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

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ARTICLE INFO

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

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

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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 β_0, β_j, β_{jj} and β_{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).