Carbohydrate Polymers 99 (2014) 319–324

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

# Extraction, purification and characterization of polysaccharides from Hawk tea

Xuejing Jia [a], Chunbang Ding [a], Shu Yuan [b], Zhongwei Zhang [b], Yang'er Chen [a], Lei Du [b], Ming Yuan [a,*]

[a] College of Life and Basic Sciences, Sichuan Agricultural University, Ya'an 625014, China
[b] College of Resources and Environmental Science, Sichuan Agricultural University, Chengdu 625014, China

ARTICLE INFO

*Article history:*
Received 4 May 2013
Received in revised form 19 July 2013
Accepted 26 July 2013
Available online 22 August 2013

*Keywords:*
*Litsea coreana* var. *lanuginose*
Polysaccharide
Response surface methodology
Antioxidant activity

ABSTRACT

In the present study, the extraction, purification and characterization of polysaccharides from Hawk mature leaf tea (HMP) were investigated. The optimal extraction parameters were obtained by using a Box–Behnken design as follows: extraction temperature 88.9 °C, extraction time 128.2 min and ratio of water to solid 11.4 mL/g. The crude HMP was sequentially purified by chromatography of DEAE-52, and two purified fractions, HMP-1 and HMP-2, were obtained. HMP-1 and HMP-2 were mainly composed of arabinose, galactose, glucose and mannose with the molecular weight of 133 and 100 kDa, respectively. For antioxidant activities in vitro, HMP-1 had strong 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity and ferric reducing activity power (FRAP). These results provide a scientific basis for the further use of polysaccharides from this traditional herb tea.



## 1. Introduction

Hawk tea is a herbal tea and one of the most popular traditional beverage in southwest of China for hundreds of years, and it is produced from buds or leaves of *Litsea coreana* Lévl. var. *lanuginosa* (Migo) Yang et P. H. Huang (*Lauraceae*). Hawk tea is widely cultivated as an undergrowth crop in the forests, which is a major type of agroforestry farming system in Ya'an and Deyang, China. Hawk tea has many biological and pharmaceutical properties, such as inhibiting hypoglycemic, lowering blood lipids and nitrosamine formation (Ji, Zhang, Du, Yang, & Wang, 2011). Hawk mature leaf tea (HM) was made from mature leaves of *Litsea coreana* var. *lanuginose*. There is abundant resource of HM in the south of China and its price just about $3 per kilogram (Jia et al., 2013). Therefore, HM is a high performance/price ratio material for application in food industry.

Response surface methodology (RSM) is less laborious and time-consuming than other approaches which are applied to optimize a process. It can reduce the number of experimental trials which need to evaluate multiple parameters and their interactions (Ye & Huang, 2012). Recently, it had been widely used to optimize processes in many researches (Poonkuzhali & Palvannan, 2011; Zinatizadeh et al., 2006). Box–Behnken design (BBD) is a type of response surface design. It is an independent quadratic design that does not contain an embedded factorial or fractional factorial design (Zhu & Liu, 2013). BBD was easy to arrange and interpret experiment in comparison with others, and had a wide range of applications in many researches (Rahmanian, Pakizeh, Esfandyari, Heshmatnezhad, & Maskooki, 2011; Zhao et al., 2012).

A great deal of attention had been paid to tea polysaccharides due to their unique bioactivities and chemical structures in recent years (Wang, Zhao, et al., 2013). Published data indicated that polysaccharides isolated from tea had antioxidant activity and could be explored as potential antioxidants (Cai, Xie, Chen, & Zhang, 2013; Chen, Zhang, Qu, & Xie, 2008). Tea polysaccharides had been reported to possess hepatoprotective activities, anticoagulant activities and anti-cancer (Cai et al., 2013; Xu, Ye, Sun, Tu, & Zeng, 2012; Yang, Chen, & Gu, 2012). However, little attention has been devoted to the extraction and monosaccharide composition of polysaccharides from Hawk tea.

In our previous studies (Jia et al., 2013), the polysaccharides from different leaf age Hawk teas exhibited good antioxidant activities and could be explored as a novel potential antioxidant. Therefore, in the present study, the extraction variables were optimized by employing BBD for maximum polysaccharide yield. Furthermore, the crude HMP was purified by chromatography of DEAE-52. Then, the crude HMP and its purified fractions (HMP-1 and HMP-2) were characterized by chemical analysis, Fourier transform-infrared spectroscopy (FT-IR), gas chromatography–mass spectrometry (GC–MS) and gel permeation chromatography (GPC). Finally, the antioxidant activities (DPPH and FRAP) in vitro of crude

* Corresponding author. Tel.: +86 835 2886124.
*E-mail address:* yuanmingsicau@126.com (M. Yuan).

0144-8617/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.carbpol.2013.07.090

**Table 1**
BBD with the experimental values and predicted values for extraction yield of HMP.

| Run | Code | | | Uncode | | | Extraction yield (%)[a] | Predicted yield (%) |
|---|---|---|---|---|---|---|---|---|
| | A Temperature | B Time | C Water/solid | $X_1$ Temperature | $X_2$ Time | $X_3$ Water/solid | | |
| 1 | −1 | −1 | 0 | 80 | 90 | 10 | 9.97 | 10.32 |
| 2 | 1 | −1 | 0 | 100 | 90 | 10 | 8.41 | 8.40 |
| 3 | −1 | 1 | 0 | 80 | 150 | 10 | 10.67 | 10.67 |
| 4 | 1 | 1 | 0 | 100 | 150 | 10 | 11.54 | 11.19 |
| 5 | −1 | 0 | −1 | 80 | 120 | 5 | 8.15 | 8.07 |
| 6 | 1 | 0 | −1 | 100 | 120 | 5 | 7.30 | 7.58 |
| 7 | −1 | 0 | 1 | 80 | 120 | 15 | 11.10 | 10.83 |
| 8 | 1 | 0 | 1 | 100 | 120 | 15 | 9.86 | 9.93 |
| 9 | 0 | −1 | −1 | 90 | 90 | 5 | 6.94 | 6.67 |
| 10 | 0 | 1 | −1 | 90 | 150 | 5 | 8.08 | 8.15 |
| 11 | 0 | −1 | 1 | 90 | 90 | 15 | 9.21 | 9.14 |
| 12 | 0 | 1 | 1 | 90 | 150 | 15 | 10.52 | 10.80 |
| 13 | 0 | 0 | 0 | 90 | 120 | 10 | 12.28 | 12.40 |
| 14 | 0 | 0 | 0 | 90 | 120 | 10 | 12.08 | 12.40 |
| 15 | 0 | 0 | 0 | 90 | 120 | 10 | 12.48 | 12.40 |
| 16 | 0 | 0 | 0 | 90 | 120 | 10 | 12.48 | 12.40 |
| 17 | 0 | 0 | 0 | 90 | 120 | 10 | 12.68 | 12.40 |

[a] Mean of triplicate measurements.

HMP and its purified fractions (HMP-1 and HMP-2) were evaluated.

## 2. Materials and methods

### 2.1. Reagents and materials

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) was purchased from the Sigma Chemical Co. (St. Louis, MO, USA). Ascorbic acid (Vc), 1,3,5-tri(2-pyridyl)-2,4,6-triazine (TPTZ) were purchased from the Sinopharm Chemical Reagent Co. (Beijing, China). Other chemicals used in this study were of analytical grade. Ultra-pure water was used throughout the experiments.

Hawk mature leaf tea (HM) was purchased from local retail shops (Yucheng District, Sichuan Province, China). The sample was ground into fine powder using a powerful mill (FW177, Taisite Instrument Co., Ltd., Tianjin, China), and screened through a 40 mesh sieve. The materials were stored at room temperature in a desiccator until use.

### 2.2. BBD for the extraction of polysaccharides

The software Design Expert (Trial Version 8.0.6, Stat-Ease Inc., Minneapolis, MN, USA) was employed for experimental design, data analysis and model building. BBD with three independent variables (extraction temperature; extraction time; ratio of water to solid) at three levels was applied to determine the best combination of extraction variables for extraction of HMP. The symbols and levels are shown in Table 1. The whole design consisted of 17 experimental points carried out in a randomized order to maximize the effect of unexplained variability in the observed response due to extraneous factors. The non-linear computer-generated quadratic model is given as below (Jiang et al., 2013):

$$Y_0 = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum \sum_{i<j} \beta_{ji} X_i X_j$$

where $Y_0$ is the estimated response, and $\beta_0$, $\beta_j$, $\beta_{jj}$ and $\beta_{ji}$ are the regression coefficients for intercept, linearity, square and interaction while $X_i$ and $X_j$ are the independent coded variables ($i \neq j$), respectively.

The polysaccharide content of crude HMP was determined by the phenol-sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). The polysaccharide yield (%) was then calculated using the following equation:

$$\text{Extraction yield}\,(\%) = \left[\frac{C \times N \times V}{W}\right] \times 100$$

where $C$ is the concentration of polysaccharide calculated from the calibrated regression equation (mg/mL); $N$ is the dilution factor; $V$ is the total volume of extraction solution (mL); and $W$ is the weight of samples (mg).

### 2.3. Preparation of crude HMP

The crude HMP was obtained under the optimum conditions when the proteins in the crude HMP were removed by the Sevag solution (chloroform:butyl alcohol, 4:1). The deproteinized solution was re-precipitated in anhydrous ethanol ten times the solution volume (Jiang et al., 2013). The precipitate was collected by centrifugation at 5000 rpm for 20 min and air-drying at 50 °C to a constant weight, affording the crude HMP.

### 2.4. Separation and purification of crude HMP

The crude HMP was purified by DEAE-52 filtration chromatography according to the reported method with little modifications (Ai et al., 2013). Briefly, the crude HMP solution (3 mL, 10 mg/mL) was applied to a column (2.6 cm × 60 cm) of DEAE-52 cellulose. Then, the column was stepwise eluted with 0, 0.2, 0.4, 0.6 and 0.8 mol/L NaCl solutions at a flow rate of 0.6 mL/min. The obtained elute (5 mL/tube) was collected automatically and the polysaccharides were detected by the phenol-sulfuric acid method. As a result, two fractions of HMP were obtained. Each fraction was collected, concentrated, dialyzed and dried for further research.

### 2.5. Characterization of HMP

#### 2.5.1. Determination of contents of carbohydrate, protein and polyphenols

The carbohydrate contents were determined by phenol-sulfuric acid colorimetric method (Dubois et al., 1956). The protein contents were measured by coomassie brilliant blue reaction (Bradford, 1976). The polyphenols contents were estimated by Folin–Ciocalteu method (Hsu, Hsu, Lin, Cheng, & Yang, 2013).

*2.5.2. Infrared spectroscopy analysis of HMP*

The organic functional groups of crude HMP and its purified fractions (HMP-1 and HMP-2) were identified using a FTIR spectrophotometer (FTIR-8400S, Shimadzu Co., Japan) within 4000–400 $cm^{-1}$ via the KBr pressed-disk method (Peng, Liu, Fang, Wu, & Zhang, 2012).

*2.5.3. Analysis of monosaccharide composition of HMP*

The monosaccharide compositions of crude HMP and its purified fractions (HMP-1 and HMP-2) were analyzed by GC–MS according to the reported method with slight modifications (Peng et al., 2013). 10 mg samples were hydrolyzed using 3 mol/L trifluoro acetic acid (TFA) at 100 °C for 6 h. The hydrolysate was then diluted with anhydrous ethanol, and the TFA was removed using a rotary vacuum evaporator at 45 °C. Anhydrous ethanol was added to the solids followed by re-evaporation. This procedure was repeated several times until the hydrolysates obtained were neutral. The reaction products were analyzed by gas chromatography–mass spectrometry (GC–MS, QP2010, Shimadzu, Japan) analyses with a fused silica capillary column (0.25 μm × 0.25 mm × 30 m) of RTX 5 ms. The operation conditions of GC–MS were as follows: flow rate of $H_2$ was 30 mL/min; the temperature of detector and inlet were 280 °C and 250 °C, respectively; the oven temperature program was set changing from 120 °C (standing for 3 min) up to 210 °C (standing for 4 min) at a rate of 3 °C/min.

*2.5.4. Molecular weight determination of HMP-1 and HMP-2*

The molecular weight of HMP-1 and HMP-2 were characterized by gel permeation chromatography (GPC) method at a flow rate of 0.6 mL/min. The calibration curve was conducted using the dextrans with various molecular weight (23,800, 80,900, 147,600 and 273,000).

*2.6. Determination of antioxidant activities in vitro of HMP*

*2.6.1. Assay of DPPH radical scavenging activity*

DPPH radical scavenging activity was determined in accordance with the reported method (Xie et al., 2012). Briefly, 1 mL sample solution with different concentrations (0.2, 0.4, 0.6, 0.8 and 1 mg/mL) was added to 3 mL (0.05 mmol/L) DPPH methanol solution in a 5 mL cuvette. The mixtures were shaken vigorously and incubated in the dark for 20 min after that the reduction of DPPH• absorption was measured at 517 nm using the spectrophotometer (UV-1750, Shimadzu). The scavenging activity on DPPH radical was calculated by the following equation.

$$\text{DPPH radical scavenging activity}\,(\%) = \left[\frac{A_{\text{DPPH}} - A_{\text{sample}}}{A_{\text{DPPH}}}\right] \times 100$$

where $A_{\text{DPPH}}$ is the absorbance of the DPPH radical solution without sample and $A_{\text{sample}}$ is the absorbance of the DPPH radical solution with tested samples.

*2.6.2. Assay of ferric reducing activity power*

The FRAP assay was performed, as previously described by Wang, Wang, Liu, Yuan, and Yue (2013) with some modifications. The fresh FRAP reagent was prepared before using, which contains 25 mL acetate buffer (300 mmol/L, pH 3.6), 2.5 mL TPTZ solutions (10 mmol/L in 40 mmol/L HCl) and 2.5 mL of $FeCl_3{\cdot}6H_2O$ solution (20 mmol/L). The reagent was warmed to 37 °C, then 500 μL were placed in a cuvette and the initiate absorbance was read. 20 μL of the sample solutions (0.2, 0.4, 0.6, 0.8 and 1 mg/mL) was added to the cuvette and the absorption value was determined at 593 nm. In this study, the reaction was followed until it reached the plateau. Values were calculated according to the calibration curve with aqueous solutions of $FeSO_4{\cdot}7H_2O$ in the range of 0–1800 μmol. The final results were expressed as the concentrations of $FeSO_4{\cdot}7H_2O$ with equivalent antioxidant activity.

**Table 2**
ANOVA for response surface quadratic model for the yield of HMP.

| Source | SS[a] | df[b] | MS[c] | *F*-value | *P*-value |
|---|---|---|---|---|---|
| Model | 58.76 | 9 | 6.53 | 59.23 | <0.0001 |
| A | 0.97 | 1 | 0.97 | 8.76 | 0.0211 |
| B | 4.94 | 1 | 4.94 | 44.78 | 0.0003 |
| C | 13.08 | 1 | 13.08 | 118.67 | <0.0001 |
| AB | 1.49 | 1 | 1.49 | 13.49 | 0.0079 |
| AC | 0.04 | 1 | 0.04 | 0.37 | 0.5644 |
| BC | 0.00749 | 1 | 0.00749 | 0.07 | 0.8018 |
| $A^2$ | 3.58 | 1 | 3.58 | 32.46 | 0.0007 |
| $B^2$ | 7.51 | 1 | 7.51 | 68.15 | <0.0001 |
| $C^2$ | 23.84 | 1 | 23.84 | 216.24 | <0.0001 |
| Residual | 0.77 | 7 | 0.11 | | |
| Lack of fit | 0.56 | 3 | 0.19 | 3.62 | 0.1230 |
| Pure error | 0.21 | 4 | 0.05 | | |
| Correlation total | 59.53 | 16 | | | |
| $R^2$ | 0.9870 | | Adj-$R^2$ | 0.9704 | |

[a] Sum of Squares.
[b] Degree of Freedom.
[c] Mean Square.

*2.7. Statistical analysis*

Analysis of the experimental design and data was carried out using Design Expert software of version 8.0.6 (Stat-Ease Inc., Minneapolis, USA). Analysis of variance (ANOVA) was carried out and the fitness of the polynomial model equation was expressed as the coefficient of determination $R^2$. The significances of the regression coefficients were tested by *F*-test, $P < 0.05$ was regarded as significant.

## 3. Results and discussion

*3.1. Model fitting and optimization for the extraction of HMP*

*3.1.1. Model fitting*

Using multiple regression analysis on experimental data by the software of Design Expert version 8.0.6, the correlation between response variables and test variables (the extraction yield of HMP) associated with the following second-order polynomial equation:

$$Y = -75.23653 + 1.40073X_1 + 0.19659X_2 + 2.30522X_3 + 0.00203X_1X_2 - 0.00201X_2X_3 + 0.00289X_1X_3 - 0.00922{X_1}^2 - 0.00148{X_2}^2 - 0.00095{X_3}^2$$

where *Y* represents the yield of HMP, $X_1$, $X_2$ and $X_3$ represent extraction temperature, extraction time and ratio of water to solid, respectively.

The variance of the quadratic regression model showed that the determination coefficient ($R^2$) was 0.9870, indicating that only 0.50% of the total variance was not explained by the model. The value of the adjusted determination coefficient (adjusted $R^2 = 0.9704$) also confirmed that the model was highly significant. Meanwhile, the coefficient of the variation (CV) was a low value (3.25), which indicated that the degree of precision was very high and the experimental data were very reliable. The model was found to be adequate for prediction within the range of experimental variables. The values of regression coefficient were listed in Table 2. The smaller the *P*-value was, more significant the corresponding coefficient was (Zhu & Liu, 2013). Accordingly, A, B, C, AB, $A^2$, $B^2$ and $C^2$ were significantly different ($P < 0.05$), while AC and BC were not significantly different ($P > 0.05$).

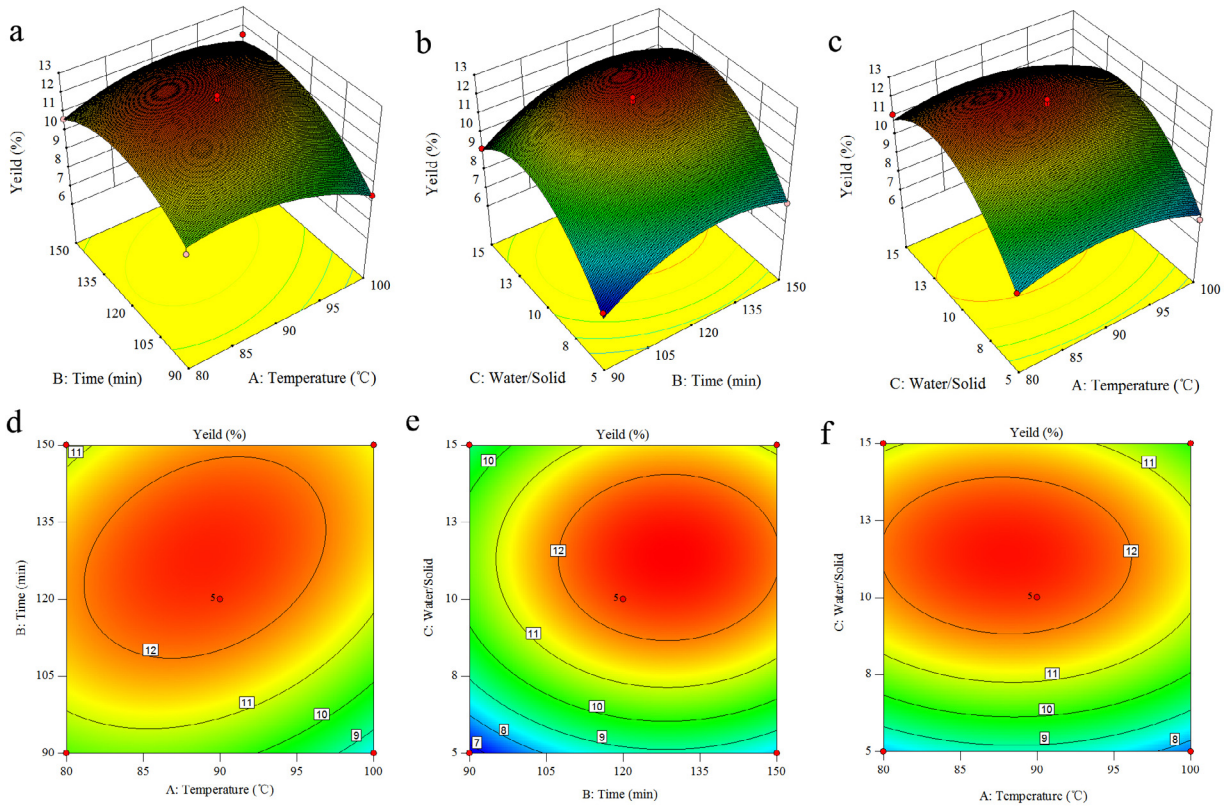


**Fig. 1.** Response surface plots (a, b and c) and contour plots (d, e and f) showing the effects of extraction temperature, extraction time and ratio of water to solid on the extraction yield of HMP and their mutual effects.

### 3.1.2. Optimization for the extraction of HMP

Process variables and experimental data are shown in Table 1. In order to understand the interactions of these variables better, the response surface plots and contour plots for the model were produced by the Stat-Ease Design-Expert software. The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between variables are significant or not. The response surface plots and contour plots in Fig. 1 were generated by using Design-Expert, which depicted the interactions between two variables by keeping the other variables at their zero levels for HMP yield. It was evident that these three-dimensional plots and their corresponding contour plots provided a visual interpretation of the interaction for two variables and facilitated the location of optimum experimental conditions. The optimum values of the tested variables for the extraction of HMP were extraction temperature 88.9 °C, extraction time 128.2 min and ratio of water to solid 11.4 mL/g, the maximum predicted extraction yield of HMP was 12.71%, which corresponded fairly well to that of real extraction (12.74 ± 0.38%, $n = 3$). This result suggested that the regression model was accurate and adequate for the prediction of HMP extraction.

## 3.2. Separation and purification of HMP

DEAE-52 cellulose chromatography column as an ion exchanger is used to isolate negatively charged polysaccharides from non-negatively charged polysaccharides (Kong et al., 2010). Polysaccharides with fewer negative charges are eluted first, followed by polysaccharides with greater quantity of negative charges. By using the optimal extraction conditions, crude HMP was obtained. Then, the crude HMP was separated through an anion-exchange column of DEAE-52 cellulose. Two fractions eluted consecutively by 0.4 mol/L NaCl and 0.6 mol/L NaCl, and coded as HMP-1 and HMP-2. As shown in Fig. 2, two fractions of HMP exhibited different peak in the profile of anion-exchange chromatogram, which implied that two fractions were separated clearly. Both of HMP-1 and HMP-2 were acidic polysaccharides. The two fractions were collected, concentrated, and freeze-dried, then purified products were obtained.

## 3.3. Characterization of HMP

### 3.3.1. Contents of carbohydrate, protein and polyphenols

Table 3 showed the contents of carbohydrate, protein and polyphenols in crude HMP and its purified fractions (HMP-1 and HMP-2). The carbohydrate contents in crude HMP, HMP-1 and

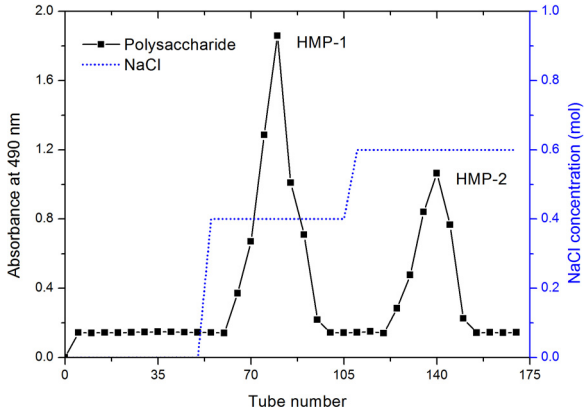


**Fig. 2.** Anion-exchange chromatogram of crude HMP detected by phenol-sulfuric acid method at 490 nm. Column: DEAE52-cellulose (2.6 cm × 60 cm); flow rate: 0.6 mL/min; fraction volume: 5 mL. HMP-1 and HMP-2 were eluted by 0.4 and 0.6 mol/L NaCl.

**Table 3**
The monosaccharide compositions and the contents of carbohydrate, protein and polyphenols for crude HMP, HMP-1 and HMP-2.

| Sample | Carbohydrate (%)[a] | Protein (%)[a] | Polyphenols (%)[a] | Sugar components (%) | | | |
|---|---|---|---|---|---|---|---|
| | | | | Glucose | Mannose | Galactose | Arabinose |
| Crude HMP | 79.19 ± 1.52b | 1.24 ± 0.12a | 1.56 ± 0.22a | 15.04 | 39.53 | 30.47 | 14.96 |
| HMP-1 | 89.24 ± 2.12a | 0.24 ± 0.08b | nd | 27.34 | 57.06 | 6.43 | 9.17 |
| HMP-2 | 91.12 ± 1.72a | 0.35 ± 0.06b | nd | 24.56 | 39.04 | 8.34 | 28.06 |

[a] Each value is expressed as means ± standard deviation ($n = 3$). Means with different letters within a column are significantly different ($P < 0.05$). nd, not detected.

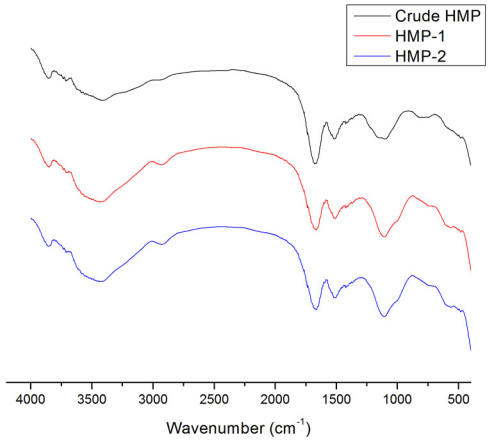


**Fig. 3.** FT-IR of polysaccharides: crude HMP, HMP-1 and HMP-2.

HMP-2 were 79.19%, 89.24% and 91.12%, respectively. In the purified fractions, no polyphenols was detected. The contents of protein in crude HMP, HMP-1 and HMP-2 were 1.24%, 0.24% and 0.35%, respectively, and more than 70% protein in crude polysaccharides was removed in the purified fractions. These results indicated that the purification process could dramatically reduce the impurities in crude HMP, such as polyphenols and proteins.

### 3.3.2. FT-IR spectrum of HMP

The characteristic absorption of crude HMP, HMP-1 and HMP-2 were identified by the FT-IR spectrum (Fig. 3). They all displayed a broad stretching intense characteristic peak at 3300–3500 $cm^{-1}$ for the hydroxyl group. A weak band at 2929–2989 $cm^{-1}$ was attributed to the C—H stretching and bending vibration. The absorption band centered at 1600–1650 $cm^{-1}$ was caused by C=O asymmetric stretching vibration. The peaks at 1000–1200 $cm^{-1}$ suggested the presence of C—O—C and C—O—H link bonds. A characteristic band at 850 $cm^{-1}$ was due to α-type glycosidic linkages.

### 3.3.3. Monosaccharide composition of HMP

The monosaccharide composition of crude HMP, HMP-1 and HMP-2 were determined by GC–MS, and the results were presented in Table 3. Crude HMP was mainly composed of glucose (15.04%), mannose (39.53%), galactose (30.47%) and arabinose (14.96%). Furthermore, the monosaccharide composition of HMP-1 and HMP-2 was different from that of crude HMP. The contents of mannose in HMP-1 were relatively higher than those in HMP-2 and crude HMP. In addition, the contents of arabinose in HMP-1 and crude HMP were lower than those in HMP-2.

### 3.3.4. Molecular weight of HMP-1 and HMP-2

For determination of the molecular weight of HMP-1 and HMP-2, GPC method was used. The average molecular weights for HMP-1 and HMP-2 were estimated to 133 and 100 kDa based on the equation of the standard curve made with a group of dextran standards.

## 3.4. Antioxidant activity in vitro of HMP

### 3.4.1. Scavenging activity on DPPH radical of HMP

The ability to act as a donor of hydrogen atoms in the transformation of the DPPH radical to its reduced form was investigated for crude HMP and its purified fractions (HMP-1 and HMP-2). The results were shown in Fig. 4a, the scavenging effect on DPPH radical of HMP-1 was higher than that of crude HMP and HMP-2, and all of them showed lower scavenging activity than Vc ($P < 0.05$). The DPPH radical scavenging activity of HMP-2 was close to crude HMP at a concentration of 1 mg/mL. At 1 mg/mL, the DPPH radical scavenging activity was 78.86, 85.26, 79.86 and 97.00% for crude HMP, HMP-1, HMP-2 and Vc, respectively. The results indicated that HMP-1 could supply more hydrogen atoms than that of crude HMP and HMP-2, and HMP-1 had a strong scavenging effect on DPPH radical.

### 3.4.2. Ferric reducing activity power of HMP

The FRAP assay is a method of measuring the ability of reductants (antioxidants) to reduce $Fe^{3+}$-$Fe^{2+}$. The formation of

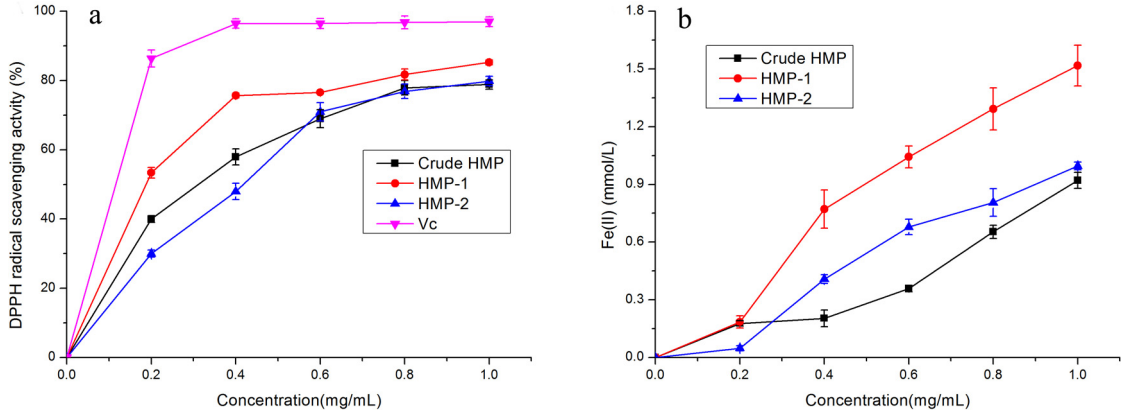


**Fig. 4.** Antioxidant activities of crude HMP, HMP-1 and HMP-2. DPPH radical scavenging activity (a); ferric reducing activity power (b). Each value is the means ± standard deviation (SD) of triplicate measurements.

blue-colored $Fe^{2+}$-TPTZ complex ($Fe^{2+}$-tripyridyltriazine) increases the absorbance at 593 nm (Kubola & Siriamornpun, 2011). The FRAP values of crude HMP, HMP-1 and HMP-2 were all increased with the increase of concentration up to 1 mg/mL (Fig. 4b). The FRAP values of HMP-1 was higher than that of crude HMP and HMP-2 ($P < 0.05$). At 1 mg/mL, the FRAP value was 0.92, 1.52 and 0.99 mmol/L for crude HMP, HMP-1 and HMP-2, respectively. These results demonstrated that HMP-1 might afford electron to $Fe^{3+}$ or bind $Fe^{3+}$ easier than HMP and HMP-2, and it possessed strong ferric reducing activity power.

Monosaccharide composition of tea polysaccharides could exert apparent effect on its antioxidant capacity (Nie & Xie, 2011). Two evaluation methods for antioxidant activity in vitro suggested that HMP-1 showed better antioxidant activity than HMP-2 and crude HMP, whatever the capacity of supplying hydrogen atoms or affording electron. The difference of their antioxidant activity might be attributed to their different monosaccharide compositions (Table 3).

## 4. Conclusion

In this study, BBD was employed to determine the optimal parameters for extraction of HMP. And the optimal extraction parameters were obtained as follows: extraction temperature 88.9 °C, extraction time 128.2 min and ratio of water to solid 11.4 mL/g. Under these optimized conditions the experimental yield agreed closely with the predicted yield. The crude HMP was further fractionated into two purified fractions of HMP-1 and HMP-2 by chromatography of DEAE-52. The average molecular weights for HMP-1 and HMP-2 were 133 and 100 kDa. No polyphenols was detected, and more than 70% protein in crude polysaccharides was removed in the purified fractions. Moreover, the crude HMP, HMP-1 and HMP-2 were characterized by FT-IR, GC–MS and antioxidant activity. The results showed that crude HMP, HMP-1 and HMP-2 had the same monosaccharide composition and similar structural characterization. Furthermore, HMP-1 exhibited strong DPPH radical scavenging activity and ferric reducing activity power. All in all, HMP could be a new source of natural antioxidants with potential value in health food.

## Acknowledgements

This work was supported by the Young Fund Project of Sichuan Provincial Department of Education (11ZB054). Sincere thanks to Shufang Yang, Le Li, Haoran Chen, Shiling Feng, Shi'an Shen and Xu Li for their support in study process.

## References

Ai, S. T., Fan, X. D., Fan, L. F., Sun, Q., Liu, Y., Tao, X. F., et al. (2013). Extraction and chemical characterization of *Angelica sinensis* polysaccharides and its antioxidant activity. *Carbohydrate Polymers*, *94*(2), 731–736.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Analytical Biochemistry*, *72*(1), 248–254.

Cai, W. R., Xie, L. L., Chen, Y., & Zhang, H. (2013). Purification, characterization and anticoagulant activity of the polysaccharides from green tea. *Carbohydrate Polymers*, *92*(2), 1086–1090.

Chen, H. X., Zhang, M., Qu, Z. S., & Xie, B. J. (2008). Antioxidant activities of different fractions of polysaccharide conjugates from green tea (*Camellia Sinensis*). *Food Chemistry*, *106*(2), 559–563.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*(3), 350–356.

Hsu, W. K., Hsu, T. H., Lin, F. Y., Cheng, Y. K., & Yang, J. P. (2013). Separation, purification, and α-glucosidase inhibition of polysaccharides from *Coriolus versicolor* LH1 mycelia. *Carbohydrate Polymers*, *92*(1), 297–306.

Ji, H. F., Zhang, L. W., Du, A. L., Yang, M. D., & Wang, Q. (2011). Effects of ultra-high pressure extraction conditions on yields and antioxidant activities of flavonoid from Laoying Tea. *International Journal of Food Engineering*, *7*(5), 6–11.

Jia, X. J., Dong, L. H., Yang, Y., Yuan, S., Zhang, Z. W., & Yuan, M. (2013). Preliminary structural characterization and antioxidant activities of polysaccharides extracted from Hawk tea (*Litsea coreana* var. *lanuginosa*). *Carbohydrate Polymers*, *95*(1), 195–199.

Jiang, G. X., Wen, L. R., Chen, F., Wu, F. W., Lin, S., Yang, B., et al. (2013). Structural characteristics and antioxidant activities of polysaccharides from longan seed. *Carbohydrate Polymers*, *92*(1), 758–764.

Kong, F. L., Zhang, M. W., Kuang, R. B., Yu, S. J., Chi, J. W., & Wei, Z. C. (2010). Antioxidant activities of different fractions of polysaccharide purified from pulp tissue of litchi (*Litchi chinensis* Sonn.). *Carbohydrate Polymers*, *81*(3), 612–616.

Kubola, J., & Siriamornpun, S. (2011). Phytochemicals and antioxidant activity of different fruit fractions (peel, pulp, aril and seed) of Thai gac (*Momordica cochinchinensis* Spreng). *Food Chemistry*, *127*(3), 1138–1145.

Nie, S. P., & Xie, M. Y. (2011). A review on the isolation and structure of tea polysaccharides and their bioactivities. *Food Hydrocolloids*, *25*(2), 144–149.

Peng, H., Zhou, M. Y., Yu, Z. P., Zhang, J. S., Ruan, R., Wan, Y. Q., et al. (2013). Fractionation and characterization of hemicelluloses from young bamboo (*Phyllostachys pubescens* Mazel) leaves. *Carbohydrate Polymers*, *95*(1), 262–271.

Peng, Z. F., Liu, M., Fang, Z. X., Wu, J. L., & Zhang, Q. Q. (2012). Composition and cytotoxicity of a novel polysaccharide from brown alga (*Laminaria japonica*). *Carbohydrate Polymers*, *89*(4), 1022–1026.

Poonkuzhali, K., & Palvannan, T. (2011). Thermostabilization of laccase by polysaccharide additives: Enhancement using central composite design of RSM. *Carbohydrate Polymers*, *86*(2), 860–864.

Rahmanian, B., Pakizeh, M., Esfandyari, M., Heshmatnezhad, F., & Maskooki, A. (2011). Fuzzy modeling and simulation for lead removal using micellar-enhanced ultrafiltration (MEUF). *Journal of Hazardous Materials*, *192*(2), 585–592.

Wang, J. G., Wang, Y. T., Liu, X. B., Yuan, Y. H., & Yue, T. L. (2013). Free radical scavenging and immunomodulatory activities of *Ganoderma lucidum* polysaccharides derivatives. *Carbohydrate Polymers*, *91*(1), 33–38.

Wang, Y. L., Zhao, Y., Yang, F., Huo, J. L., Yang, C., & Xiao, J. B. (2013). Tea polysaccharides as food antioxidants: An old woman's tale? *Food Chemistry*, *138*(2–3), 1923–1927.

Xie, J. H., Shen, M. Y., Xie, M. Y., Nie, S. P., Chen, Y., Li, C., et al. (2012). Ultrasonic-assisted extraction, antimicrobial and antioxidant activities of *Cyclocarya paliurus* (Batal.) Iljinskaja polysaccharides. *Carbohydrate Polymers*, *89*(1), 177–184.

Xu, R. J., Ye, H., Sun, Y., Tu, Y. Y., & Zeng, X. X. (2012). Preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia sinensis*). *Food and Chemical Toxicology*, *50*(7), 2473–2480.

Yang, J. J., Chen, B., & Gu, Y. (2012). Pharmacological evaluation of tea polysaccharides with antioxidant activity in gastric cancer mice. *Carbohydrate Polymers*, *90*(2), 943–947.

Ye, C. L., & Huang, Q. (2012). Extraction of polysaccharides from herbal *Scutellaria barbata* D. Don (Ban-Zhi-Lian) and their antioxidant activity. *Carbohydrate Polymers*, *89*(4), 1131–1137.

Zhao, X. J., Liu, J. G., Hu, Y. L., Fan, Y. P., Wang, D. Y., Yuan, J., et al. (2012). Optimization on condition of glycyrrhetinic acid liposome by RSM and the research of its immunological activity. *International Journal of Biological Macromolecules*, *51*(3), 299–304.

Zhu, C. P., & Liu, X. L. (2013). Optimization of extraction process of crude polysaccharides from Pomegranate peel by response surface methodology. *Carbohydrate Polymers*, *92*(2), 1197–1202.

Zinatizadeh, A. A. L., Mohamed, A. R., Abdullah, A. Z., Mashitah, M. D., Hasnain Isa, M., & Najafpour, G. D. (2006). Process modeling and analysis of palm oil mill effluent treatment in an up-flow anaerobic sludge fixed film bioreactor using response surface methodology (RSM). *Water Research*, *40*(17), 3193–3208.