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Microwave-assisted extraction, chemical characterization of polysaccharides from *Lilium davidii* var. *unicolor* Salisb and its antioxidant activities evaluation

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ABSTRACT

Microwave-assisted extraction (MAE) of polysaccharides from *Lilium davidii* var. *unicolor* Salisb (LP_{MAE}) was studied. The four parameters, extraction time, microwave power, extraction temperature, extraction temperature and the ratio of solid to water, were optimized using the Box–Behnken design (BBD) with a quadratic regression model built by using response surface methodology (RSM). The optimal extraction conditions for LP_{MAE} were determined as follows: microwave power 597 W, extraction time 60 min, ratio of raw material to liquid 1:65, extraction temperature 50 °C, where the highest yield of LP_{MAE} 36.55 ± 1.1% was achieved. The resulted LP_{MAE} was characterized by FT-IR. The peaks at 3406 cm^{-1}, 2930 cm^{-1}, 1250 cm^{-1}, 1060, 812 cm^{-1} indicated that LP_{MAE} possesses typical absorption peak of polysaccharides. Monosaccharide composition was determined by GC–MS method. LP_{MAE} was mainly composed of glucose and mannose with the molar ratio of 5.17:4.82. The weight average molar mass (Mw) determined by SEC–LLS was 1.193×10^5. In addition, the antioxidative activity of LP_{MAE} was investigated by measuring its scavenging ability on DPPH, hydroxyl radicals and superoxide radical, Chelating activity on ferrous ion and reducing power in vitro. The results indicated that LP_{MAE} has good antioxidant activity.

1. Instruction

Free radicals, particularly reactive oxygen species (ROS) and reactive nitrogen species (RNS), are involved in the pathogenesis of several chronical and degenerative diseases such as inflammation, cardiovascular diseases, neurodegenerative diseases, cancer and aging-related disorders. One mechanism of injury involves the generation and reactions of reactive oxygen species (ROS), such as superoxide anions (O_2), hydroxyl radical (OH), hydrogen peroxide (H_2O_2) and singlet oxygen (O) (Zhang, Hub, & Yao, 2010). A number of synthetic antioxidants, such as 2- and 3-tert-butyl-4-methoxyphenol (i.e. butylated hydroxyanisole, BHA), 2,6-di-tert-butyl-4-methylphenol (i.e. butylated hydroxytoluene, BHT) and tert-butylhydroquinone (TBHQ) have been added to foodstuffs but, because of toxicity issues, their use is being questioned (Amarowicz, Pegg, Rahimi-Moghaddam, Barld, & Weil, 2004). Attention has therefore been directed toward the development/isolation of natural antioxidants from botanical sources, especially edible plants.

Lily has been used in traditional Chinese medicine for many centuries, as its curative effects on tuberculosis, pertussis, chronic bronchitis, chronic gastritis, etc. Bulbs and flowers of this plant have been used for the treatment of ulcers, furuncles, finger ulcers, reddened skin, burns and injuries (Mimaki, Satou, Kuroda, Sashida, & Hatakeyama, 1994). Lily extracts strongly inhibited HSV-1 and slightly HSV-2. None of the extracts showed significant cytotoxic effect on uninfected Vero cells even at a concentration of 250 mg/mL (CC50 > 400 mg/mL) (Yarmolinsky, Zaccai, Ben-Shabat, Mills, & Huleihel, 2009). Bulb of lily is not only a good source of nutrient substances including starch, protein and dietary fiber, but also contains a variety of bioactive substances, such as polysaccharides, saponin, and colchicine (You, Xie, Liu, & Gu, 2010). *Lilium davidii* var. *unicolor* Salisb (*L. davidii* var), belongs to the genus *Lilium* of the family, commonly known as “the only sweet lily”, and is a well-known edible and medicinal plant, distributed in China and cultivated in the city of Lanzhou of Gansu Province. As the bulbs of L. davidii contain a high amount of polysaccharides and have been considered as having some medicinal value, it becomes very important to characterize their physical and chemical properties

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and chemical structure for efficient use of this plant species (Zhang, Gao, Zhou, Hu, & Xie, 2010). In recent years, *L. davidii* has gained increasing attention due to its unique biological activities. Conventional techniques to obtain polysaccharides, such as heating, boiling, or refluxing, usually require long extraction time and high extraction temperature, but the extraction efficiency was low. Compared with traditional methods, microwave-assisted extraction approach has many advantages, such as shorter extraction time, use of less solvent and higher extraction rate. So far, it has been widely employed to extract polysaccharides from different materials with great extraction Efficiency.

To the best of our knowledge, there were no reports available in the literature regarding the optimization of microwave-assisted extraction of polysaccharides from the Bulb of *L. davidii* var by RSM. In this study, the microwave-assisted extraction parameters (microwave-assisted power) ratio of liquid to solid, (extraction temperature and time) of polysaccharides from the Bulb of *L. davidii* var was firstly investigated and optimized using a three-level, Four-variable Box–Behnken design (BBD). Antioxidant properties of the polysaccharides were investigated by various in vitro assays.

2. Materials and methods

2.1. Materials and chemicals

Fresh bulbs of *L. davidii* var. *unicolor* Salisb were obtained from the local farmers' market of Lanzhou, Gansu province, China, washed, dried at 65 °C for 24 h, and powdered for this study.

1,1-Diphenyl-2-picrylhydrazyl (DPPH), inositol, pyridine, butyl hydroxy anisd (BHA), dihydronicotineamid adenine dinucleotide (NADH), phenazine methosulfate (PMS), 2,2-azinobis-3-ethylbenzthiazoline-6-sulfonate (ABTS), nitro blue tetrazolium (NBT), ethylene diamine tetraacetic acid were (EDTA), trifluoroacetic acid (TFA), acetic anhydride and ascorbic acid (Vc) were purchased from Sigma–Aldrich (St. Louis, MO, USA). The solvents for GC–MS were of chromatographic purity. All other reagents used were of analytical grade.

2.2. Extraction and isolation of LP using microwave-assisted extraction (MAE)

L. davidii var bulb powder was put into a 3000 mL PTFE extraction vessel and extracted under different MAE conditions. MAE was performed on microwave apparatus using vessel system (NJC 03-2, Microwave Experiment Equipment, Nanjing, China). After extraction, the vessel was allowed to cool at room temperature. The suspension was centrifuged (4000 × *g*, 10 min) and the insoluble residue was treated again for 2 times as mentioned above. The supernatant was incorporated and concentrated to one-fifth of the initial volume using a rotary evaporator at 50 °C under vacuum. The supernatant was precipitated by the addition of anhydrous ethanol to a final concentration of 80% (v/v) and the precipitates as crude extract were collected by centrifugation (4500 × *g*, 10 min). After being washed three times with anhydrous ethanol, the precipitate was freeze-dried to obtain crude LP_{MAE}. The content of the polysaccharides was measured by phenol–sulfuric method.

The polysaccharides yield (%) is calculated as follows:

$$\text{polysaccharides yield}(\%) = \frac{\text{the polysaccharides content of extraction}(g)}{\text{weight of } L.\ davidii \text{var powder}(g)}$$

2.3. Experimental design and statistical analysis

BBD was employed to statistically optimize the formulation parameters and evaluate main effects, interaction effects and quadratic effects of the formulation ingredients on the yields of polysaccharide (Table 1). According to the principle of BBD, ratio of raw material to water, microwave power, extraction temperature and extraction time, which were identified to have strong effects on the yields were taken as the variables tested in a 29-run experiment. As shown in Table 1, the four factors chosen for this study were designated as X_1, X_2, X_3, and X_4 and were prescribed into three levels, coded +1, 0, and −1 for high, intermediate and low value, respectively. Test variables were coded according to the following equation:

$$x_i = \frac{X_i - X_0}{\Delta X}$$

where x_i was the coded value of an independent variable; X_i was the actual value of an independent variable; X_0 was the actual value of an independent variable at center point; ΔX was the step change value of an independent variable. All experiments were performed in triplicate and the averages of polysaccharide yields were taken as response. For predicting the optimal point, a second order polynomial model was fitted to correlate relationship between independent variables and response (polysaccharide yield). For the three factors, the equation was

$$Y = A_0 + \sum A_{ix} i + \sum A_{ii} X_{i2} + \sum A_{ij} X_i X_j$$

where Y was the response variables (yields of polysaccharides in real values). A_0, A_i, A_{ii}, A_{ij} were the regression coefficients of variables for intercept, linear, quadratic and interaction terms, respectively. X_i and X_j were independent variables ($i \neq j$). The fitness of the polynomial model equation was expressed by the coefficient of determination R^2. According to the analysis of variance, the effect and regression coefficients of individual linear, quadratic and interaction terms were determined. The regression coefficients were then used to make statistical calculation to generate dimensional and contour maps from the regression models. Statistica (Version 8.0, USA) software package was used to analyze the experimental data. *P*-values of less than 0.05 were considered to be statistically significant.

2.4. Structural characterization of LP_{MAE}

2.4.1. Components analysis

The carbohydrate contents of LP_{MAE} were determined by the phenol–sulfuric acid method, using glucose as the standard. The protein contents were measured according to Bradford's method, using bovine serum albumin (BSA) as the standard. The composition was analyzed according to the earlier report from our laboratory (Wang et al., 2010). Briefly, 4 mg of sample were dissolved in 4 mL of 4 M trifluoroacetic acid (TFA) in a test tube and then hydrolyzed at 120 °C for 10 h under air-tight conditions. TFA was then evaporated through decompression and distillation. When the tube was dry, 10 mg of ammonium hydrochloride and 0.5 mg pyridine were added and allowed to react in a 90 °C water bath for 30 min. Then 0.5 mL of cold (kept at 4 °C in a refrigerator) acetic anhydride was added to the test tube and the mixture was incubated in the