Homogenate extraction of polysaccharides from loquat leaves by response surface methodology

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Homogenate extraction method was applied to fast extraction of polysaccharides from loquat leaves. Factors which affected extraction yield were investigated and optimized by response surface methodology (RSM). Results showed that optimum technological conditions were as follows: ratio of liquid to material, 25 ml: 1g; extraction time, 100 s; and pH of extracting solvent, 11. Under optimum conditions, yield of polysaccharides was (1.19±0.11)%. Compared with traditional hot-water extraction, homogenate extraction has notable advantages in good extraction yield and short extraction time.

Keywords: Homogenate extraction, Loquat leaves, Polysaccharides, Response surface methodology

Introduction

Loquat (Eriobotrya japonica), belongs to the trees of the Rosaceae family, is an Asian fruit and an important food crop in China1. Loquat leaves are widely used in preparation of oriental herbal teas. In folk medicine, loquat leaves are used against various skin diseases, cough, nausea, and itching 2. Loquat leaves contain active ingredients of phenols, triterpenes and polysaccharides which exhibit beneficial effects on health. In recent years, polysaccharides of plant origin have emerged as an important class of bioactive natural products. A wide range of polysaccharides has been reported to exhibit a variety of immunological activities of anti-tumor3, immunomodulating 4, anti-complementary5,6, anti-inflammatory7, and anti-coagulant 8. As reported by Huang et al.9, polysaccharides of loquat leaves can significantly improve anti-fatigue ability in mice.

Extraction of polysaccharides from plants and fungus is an important process for their application or further research. Dong et al.10 have optimized the hot water extraction of polysaccharides from cultured mycelium of Cordyceps sinensis using a Box-Behnken design (BBD). Yang et al.11 have employed ultrasound technology to extract polysaccharides from longan fruit pericarp and determined the optimal extraction conditions by response surface methodology (RSM). They find that the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging activity of the polysaccharides could be improved by application of ultrasound treatment. Qiao et al.12 use RSM to optimize the conditions for hot water extraction of polysaccharides from Hyriopsis cumingii. Cai et al.13 have studied the effects of hot water extraction parameters on the yield of polysaccharides from Opuntia milpa alta and established the optimal extractions conditions. In general, hot-water extraction is the most widely used technology for polysaccharide extraction, but it should be noted that hot-water extraction of polysaccharides is associated with lower yields, long extraction times and high temperatures, so it is desirable to find a novel extraction technology for polysaccharides that avoid the disadvantages of hot water extraction. Homogenate extraction is an alternative to conventional extraction methods, through which chemical compositions are extracted from materials in solvent by high-speed mechanical shearing, mixing, fluid cutting action and smashing without heating and pressure14. This method has been proved to be effective to extract gardenia yellow pigment from Gardenia Jasminoides Ellis fruit14, extract camptothecine and hydroxycamptothecin from Camptotheca acuminata leaves15, extract isoflavones from soybean meal16. However, application of homogenate extraction on extraction of polysaccharides from loquat leaves has never been reported. In this study,
homogenate extraction method was used to extract polysaccharides from loquat leaves. Effects of various extraction parameters such as ratio of liquid to material, extraction time and pH of extracting solvent on yield of polysaccharides were investigated by RSM, an effective tool for optimizing process. Homogenate extraction method was compared with traditional hot-water extraction method.

**Experimental Section**

**Materials and Apparatus**

Loquat leaves were collected in Hangzhou City, Zhejiang Province, China, and were identified in College of Pharmaceutical Sciences, Zhejiang University of Technology. A voucher specimen (EJL110727) was deposited in College of Pharmaceutical Sciences, Zhejiang University of Technology. Leaves were dried at 60°C, pulverized into to a certain particle size (average diam. 2 mm) by a disintegrator, and kept in a dry place until used. Standard glucose (purity, 98%) was purchased from Shanghai Yuanye Biotechnology Co. Ltd. Homogenate extractor (JHBE-50A, maximum rotary velocity of 10000 r/min) was purchased from Golden Star Technology, Inc., Ltd. (Zhengzhou, China).

**Homogenate Extraction of Polysaccharides from Loquat Leaves**

Dried loquat leaves (5.0 g) and alkaline water (pH was adjusted with NaOH) were put into homogenate extractor for extraction in a designed extraction time, ratio of liquid to material and pH of extracting solvent at room temperature. After extraction, mixture was centrifuged at 4700 rpm for 10 min. Supernatant was collected and concentrated by rotary evaporator. Concentrated solution was precipitated by adding fourfold volume of anhydrous ethanol and then incubated at 4°C for 24 h. After centrifugation and vacuum drying, polysaccharides was obtained and analyzed by UV-Vis spectrophotometer test. Each experimental group was repeated three times. Yield of polysaccharides was expressed as follows:

\[ \text{Yield (\%) = (m / M) \times 100\%} \]  

(1)

where \( m \) was weight of polysaccharides analyzed by UV-Vis analysis (g), and \( M \) was weight of loquat leaves (g).

**Traditional Hot-water Extraction of Polysaccharides from Loquat Leaves**

According to optimal conditions, dried loquat leaves (5.0 g) and alkaline water (100 ml, pH 11) were put into a reflux apparatus for extraction at 80°C for 2 h. This process was repeated for two cycles. Extracting solution was collected together and centrifuged (4600 rpm, 10 min). Latter steps were same as homogenate extraction method.

**Experimental Design**

Response surface methodology is an empirical modeling technique used to estimate relationship between a set of experimental control factors and observed results. In this study, a BBD was applied to optimize extraction conditions by using software Design-Expert (Trial Version 7.1.6, Stat-Ease Inc., Minneapolis, MN, USA). According to preliminary results, ratio of liquid to material, extraction time and pH of extracting solvent were chosen as key parameters and were designated \( X_1, X_2 \) and \( X_3 \), respectively. Ranges of variables and their levels were shown in Table 1.

A total of 17 experimental runs were employed and experiments were performed in a randomized order. Variables were coded according to following equation:

\[ x_i = (X_i - X_o)/\Delta X \quad i = 1, 2, 3 \]  

(2)

where \( x_i \) was dimensionless value of an independent variable, \( X_i \) was real value of an independent variable, \( X_o \) was real value of an independent variable at center point, \( \Delta X \) is step change. Based on experimental data, regression analysis was performed and was fitted into an empirical second-order polynomial model:

\[ Y = A_0 + \sum_{i=1}^{3} A_i X_i + \sum_{i=1}^{2} A_{ij} X_i X_j + \sum_{i=1}^{3} A_{iij} X_i X_j X_k \]  

(3)

where \( Y \) was dependent variable, \( A_0, A_i, A_{ij}, A_{iij} \) are regression coefficients of variables for intercept, linear, quadratic and interaction terms respectively, and \( X_i, X_j, X_k \) were levels of independent variables. They represented

| Table 1—Independent variables and their levels used in response surface design |
|-----------------------------|-----------------|-----------------|
| Level                        | Levels\(^a\)    |
| Ratio of liquid to material  | 15, 20, 25      |
| Extraction time (S)          | 60, 90, 120     |
| pH of extracting solvent (S) | 9, 10, 11       |

\(^a\)\( x_i = (X_i - 20)/5; x_j = (X_j - 90)/30; x_k = (X_k - 10)/1 \).
linear, quadratic, and cross-product effects of $X_1$, $X_2$, and $X_3$ factors on response, respectively. Effects of each independent variable were evaluated by model via response. Analysis of experimental design data and calculation of predicted responses were showed in Table 2. Three additional checking experiments were performed later to verify validity of statistical experimental strategies. All experiments were repeated three times.

### Results and Discussion

#### Preliminary Results

In order to determine main factors and the appropriate ranges for the BBD, preliminary experiments were performed. Various parameters such as extracting solvent, rotary velocity of homogenate extractor, ratio of liquid to material, extraction time, extraction temperature and so on might potentially influence extraction process. Results of preliminary experiments showed that, extraction temperature had no significant effect on yield of polysaccharides, and room temperature (25 °C) could be selected as suitable temperature. Maximum of homogenate extractor rotary velocity was 10000 r/min. Yield of polysaccharides increased with increasing rotary velocity. When rotary velocity was over 5000 r/min, it had no apparent effect on yield of polysaccharides. So 5000 r/min was used as an appropriate rotary velocity. For extraction of polysaccharides, water is a preferred solvent. As found in our experiments, pH of extracting solvent could obviously affect yield of polysaccharides. Among variables screened, ratio of liquid to material, extraction time, and pH of extracting solvent were most significant variables to be further determined.

Effect of ratio of liquid to material (10 ml: 1 g - 30 ml: 1 g) on yield of polysaccharides was studied at: extraction time, 90 s; pH of extracting solvent, 8. As seen from Fig. 1a, when ratio of liquid to material reached 20 ml: 1g, extraction yield was optimum. 15 - 25 (ml: g) was chosen as appropriate range of ratio of liquid to material in Box-Behnken design process. On basis of optimal ratio of liquid to material, effect of extraction time (30, 60, 90, 120, and 150 min) on yield of polysaccharides was studied at: ratio of liquid to material, 20 ml: 1 g; pH of extracting solvent, 8. When extraction time reached 90 s, extraction yield reached relatively high value (Fig. 1b). According to results, 60 - 120 s was chosen as optimum range of extraction time.

Effect of pH of extracting solvent (7, 8, 9, 10, and 11) on yield of polysaccharides was investigated at: ratio of liquid to material, 20 ml: 1 g; pH of extracting solvent, 8. When extraction time reached 90 s, extraction yield reached relatively high value (Fig. 1b). According to results, 60 - 120 s was chosen as optimum range of extraction time.

Effect of pH of extracting solvent (7, 8, 9, 10, and 11) on yield of polysaccharides was investigated at: ratio of liquid to material, 20 ml: 1 g; pH of extracting solvent, 8. Fig. 1c revealed that when pH of extracting solvent reached 10, extraction yield was optimum. Therefore, in Box-Behnken design process, pH 9-11 was chosen as appropriate range of pH of extracting solvent.

### Optimization of Homogenate Extraction Operating Parameters

Further optimization of homogenate extraction conditions was conducted by employing BBD. Data were analyzed using Design Expert 7.1.6 software for statistical analysis of variance (ANOVA), regression
coefficients and regression equation. Polynomial equations, describing yield of polysaccharides ($Y$) as a simultaneous function of ratio of liquid to material ($X_1$), extraction time ($X_2$), pH of extracting solvent ($X_3$), were shown in equation:

$$Y = 1.13 + 0.090X_1 + 0.099X_2 + 0.12X_3 + 0.015X_1X_2 - 0.040X_1X_3 + 0.018X_2^2X_3 - 0.029X_1^2 - 0.15X_2^2 - 0.12X_3^2 \ldots (4)$$

To evaluate optimal extraction conditions of homogenate extraction for polysaccharides and relationship between response and significant variables, analysis of variance (ANOVA) for model was performed. As shown in Tables 3, experimental data fitted well to quadratic models. ANOVA for response surface quadratic regression model showed that model was highly significant ($P<0.0001$) with a high F-value of 31.61. It could be seen that linear terms of ratio of liquid to material ($X_1$), extraction time ($X_2$), pH of extracting solvent ($X_3$), extraction time ($X_2^2$), pH of extracting solvent ($X_3^2$) were significant.

Regression analysis of data showed coefficient of determination ($R^2$) values for polysaccharides was 0.9760, suggesting that model was significant. Adjusted determination coefficients (Adj $R^2 = 0.9451$) was also satisfied to confirm significance of model. This indicated that equation (4) was suitable to describe response of experiment to extract polysaccharides from leaves of loquat. At same time, lack-of-fit statistics, which was used to test adequacy of model, indicated that P-value for polysaccharides (0.5581) were not significant. No abnormality was obtained from diagnoses of residuals. Thus, it could be concluded that model was statistically sound. To depict interactive effects of operational variables on responses, one variable was kept constant and other two variables varied in defined ranges. Shapes of response surfaces and contour plots indicated nature
and extent of interaction between different variables. By solving inverse matrix, optimal parameters (ratio of liquid to material, 25 ml: 1 g; extraction time, 102.28 s; pH of extracting solvent, 10.35) were obtained on basis of response surface. Under optimal conditions, yield of polysaccharides was 1.23%.

Verification Experiments

Adequacy of model equation for predicting optimum response values was tested using selected optimal conditions. Verification experiments were conducted and yield of polysaccharides was (1.21±0.13)%, which was not significant different from predicted value of 1.23%. Good correlation between these results confirmed that response model was adequate to reflect expected optimization. Taking account of rationality and practicability of operation, optimal extraction parameters were revised to: ratio of liquid to material, 25 ml: 1 g; extraction time, 100 s; pH of extracting solvent, 11. In this conditions yield was (1.19±0.11)%, which was little differed from that in optimal conditions, so revised conditions were rational.

Comparison of Different Extraction Methods

Homogenate extraction and traditional hot-water extraction were compared for extraction of polysaccharides from leaves of loquat in optimized conditions. Extraction conditions and yield were listed in Table 4. Yield of polysaccharides using homogenate extraction was 0.15% higher than that in hot-water extraction. Extraction time of homogenate extraction was significantly shortened. Hence, it was worth noticing that homogenate extraction is a good alternative to extraction of polysaccharides from leaves of loquat in practical production.

Conclusions

In this study, homogenate extraction technology was used to extract polysaccharides from loquat leaves. Optimal parameters were determined by RSM as follows: ratio of liquid to material, 25 ml: 1 g; extraction time, 100 s; pH of extracting solvent, 11. Under these conditions, extraction yield of polysaccharides was higher than hot-water extraction method, and extraction time of homogenate extraction was significantly shortened. All these showed that homogenate extraction method was a more efficient way for extracting polysaccharides from loquat leaves.

References


