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Extraction optimization, preliminary characterization and immunological activity of polysaccharides from figs

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ABSTRACT

Complex enzyme extraction (CEE), purification, characterization of fig polysaccharides (FPs) from dried figs were investigated. Orthogonal experiment was used to optimize the concentration of cellulose, pectinase and papain. Response surface methodology (RSM) was employed to optimize extraction conditions. The optimum extraction conditions were: enzyme concentration of 1.5%, 1.5%, 0.5% (wt%) of pectinase, papain, cellulose, ratio of water to raw material 40.3 mL/g, extraction time 54.1 min, temperature 34.15 °C and pH 3.8. Under these conditions, the experimental yield was 7.98 ± 0.17%. Two homogeneous heteropolysaccharides (FPs-1-1, FPs-2-1) were purified by DEAE-Sepharose and Sephadex G-200 chromatography, which were composed of rhamnose, arabinose, xylose, mannose, glucose and galactose with molecular weight of 1.52×10^6 and 4.75×10^5 Da, respectively. The bioactivity assay showed that FPs-1-1 and FPs-2-1 could more significantly enhance splenic lymphocyte proliferation, phagocytosis and NO production of macrophages, could be explored as potential immunopotentiating agent for use in functional food or medicine.

1. Introduction

The polysaccharides isolated from plants, animals, fungi, yeasts, algae and lichens, have attracted more attention recently in the biochemical and medical areas due to their immunomodulatory [1], anti-cancer [2], anti-oxidation [3], anti-inflammatory [4], etc. Many polysaccharides exhibit superior immunomodulatory activities with no significant side effects and low toxicity could be ideal immunomodulator agents to prevent and treat cancer [5]. The traditional extraction method of polysaccharides from plant tissues is heat reflux extraction (HRE). HRE requires long time, high temperature and high energy consumption with low extraction yield. Moreover, high temperature and long extraction time might lead to the degradation of polysaccharides and the decrease of the pharmacological activities of polysaccharides [6]. Therefore, an ideal extraction method should be capable of producing high quantities of the polysaccharide and be non-destructive with a shorter extraction time. In recent years, various novel extraction techniques have been explored for the extraction of polysaccharide from plant, including enzyme-assisted extraction [7], ultrasound-assisted extraction [8], microwave-assisted extraction [9], etc.

RSM is an effective statistical technique for optimizing complex processes, because it allows more efficient and easier arrangement and interpretation of experiments compared to other approaches [10]. At the same time, it is less the number of experiments which are required to evaluate multiple parameters and their interactions. It is widely used in optimizing the extraction process variables of bioactive components, such as polysaccharides, flavonoids, anthocyanins, phenolics, etc. [11–13].

Fig. (*Ficus carica* L.) is a sort of deciduous tree which belongs to the moraceae family. Its fruit is generally referred as figs which have been used as food and medicine for several centuries [14]. The figs are rich in polysaccharides [15], phenols [16] and flavonoids, etc. [17], which have antioxidant and immunity activity [18].

In this study, the concentration of complex enzyme was optimized using an orthogonal experiment, the CEE parameters were further investigated and optimized using a three-level, four-variable Box-Behnken design (BBD). Two polysaccharides fractions from FPs were purified, and preliminary characterizations were carried out. The immunological activities of two fractions were also evaluated. The objective of the work is to establish novel extraction methods with higher extraction yields and bioactivities

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for development and application of the polysaccharide resource used in food or medicine.

2. Materials and methods

2.1. Materials

Dried figs were purchased from market located in Jilin, China (crush and degrease before use). Papain (activity: 500 U/mg), pectase (activity: 6 U/mg), cellulase (activity: 15 U/mg) were purchased from Sinopharm Chemical Reagent Co. Ltd. Concanavalin A (ConA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), lipopolysaccharide (LPS), sulfanilamide, N-(1-naphthyl) ethylenediamine dihydrochloride, L-Arabinose (Ara), D-glucose (Glc), L-rhamnose (Rha), D-galactose (Gal), D-mannose (Man), D-xylose (Xyl), D-glucuronic acid (GlcA), Dextran standards (T-50, T-100, T-500, T-1000, T-2000) were purchased from Sigma Chemical Co. (St. Louis, MO, USA.). DEAE-Sepharose and Sephadex G-200 resin were purchased from Amersham Biosciences Co. RPMI-1640 medium was purchased from Gibco Invitrogen Co. Bovine serum albumin (BSA) was purchased from Kaiyang Biochemistry Co. (Shanghai, China). All other reagents and solvents were of analytical purity (AR) grade.

2.2. Extraction procedure of FPs

The samples (10.0 g) were extracted with distilled water (ratio of water to raw material of 40 mL/g) in a round bottom flask with a designated pH (pH of the mixtures were adjusted with 0.1 M HCl), temperature, time and complex enzyme concentration. After extraction, the extracted slurry was filtered through a filter paper under vacuum and inactivated the enzyme activity in boiling water for 10 min. The mixture was centrifuged (4000 r/min for 10 min). The supernatant was concentrated to one-fifth of the initial volume using a rotary evaporator at 60 °C under vacuum, and then precipitated by adding 95% ethanol to reach a final concentration of 80% and kept overnight at 4 °C. The precipitate was collected by centrifugation (4000 r/min for 10 min), removed proteins with the Sevag method [19], dialyzed against water for 48 h and lyophilized to get the crude polysaccharides (FPs). All experiments were performed for three times.

The polysaccharide contents of FPs were measured by the phenol-sulfuric acid method using Glc as standard [20]. The extraction yield (Y, %) of FPs was calculated with the formula of Y (%) = $(m/w) \times 100$, where m is FPs weight (g) of extraction, and w is the weight of dried sample (10 g).

2.3. Orthogonal test design of complex enzyme concentration

An orthogonal $L_9(3)^3$ test design was applied to investigate the optimal concentration of pectinase (A), papain (B) and cellulose (C), with fixed other extraction conditions as: ratio of water to raw material 40 mL/g, extraction temperature 30 °C, extraction time 60 min and pH 4.0. Nine extractions were carried out at enzymes concentration of A (pectinase of 0.5%, 1.0% and 1.5%, wt%), B (papain of 0.5%, 1.0% and 1.5%, wt%) and C (cellulase of 0.5%, 1.0% and 1.5%, wt%). The extracts were collected, processed as the method in Section 2.2.

2.4. Single factor experiment

In order to confirm the center point of the BBD, depend on the results of orthogonal experiment about the concentration of complex enzyme; the effects of ratio of water to raw material, time, temperature and pH were evaluated by determining the yield of FPs, respectively.

2.5. Response surface method (RSM) design and statistical analysis

After determining the preliminary range of the extraction variables through single-factor test, a three-level-four-factor, BBD was applied to determine the best combination of extraction variables for the yields of FPs. Ratio of water to raw material (X_1), extraction time (X_2), extraction temperature (X_3) and pH (X_4) were the independent variables and selected to be optimized for the extraction yield. Extraction yield (Y) was taken as the response of the design experiments. The coded and uncoded (actual) levels of the independent variables and the results of 29 runs using BBD design are presented in Table 1, that include the design, experimental values. Five replicates at the center point were used for estimation of a pure error sum of squares. Triplicate determinations were performed at all design points in randomized order.

In order to predict the optimized conditions, the results were analyzed by using software Design-Expert (Version 7.0, USA) and fitted to a second-order polynomial model:

$$Y = \beta_0 + \sum_{i=1}^{4} \beta_i X_i + \sum_{i=1}^{4} \beta_{ii} X_i^2 + \sum_{i=1}^{4} \sum_{j=1}^{4} \beta_{ij} X_i X_j$$

where Y is the response variable (experimental values); β_0 is the constant coefficient; β_i is the linear coefficient; β_{ii} is the squared coefficient; and β_{ij} is the interaction coefficient; X_i and X_j are the coded independent. The terms X_iX_j and X_i^2 represent the interaction and quadratic terms, respectively.

Analysis of the experimental design data and calculation of predicted responses were carried out using Design Expert software (version 7.0, USA). Analyses of variance were performed by ANOVA procedure. The fitness of the polynomial model equation was expressed by the coefficient of determination R^2 and the values of adjusted-R^2 of models. Its statistical significance was checked by F-test at a probability (P) of 0.0001, 0.01 or 0.05. The significances of the regression coefficients were also tested by F-test. The regression coefficients were then used to make statistical calculation to generate contour and dimensional maps from the regression models.

2.6. Isolation and purification of FPs

FPs were redissolved in distilled water, centrifuged at 5000 rpm for 10 min, and the supernatants were loaded onto a DEAE-Sepharose fast flow anion-exchange chromatography column (10 × 300 mm), equilibrated with Tris-HCl buffer solution and then eluted with a linear gradient from 0 to 0.8 M NH_4HCO_3 solution at a flow rate of 1.0 mL/min, respectively. The elutates were collected every 5 mL/tube and monitored by the phenol-sulfuric acid method [20] for polysaccharide content. The appropriate fractions were combined, dialyzed against deionized water and lyophilized to give two polysaccharide conjugate fractions, coded FPs-1 and FPs-2. Then FPs-1 and FPs-2 were purified by size-exclusion chromatography on a Sephadex G-200 column (20 × 500 mm) eluted with 0.1 M NaCl at a flow rate of 1 mL/min to yield two main fractions. Two fractions were collected, dialyzed and lyophilized to give purified polysaccharide fractions FPs-1-1 and FPs-2-1, which were subjected to the subsequent analyses.

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