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Ultrasound-assited extraction and structural identification of polysaccharides from *Isodon lophanthoides* var. gerardianus (Bentham) H. Hara

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ABSTRACT

Ultrasound-assisted extraction was employed to prepare polysaccharides from *Isodon lophanthoides* var. gerardianus (Bentham) H. Hara (*Xihuangcao*). Response surface methodology was used to optimize the ultrasound-assisted extraction parameters. Central-composite design was applied to estimate the effects of ultrasonic time, liquid/solid ratio and pH on the yield of the polysaccharides from *I. lophanthoides* var. gerardianus (Bentham) H. Hara (ILHP). A mathematical model with high fitness was obtained. Ultrasonic time, liquid/solid ratio and pH exhibited independent and interactive effects on ILHP yield. Gas chromatography analysis suggested that ILHP-3 comprised Rha, Man, Glc with relative molar percentages of 3.9%, 8.0% and 88.1%, respectively. The assay of glycosidic linkage showed that ILHP-3 was consisted of →2)-Man-(1→, →6)-Glc-(1→, →3)-Rha-(1→ with a molar proportion of 1.9:26.5:1. The average molecular weight of ILHP-3 was measured to be 247 kDa by high-performance gel permeation chromatography. Furthermore, a good antioxidant activity was observed for ILHP-3.

1. Introduction

Isodon lophanthoides var. gerardianus (Bentham) H. Hara, namely '*Xihuangcao*' traditionally in Chinese, belongs to a perennial herb of the *Labiatae* family. It has been popularly used for treatment of arthritis, enteritis, jaundice, hepatitis, lepromatous leprosy, ascariasis and acute cholecystitis (Jiang, Lu, Zhang, Zhao, & Sun, 2000). *I. lophanthoides* contains many kinds of bioactive chemicals, primarily including terpenoids, flavonoids, phenolics and polysaccharides (Lin et al., 2008; Xu, Ma, Zhou, & Sun, 1988; Xu, Wang, Li, & Fu, 1989).

In the past decades, it has been found that the polysaccharides in plants, epiphyte and animals are a potential source of bioactive products (Dourado et al., 2004). They play an important role in the growth and development of living organisms, and have attracted much attention in recent years, due to their strong biological activities (Yang et al., 2009). Published literatures have indicated that plant polysaccharides in general have strong antioxidant activities and can be explored as novel potential antioxidants (Chen, Zhang, & Xie, 2005; Wang & Luo, 2007).

Conventional extraction of bioactive compounds from plant materials requires long time, high temperature and exhibits low efficiency (Li, Ding, & Ding, 2007). Therefore, it is desirable to find an effective and economical method for bioactive compounds extraction. Ultrasound-assisted extraction has been employed for preparing bioactive compounds from different plant materials in recent years, which has been proved to be effective (Hemwimon, Pavasant, & Shotipruk, 2007; Prasad, Yang, Zhao, Ruenroengklin, & Jiang, 2009; Prasad et al., 2010; Yang, Jiang, Zhao, Shi, & Wang, 2008). The great extraction efficiency by ultrasonic treatment is mainly due to the breakage of the cell wall, and enhancement of mass transfer through the cell walls as a result of the cavitation effect (Entezari, Hagh Nazary, & Haddad Khodaparast, 2004).

Up to now, information regarding ILHP is limited. Therefore, the aim of this work was to research ILHP further and promote the exploitation of *I. lophanthoides*. In this work, the ultrasound-assisted extraction for ILHP was investigated, and then the extraction conditions were optimized using response surface methodology. Furthermore, the structural characteristics and antioxidant activity of ILHP were measured.

2. Materials and methods

2.1. Chemicals and reagents

1,1-Diphenyl-2-picryldydrazyl (DPPH), standards of xylose (Xyl), arabinose (Ara), glucose (Glc), galactose (Gal), fructose (Fru), mannose (Man), fucose (Fuc), galacturonic acid (GalA), and glucuronic acid (GlcA), and dextrans standards were purchased from Sigma Chemical Company (St. Louis, MO, USA). Sulphuric acid,

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phenol, hydrochloric acid and sodium hydroxide were obtained from Guangzhou Reagent Co. (Guangzhou, China). Other chemicals used were of analytical grade.

2.2. Plant materials

Dried stems and leaves of wild *I. lophanthoides* was obtained from Qingyuan, Guangdong province, China in September, 2009, ground into fine powder by laboratory mill (FW100, Taisite Instrument Co., Ltd., Tianjin, China), and screened through a 60-mesh sieve. The materials were stored at room temperature in a desiccator until use.

2.3. Preparation of polysaccharides

Dried *I. lophanthoides* powder was refluxed with 95% ethanol at 70 °C in a water bath for 2 h, to remove ethanol-soluble substances. Then the mixture was filtered through Whatman No. 1 filter paper, and the residue was dried at 45 °C for 12 h.

The dried residues (4.00 g) were exactly weighed and mixed with a specified amount of distilled water. The extraction process was performed using an ultrasonic cell disrupter (XO-650D, Xian'ou Biological Technology Co., Ltd., Nanjing, China, 24 kHz), with different ultrasonic times, liquid/solid ratios, pH values at the ultrasonic power of 450 W. The extract was centrifuged (8000 × *g*, 15 min), then filtered through Whatman No. 1 filter paper, and pH value of the supernatant was regulated to neutral with 2 M NaOH or HCl before concentrating to 20 mL with a rotary evaporator (RE52AA, Yarong Equipment Co., Shanghai, China) under reduced pressure at 55 °C. Four volumes of anhydrous alcohol were added to a concentration of 80%. The extract was kept overnight at 4 °C, and then centrifuged (8000 × *g*, 30 min) to obtain crude ILHP.

2.4. Central composite design

The software Design Expert (Trial Version 7.0.3, Stat-Ease Inc., Minneapolis, MN, USA) was applied to experimental design, data analysis, and model building. Central composite design was employed for optimization. Ultrasonic time (X_1), liquid/solid ratio (X_2), and pH value (X_3) were set as independent variables, while the extraction yield of ILHP (Y) was the dependent variable. The symbols and levels are given in Table 1. There were twenty experimental points in the whole design, which were carried out in a randomized order. The nonlinear computer-generated quadratic model is given as follows:

$$Y = \beta_0 + \sum_{j=1}^{K} \beta_j X_j + \sum_{j=1}^{K} \beta_{jj} X_j{}^2 + \sum \sum_{i<j} \beta_{ij} X_i X_j$$

where Y is the estimated response; β_0, β_j, β_{jj}, and β_{ij} are the regression coefficients for the intercept, linearity, square, and interaction; X_i and X_j are the independent coded variables.

2.5. Control experiment

Heat reflux extraction was also employed for ILHP preparation as a control experiment. The dry residue (4.00 g) were extracted for 2 h with 80 mL of distilled water through refluxing and then centrifuged (8000 × *g*, 15 min), filtered through Whatman No. 1 filter paper filter paper. The subsequent extraction of ILHP was the same as the above-mentioned procedures.

ILHP was also prepared under high pressure. The dry residues (4.00 g) were mixed with 80 mL of distilled water, and extracted for 2 h at 121 °C in vertical heating pressure steam sterilizer (LDZX-30KBS, Shen'an Medical Instrument Co., Shanghai, China). The subsequent extraction of ILHP was the same as above-mentioned.

Table 1
Central composite design and the responses for the extraction yield of ILHP.

Experiment	Variable levels			Responses
	X_1 Ultrasonic time (min)	X_2 Liquid/solid ratio (mg/mL)	X_3 pH	Y Yield (mg GE/g DW)
1	55.00	17.50	8.50	75.58
2	40.00	10.00	6.00	66.64
3	55.00	17.50	8.50	73.86
4	55.00	17.50	8.50	74.22
5	70.00	10.00	6.00	66.29
6	55.00	17.50	11.79	65.69
7	55.00	27.37	8.50	58.66
8	74.74	17.50	8.50	77.50
9	40.00	25.00	6.00	52.96
10	70.00	25.00	11.00	74.10
11	35.25	17.50	8.50	64.79
12	40.00	25.00	11.00	52.95
13	55.00	17.50	8.50	76.28
14	55.00	17.50	5.21	66.97
15	40.00	10.00	11.00	48.84
16	55.00	7.63	8.50	57.85
17	55.00	17.50	8.50	75.94
18	70.00	25.00	6.00	65.66
19	70.00	10.00	11.00	56.05
20	55.00	17.50	8.50	73.99

The polysaccharide content of ILHP was determined by the phenol-sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956), using glucose as standard, and the extraction yield of ILHP was expressed as milligram of glucose equivalent (GE) per gram of *I. lophanthoides* powder on dry weight (DW) basis.

2.6. Preliminary purification of ILHP

The crude ILHP was obtained under the optimum conditions of central composite design, and the proteins in crude ILHP were removed by Sevag reagent (Navarini et al., 1999). The water phase was collected and mixed with four volumes of anhydrous alcohol overnight at 4 °C to precipitate polysaccharides. After removing the precipitate, macroporous resin AB-8 (Haiguang chemical Co., Ltd., Tianjin, China) was applied to static adsorption test for decoloration of ILHP. The test was performed as follows: 200 mL of 10 mg/mL ILHP and 80 g of AB-8 (wet-weight) were mixed in a conical flask, the flask was shaken at 150 rpm and 40 °C for 12 h. Then the resin was isolated from sample solution by filtration, and four volumes of anhydrous alcohol were added to precipitate polysaccharides. The crude ILHP, ILHP after deproteinization and decoloration sequentially were dried by freeze drying, to obtain ILHP-1, ILHP-2 and ILHP-3, respectively.

2.7. Analysis of monosaccharide composition

ILHP-3 (10 mg) were hydrolyzed by 10 mL of 2 M trifluoroacetic acid at 120 °C for 2 h. Derivatization of the released monosaccharides was then carried out by the trimethylsilylation reagents according to the method described by Guentas et al. (2001). The hydrolyzed ILHP-3 mixture was dried at low pressure by a rotary evaporator (RE52AA, Yarong Instrument Co., Shanghai, China) at 60 °C. Two milliliters of pyridine, 0.4 mL of hexamethyldisilazane, and 0.2 mL of trimethylchlorosilane were added and kept at room temperature for 5 min. The trimethylsilylated derivatives (1 μL) were centrifuged at 12,000 × *g* for 15 min, and the supernatant was loaded onto a GC-2010 gas chromatography system (Shimadzu, Shanghai, China) equipped with a RTX-5 capillary column and a

flame ionization detector. The following program was adopted for gas chromatography analysis: injection temperature: 230 °C; detector temperature: 230 °C; column temperature was kept to 130 °C for 1 min, and programmed from 130 to 180 at 2 °C/min, holding for 3 min at 180 °C, then increasing to 220 at 10 °C/min, and finally holding for 3 min at 220 °C. The model of splitless was adopted. Inositol was used as the internal standard to quantify the monosaccharide content. According to the contrast of the peak position of sample with standard substance, the monosaccharide composition of ILHP-3 was identified.

2.8. Methylation analysis

Methylation of ILHP was determined by the method of Needs and Sevendran (1993) with some modification. Dry ILHP-3 (10.0 mg) were weighed precisely and dissolved in 5.0 mL of dimethyl sulphoxide before 200 mg of NaOH was added. The mixture was then treated by ultrasonic wave with an ultrasonic cleaner (KQ-300DE, Kunshan Ultrasonic Equipment Co., Kunshan, China, 40 kHz) for 10 min. After reaction for 1 h at room temperature (25 °C), 1.5 mL of methyl iodide was added for ILHP methylation. Distilled water (4.0 mL) was used to decompose methyl iodide remained and to terminate the reaction. The methylated ILHP were extracted by 4 × 3 mL of chloroform, and then washed twice with 10 mL of distilled water. The organic phase was dried at low pressure by a rotary evaporator (RE52AA, Yarong Instrument Co., Shanghai, China) at 60 °C. After hydrolysis by 10 mL of 2 M trifluoacetic acid, the ILHP hydrolysates were dissolved into 4 mL of distilled water and then 20 mg of $NaBH_4$ was added to reduce the hemiacetal bond. After reaction at room temperature for 6 h, 0.1 mL of glacial acetic acid was used to terminate the reduction. The sample was dried under low pressure and then acetylated by 2 mL of acetic anhydride and 2 mL of pyridine. Two milliliters of distilled water was used to decompose the remained acetic anhydride. The acetylated derivatives were extracted by 4 mL of methylene chloride. A gas chromatography/mass spectrometer (GCMS-QP 2010, Shimadzu, Kyoto, Japan) was used to analyze glycosidic linkage. The acetylated derivatives were loaded into an HP-1 capillary column. The temperature program was set as follows: the initial temperature of the column was 150 °C and held for 3 min, then increased to 260 at 10 °C/min, and held for 5 min at 260 °C; the flow rate was 1 mL/min; the injection temperature was 260 °C. The ion source of the mass spectrometer was set at 280 °C. One microliter of sample was injected, and the split ratio was 20:1.

2.9. Molecular weight determination of ILHP-3

Molecular weight distribution of ILHP was determined by a high-performance gel permeation chromatography (HP-GPC) with a Waters HPLC apparatus (Waters 1525, Waters Co., Ltd., USA), which was equipped with TSK-GEL Guard Column (PWXL 6.0 × 40 mm), TSKGEL4000K gel column (PWXL 7.8 mm × 300 mm) and TSK-GEL2500 K gel column (PWXL 7.8 mm × 300 mm) (TOSOH Co., Ltd., Japan), and Waters 2414 Refractive Index Detector. The detailed operation conditions were as follows: mobile phase: 0.2 M phosphate buffer (pH 7.0); flow rate: 0.6 mL/min; column temperature: 35 °C; injection volume: 30 μL; running time: 35 min.

The dextrans with various molecular weight (5200, 11,600, 23,800, 48,600, 148,000, 273,000, 410,000, 668,000, 1,400,000) were used as standard to calibrate the column and to fit the regression curve. Breeze GPC software was employed to calculate molecular weight. According to the equation of elution volume and the logarithm of their molecular weights, the molecular weight was determined.

2.10. Assay of DPPH radical scavenging activity

The DPPH radical scavenging activity was measured according to the method described by Blois (1958), with some modification. Different samples were accurately weighed and dissolved in distilled water to obtain different final concentrations (50, 100, 150, 200, 250 and 300 μg/mL). Two milliliters of 0.2 mM DPPH in ethanol were added to 2 mL of the sample solution. After vortex, the fluid was kept in dark at room temperature for 30 min. The absorbance was measured at 517 nm. The control was carried out with water instead of sample solution, while ethanol was used as the blank, Vc was used as positive standard. The scavenging activity of DPPH radicals scavenging was expressed as:

$$\text{scavenging rate}\ (\%) = \left(1 - \frac{\text{As} - \text{Ac}}{A}\right) \times 100$$

where As is the absorbance of the reaction solution, Ac is the absorbance of the solution including 2 mL of sample and 2 mL of ethyl alcohol, and *A* is the absorbance of the solution including 2 mL of DPPH and 2 mL of ethyl alcohol.

2.11. Statistical analysis

All the data were analyzed for significance by one-way ANOVA using SPSS 11 (SPSS Inc., Chicago, USA). *P*-values < 0.05 were regarded as significant.

3. Results and discussion

3.1. Effects of ultrasonic time, liquid/solid ratio and pH value on the yield of ILHP

The effects of ultrasonic time, liquid/solid ratio and pH value on the yield of ILHP and their interactions are shown in Fig. 1. As shown in Fig. 1a, ultrasonic time had a positive impact on the extraction yield of ILHP. There was an increase in the extraction yield of ILHP with the extension of ultrasonic time. However, the extraction yield of ILHP was found to increase rapidly with the increase of liquid/solid ratio from 10 to 18. However, beyond 18, the yield of ILHP decreased with increasing ratio of water to raw material. A similar phenomenon was also found for pH used in this study. The yield of ILHP was increased at low pH level and then decreased with increasing pH value at the same ultrasonic time, and reached the maximum during pH of 8.0 to 9.0 (Fig. 1b). But the ILHP yield increased with the extending ultrasonic time under the same pH. As displayed in Fig. 1c, the yield of ILHP decreased slightly with increasing pH when a low liquid/solid ratio was used. However, the yield of ILHP increased slightly with increasing pH at a high liquid/solid ratio.

The propagation of ultrasonic wave and cavitation phenomena are cited as the key factors leading to the enhancement of extraction efficiency. High shear force causes increased mass transfer of extractants (Ji, Lu, Cai, & Xu, 2006). Ultrasonic cavitation can cause superhigh temperature and pressure at micro-environment level (Kanthale, Gogate, Pandit, & Wilhelm, 2003), and free radicals (Paniwnyk, Beaufoy, Lorimer, & Mason, 2001; Vilkhu, Mawson, Simons, & Bates, 2008), which may accelerate or trigger chemical reactions of extracted compounds, and result in further increase of mass transfer. It was inferred that a higher extraction efficiency of polysaccharides at a longer ultrasonic time was due to the cavitation effect. It facilitates the diffusion of extracts by improving the osmotic pressure difference between inside and outside of the cell (Sun, Liu, Chen, Ye, & Yu, 2011). The phenomenon of higher extraction yield at suitable liquid/solid ratio was also observed by Vongsangnak, Gua, Chauvatcharin, and Zhong (2004), who found that a larger solvent volume did not lead to a higher saponin yield

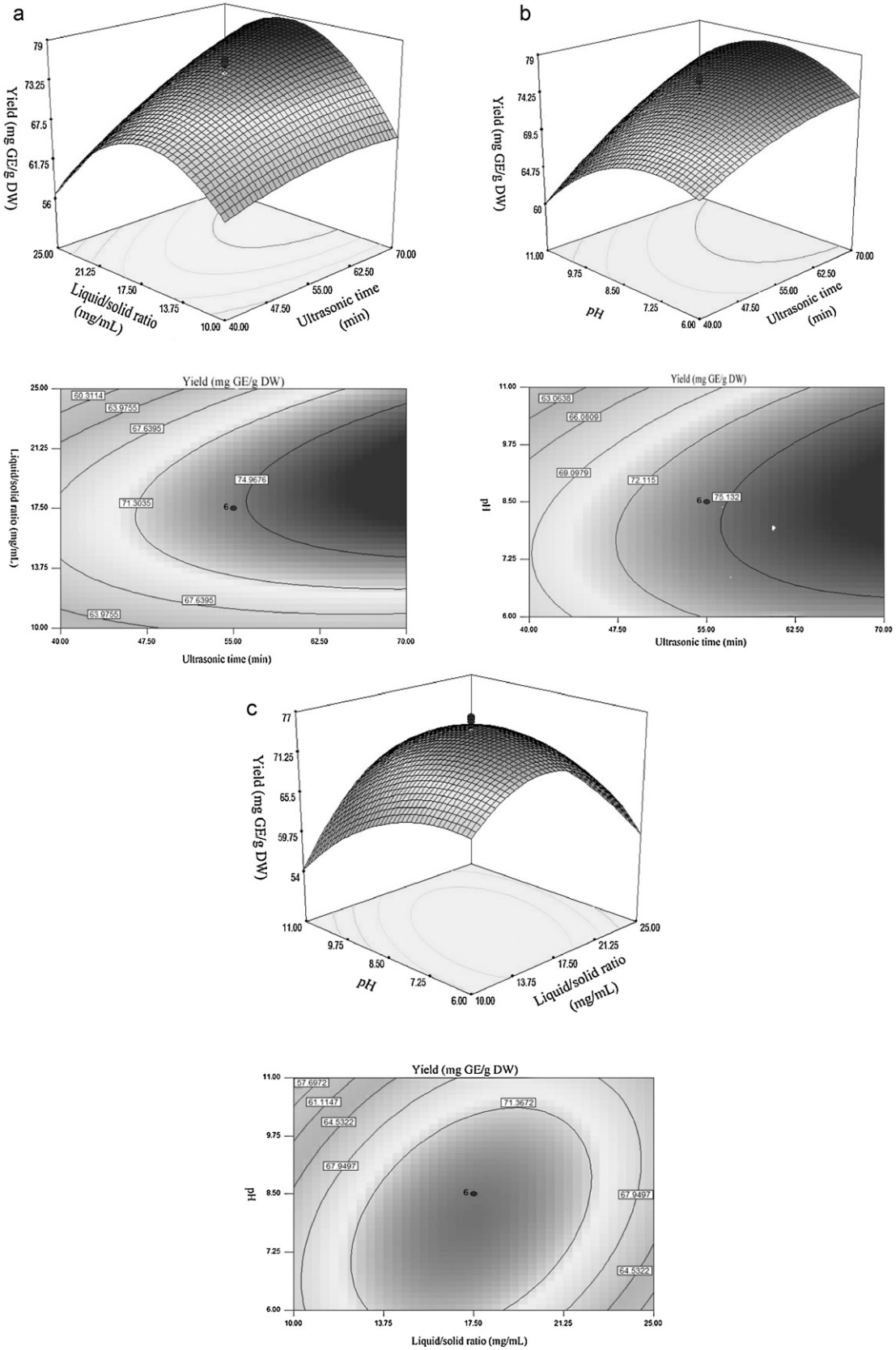

Fig. 1. Response surface plots and countour plots showing effects of ultrasonic time, liquid/solid ratio and pH on the yield of ILHP and their interaction. (a) The pH was constant at 8.50. (b) The liquid/solid ratio was constant at 17.50 mg/mL. (c) The ultrasonic time was constant for 55.00 min.

Table 2
Analysis of variance for the response surface quadratic model for the yield of ILHP.

Source	Degrees of freedom	Sum of squares	Mean square	*F*-value	*P*-value prob > *F*
Model	9	1519.36	168.82	70.32	<0.0001
Residua	10	24.01	2.40		
Lack of fit	5	18.22	3.64	3.15	0.1167
Pure error	5	5.78	1.16		
Total	19	1543.37			
X_1	1	287.77	287.77	119.87	<0.0001
X_2	1	6.93	6.93	2.89	0.1201
X_3	1	39.55	39.55	16.48	0.0023
X_1X_2	1	91.06	91.06	37.93	0.0001
X_1X_3	1	32.04	32.04	13.35	0.0044
X_2X_3	1	166.26	166.26	69.25	<0.0001
X_1^2	1	14.51	14.51	6.05	0.0337
X_2^2	1	550.10	550.10	229.14	<0.0001
X_3^2	1	124.27	124.27	51.76	<0.0001

from cells of *Panax notoginseng* by microwave extraction. The interaction effect of ultrasonic time and pH value on the yield of ILHP was supported by the polynomial equation of the model, where in higher extraction time and lower pH is more favorable for extraction of ILHP. However, in a certain range, with increasing pH, polysaccharides were easier to dissolving out, and the yield of ILHP increased.

3.2. Model fitting

The experimental conditions and the results of extraction yield of ILHP according to the factorial design are shown in Table 1, which indicated that there was a considerable variation on the yield of ILHP within the range of the extraction conditions. The multiple regression analysis was applied to the experiment data, the response variable and the test variables can be expressed by the following second-order polynomial equation:

$$Y = +74.58 + 5.01 \times X_1 + 0.78 \times X_2 - 1.86 \times X_3 + 3.37 \times X_1 \times X_2 + 2.00 \times X_1 \times X_3 + 4.56 \times X_2 \times X_3 - 1.44 \times X_1^2 - 8.89 \times X_2^2 - 4.22 \times X_3^2$$

where Y is the extraction yield of ILHP, X_1, X_2, X_3 are the coded variables for ultrasonic time, liquid/solid ratio and pH, respectively.

Generally, there may be poor or misleading result in the exploration and optimization of a fitted response surface, unless the model exhibits a good fitness, which makes the model adequacy investigation essential (Liyana-Pathirana & Shahidi, 2005). The *P*-value of the model was significant ($P < 0.0001$), while the lack of the fitted value of the model was 0.1167 ($P > 0.05$, not significant). In addition, coefficient (R^2 of determination is defined as the ratio of the explained variation to the total variation and is a measurement of the degree of fitness (Nath & Chattopadhyay, 2007). A small value of R^2 indicates a poor relevance of the dependent variables in the model. In this study, the value of R^2 (0.9844) indicated a good agreement between the predicted and veritable values of ILHP yield. The value of the Adj-R^2(0.9704) also suggested that the model was highly significant. There was only about 3% of the total variation could not be explained by the model. And the "Pred R-Squared" of 0.9120 is in reasonable agreement with the "Adj R-Squared" of 0.9704. All these values indicated that the model exhibited a good fitness to the true behavior of the system.

The coefficient estimates of the model equation, along with the corresponding *P*-values and *F*-values, are listed in Table 2. The *P*-value is a parameter to check the significance of each coefficient, which also indicates the interaction between each independent variable. A small *P*-value less than 0.05 indicates a significant effect of the term.

3.3. Validation of the model and control experiment

According to the result of response surface, the optimal conditions were ultrasonic time 66.04 min; liquid/solid ratio 17.76 mL/g; pH 8.30. In order to assure the adequacy of the model equation, a verification experiment was carried out under the optimal conditions. The model predicted a maximum response of 77.58 mg GE/g DW. In the verification experiment, the yield of ILHP was 77.15 ± 1.02 mg GE/g DW ($n = 3$). There was no significant difference between the predicted value and practical value within the 95% confidence interval. This good correlation confirmed that the response model was adequate for reflecting the expected optimization. The results also indicated that the model was adequate for the extraction process.

In the control experiment, the yield of ILHP obtained by heat reflux extraction and high pressure extraction were 40.39 ± 0.34 and 50.75 ± 0.52 mg GE/g DW, respectively. Compared to the optimal condition of ultrasound-assisted extraction, the yield of ILHP was much higher than the control. Moreover, ultrasound-assisted extraction was very time-saving. The result was in agreement with previous investigation (Hemwimol, Pavasant, & Shotipruk, 2006; Hromadkova, Ebringerova, & Valachovic, 1999).

3.4. Monosaccharide composition and glycosidic linkages of ILHP-3

The ILHP-3 was hydrolyzed by trifluoracetic acid into individual monosaccharides. They were further trimethyl silylated for gas chromatography analysis. The experimental results are summarized in Table 3. Three monosaccharides, including rhamnose, mannose and glucose, were found to be present in ILHP-3 after comparison of the retention time with monosaccharide standards. And their molar percentages were 3.89%, 8.02% and 88.09%, respectively. The results indicated that D-glucose was the predominant monosaccharide and constructed the backbone for ILHP-3, and a similar result was found in the polysaccharides isolated from ganoderma (Chen, Xie, Nie, Li, & Wang, 2008). Methylation by methyl

Table 3
The monosaccharide composition and glycosidic linkages of ILHP-3.

Monosaccharide	Relative molar percentages (%)
Rha	3.9 ± 0.17
Man	8.0 ± 0.25
Glc	88.1 ± 0.37
Glycosidic linkage	**Molar proportion**
→2)-Man-(1→	1.9
→6)-Glc-(1→	26.5
→3)-Rha(1→	1

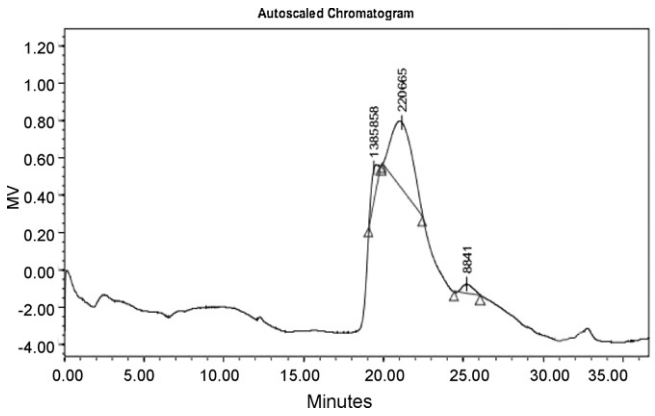

Fig. 2. The molecular weight distribution of ILHP-3.

iodide is a classical method to form a methoxyl group on the polysaccharide chain. And methylation analysis of ILHP-3 by GC-MS revealed three types of glycosidic linkages, which were →2)-Man-(1→, →6)-Glc-(1→, →3)-Rha-(1→ at a molar ratio of 1.9:26.5:1 corresponding to the peak areas (Table 3). The results indicated that there was no branch in ILHP-3.

3.5. Molecular weight of ILHP-3

Gel permeation chromatography has been proved to be an effective method for the polysaccharide molecular weight determination (Dreher, Hawthorne, & Grant, 1979). In this study, high-performance gel permeation chromatography was applied to elucidate the molecular weights (Mw) of ILHP-3. The equation of the standard curve was: $\text{Log Mw} = 37.8 - 5.81\,V + 0.363\,V^2 - 0.00836\,V^3$ (where Mw represents the molecular weight, while V represents elution volume) with a correlation coefficient of 0.996. The ILHP-3 was eluted as a main peak followed by two small peaks, which could be seen in Fig. 2. The average molecular weight of ILHP-3 was estimated to be 247 kDa.

3.6. DPPH radical scavenging activity of ILHP

The method of scavenging the DPPH radical is commonly used for rapidly evaluating antioxidant activity in a relatively short time compared with other methods (Yuan et al., 2005). In this study, the DPPH radical scavenging activities of ILHP solutions at different concentrations were measured. As shown in Fig. 3, ILHP-3 exhibited obvious scavenging activity on DPPH radical in a concentration-dependent manner. The scavenging capacity were proportionally improved with increasing concentration of ILHP-3, and the highest DPPH radical scavenging activity of ILHP-3 was 62.92% at 300 μg/mL. However, the DPPH radical scavenging activity of ILHP-1 and ILHP-2 increased slightly when increasing concentration from 50 to 100 μg/mL and then remained relatively constant. The possible reason was attributed to the steric hindrance effect. The calculation of IC_{50} (concentration of 50% scavenging activity) of ILHP-3 and Vc were 211.93 and 12.05 μg/mL, respectively. The oligosaccharides from longan fruit pericarp has been found to exhibit a good antioxidant activity against DPPH radicals with IC_{50} value of 177.09 μg/mL (Jiang et al., 2009). The antioxidant properties of polysaccharides from *Ganoderma tsugae* indicated a low IC_{50} value of 2.84 mg/mL against DPPH radical (Tseng, Yang, & Mau, 2008). DPPH, a relatively stable nitrogen-centered free radical, is noticeable as color change from purple to yellow when reduced by hydrogen donation. The mechanism of DPPH radical scavenging activity is based on the reduction of DPPH$^{\bullet}$ to DPPH-H in the presence of a hydrogen-donating antioxidant, leading to the fading of purple color and therefore to inhibit the propagation of oxidizing reaction. It is widely accepted that hydrogen-donating ability of the antioxidant is responsible for its free radical-scavenging activity (Sentandreu, Navarro, & Sendra, 2008). In this study, ILHP showed a good antioxidant activity against DPPH radicals, which was probably due to their hydrogen donation power to the free radicals, thereby terminating the radical chain reaction further (Lai, Wen, Li, Wu, & Li, 2010).

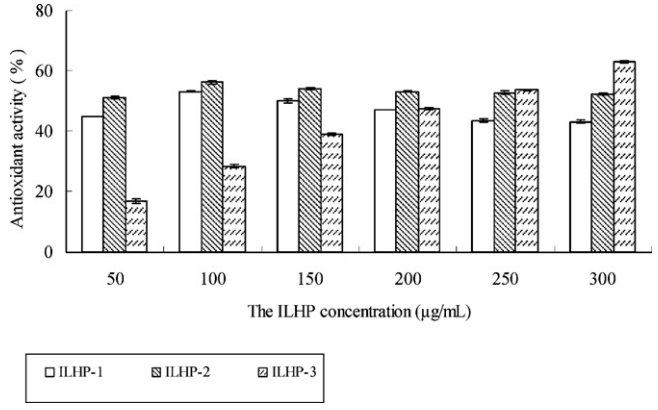

Fig. 3. DPPH radical scavenging activities of ILHP. Each value represents the mean ± standard deviation ($n = 3$). For each activity, the values having the same letter are not significantly different ($P > 0.05$).

4. Conclusions

In this study, the response surface methodology was proved to be useful for the optimization of the polysaccharides extraction from *I. lophanthoides*. And a mathematical model with high fitness was constructed. In contrast with heat reflux extraction and high pressure extraction, ultrasound-assisted extraction showed a higher extraction efficiency. ILHP-3 comprised of Rha, Man, Glc with molar percentages of 3.9%, 8.0% and 88.1%, respectively. The assay of glycosidic linkage showed that ILHP-3 consisted of →2)-Man-(1→, →6)-Glc-(1→, →3)-Rha-(1→with a molar proportion of 1.9:26.5:1. The average molecular weight of ILHP-3 was measured to be 247 kDa. In addition, ILHP-3 showed good antioxidant potential according to the *in vitro* evaluation of DPPH radical scavenging activity. Further purification and bioactivity investigation of ILHP is worthy to do in the future work for better exploiting this traditional Chinese medicine.

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