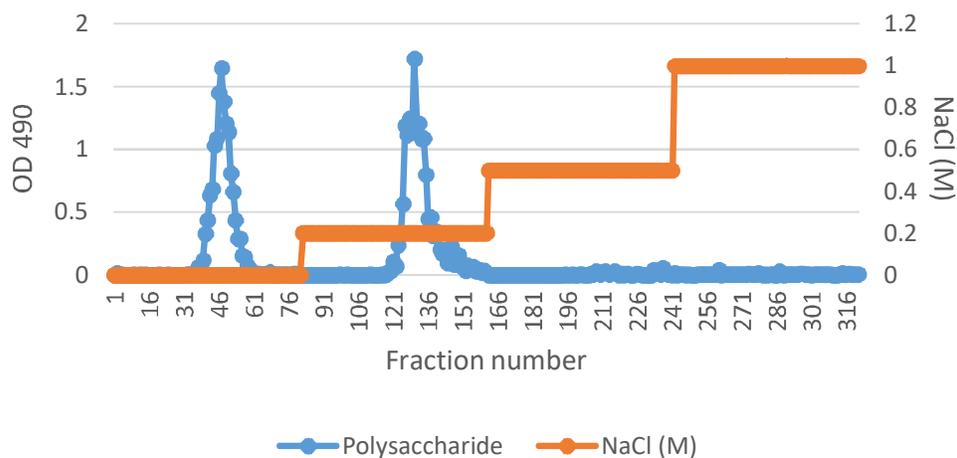
**Figure 1.** ESI-MS spectra of the insoluble  $\beta$ -glucan *P. sajor-caju*. (A) and *Ps*-GOS (B) obtained from enzymatic digestion of insoluble  $\beta$ -glucan *P. sajor-caju*.

### Purification of crude *Ps*-GOS with chromatography techniques

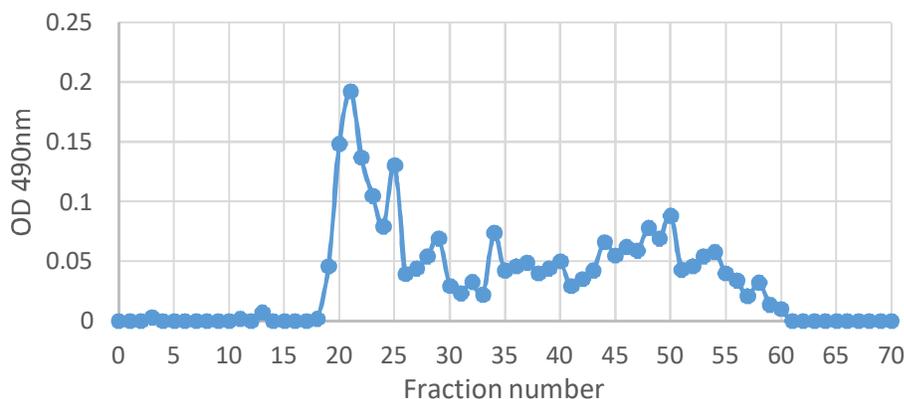
Result from anion-exchange chromatography shown that crude *Ps*-GOS exhibit a 2 types of polysaccharides, bound and un-bound fractions (Figure 3A). Unbound fraction was eluted with distilled water and it was called as a partial purified *Ps*-GOS, which FT-IR spectrum exhibit the peak wavenumber ( $\text{cm}^{-1}$ ) at 995.91 and 1076.26 as  $\beta$ -(1,6)-glucan and  $\beta$ -(1,3)-glucan, respectively (Figure 2). Whereas, bound fraction, which was identified as a contaminants from *Hevea* latex serum (data not show), was eluted with 0.2 M NaCl solution. In addition, the range of molecular weight of partial purified *Ps*-GOS was vary from 1.5 kDa to 20 kDa after purification with gel filtration chromatography (Figure 3B).



**Figure 2.** FT-IR spectrum of *Ps*-GOS obtained from enzymatic digestion of the insoluble  $\beta$ -glucan *P. sajo*-*caju*.



**Figure 3A.** Elution profile of crude *Ps*-GOS on a DEAE-Cellulose column



**Figure 3B.** Elution profile of partial purified *Ps*-GOS on a Bio-Gel P-10 column

## CONCLUSION

This study demonstrated that *Ps*-GOS derived from *Hevea* glucanase digestion of insoluble  $\beta$ -glucan *P. sajor-caju* was water soluble and its size was decreased from 20813 Da to 5151 Da. The partial purified *Ps*-GOS, which is unbound fraction obtained from anion-exchange chromatography, shown a various size of gluco-oligosacchride (its molecular weight range from 1.5 kDa to 20 kDa) when subjected to Bio-Gel P-10 column, respectively. However, further investigation of anti-inflammation in lipopolysaccharide-induced RAW264.7 macrophage is needed to be done.

## ACKNOWLEDGEMENTS

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