Carbohydrate Polymers 86 (2011) 1175–1180

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Extraction of *Polygonatum odoratum* polysaccharides using response surface methodology and preparation of a compound beverage

Gaoshuang Lan, Haixia Chen*, Zhaoshuai Wang, Wenjing Zhang, Likang Zhang

Tianjin Key Laboratory for Modern Drug Delivery & High-Efficiency, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin 300072, PR China

ARTICLE INFO

Article history:
Received 29 March 2011
Received in revised form 27 May 2011
Accepted 6 June 2011
Available online 12 June 2011

Keywords:
Polygonatum odoratum
Polysaccharides
Response surface methodology
Orthogonal test
Beverage

ABSTRACT

An ultrasonic procedure for the extraction of *Polygonatum odoratum* (Mill.) Druc (*P. odoratum*) polysaccharides was established. Response surface methodology (RSM) was applied to optimize the ultrasound-assisted extraction parameters (ultrasonic time (X_1), extraction times (X_2), and ratio of solvent to raw material (X_3)) for enhancing the forward extraction efficiency of polysaccharides. The optimum extraction conditions were found to be ultrasonic time 40 min, extraction times 3, and ratio of water to raw material 80. Under these conditions, the yield of *P. odoratum* polysaccharides can increase from 11.40% to 15.15%. The physicochemical properties of *P. odoratum* polysaccharides were characterised. The results of monosaccharide composition by gas chromatography (GC) showed that the polysaccharides consisted of fucose, mannose, galactose, with the molecular ratio of 4.72:3.90:1.00. Four factor, three-level designed orthogonal experiment was developed to better understand how flavor of compound beverage is affected by different factors. The optimal combination parameters of the processing technology were *P. odoratum polysaccharide* solution (45.4%), hawthorn juice (18.2%), apple juice (36.4%), glucose (4%) and citric acid (0.2%).

1. Introduction

Polygonatum odoratum (Mill.) Druc (*P. odoratum*, Yu Zhu in Chinese) belongs to Polygonatum, Liliaceae large family, which grows wildly and is cultivated in the southern area of China. In addition to China, *P. odoratum* grows in Thailand and Vietnam. It can also be found growing throughout the southern United States. *P. odoratum* has a long history of indigenous use such as a condiment and it has also been used as a crude medicinal agent in the treatment of analeptic (Tomoda, Yoshiko, Tanaka, & Uno, 1971). In China it has been used as functional foods and well-known Chinese traditional medicine with the functions of removing dryness, promoting secretion of fluid and quenching thirst, treatment of diverse diseases for example diabetes etc. (Liu, Fu, & Cui, 1998; Zhou, Tang, Gao, & Zhou, 2005). *P. odoratum* was reported to reduce significantly hyperglycemia caused by starch loading in normal and diabetic mice and the effect was similar to that of acarbose (Chen, Feng, Guo, Sun, & Jiang, 2001). In southern China, people like to cook it with meats or porridges as health foods. *P. odoratum* is attracting more and more attention for its healthy function value.

There are many types of compounds that exists in *P. odoratum*. Compounds that have been previously identified in *P. odoratum* include quercitol (Lazer, Gheta, & Grigorescu, 1971), flavonoids (Yang, Chen, Chen, Yang, & Liu, 2005), azetidine 2-carboxylic acid (Fowden, 1956), mucous polysaccharides (Tomoda et al., 1971), and steroidal compounds (Lin, Han, & Liao, 1994; Sugiyama, Nakano, Tomimatsu, & Nohara, 1984). Among them, polysaccharide is one of the main bioactivity components of *P. odoratum* with the hypoglycemic, antioxidant, antitumor and hypotense activities. There were some reports on the extraction and activities of polysaccharides of *P. odoratum* (POPS) in recent years. Ultrasound extraction is the new technology that attracts much more attention in the department of separation and extraction. The application of ultrasound-assisted extraction offers many advantages including the reduction of solvents, temperature and the time for extraction. But there was no information on ultrasonic assisted extraction of *P. odoratum* polysaccharide (POPS) using response surface methodology (RSM) and there was no report on the beverage about *P. odoratum* polysaccharide till now.

In the present study, RSM was employed to estimate effects of different extraction parameters on yields of polysaccharide from *P. odoratum*. At the same time, an orthogonal test was employed to estimate optimal combination parameters of three raw materials (hawthorn juice, *P. odoratum* polysaccharide juice, and apple juice) for the preparation of a compound beverage.

* Corresponding author. Tel.: +86 22 2740 1483; fax: +86 22 2789 2025.
E-mail address: chennhxx@yahoo.com.cn (H. Chen).

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doi:10.1016/j.carbpol.2011.06.009

2. Materials and methods

2.1. Materials

Rhizoma of *P. odoratum* was collected in November 2009 from natural habitat in Kuandian County (Liaoning, China) and authenticated by associated Professor Haixia Chen, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, where a voucher specimen (TJC2009003) has been deposited. Hawthorn and apple were obtained from the local fruit market of Tianjin, China. White granulated sugar and citric acid were food-grade. All other chemicals were of analytical grade.

2.2. Experimental design

A three-level-three-factor, Box-Behnken factorial design (BBD) was employed in this optimization study. Ultrasonic time (X_1), extraction times (X_2), and ratio of water to raw material (X_3) were the independent variables selected to be optimized for the extraction of *P. odoratum*. Extraction yield (Y) was taken as the response of the design experiments. Seventeen experiments were augmented with three replications were carried out at the center points to evaluate the pure error.

Once the experiments are performed, the response variable (extraction yield) was fitted a second-order model in order to correlate the response variable to the independent variable. The general form of the second-order polynomial equation is as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} {X_i}^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

where Y is the predicted response; X_i and X_j are input variables which influence the response variable Y; β_0 is a constant; β_i is the linear coefficient; β_{ii} is the quadratic coefficient and β_{ij} $(i \neq j)$ is the linear- by-linear interaction between X_i and X_j. The test variables were transformed to range between −1 and 1 for the appraisals of factors.

2.3. Ultrasonic extraction (UAE) of crude polysaccharides (UPS)

Dried ground samples (10 g) were extracted with water at the corresponding ultrasonic conditions. The water extraction solutions were obtained by centrifugation (2000 × *g* for 10 min, at 20 °C), and then concentrated and precipitated by the addition of dehydrated alcohol to a final concentration of 75% (v/v). The precipitates collected by centrifugation (2000 × *g* for 10 min, at 20 °C) were washed by dehydrated alcohol for three times and freezing dried. The sugar content was measured by phenol–sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using D-glucose as a standard (R^2 = 0.9949), the purity (%) was calculated as the glucose content of extraction/dried crude polysaccharide weight.

2.4. Hot water extraction (HWE) of crude polysaccharides (PS)

Dried ground *P. odoratum* samples (10 g) were extracted with water at 100 °C (1:56 (w/v), 3 h, 3 times). The water extraction solutions were separated by centrifugation (2000 × *g* for 10 min, at 20 °C), and then were concentrated and precipitated by the addition of dehydrated alcohol to a final concentration of 75% (v/v). The precipitates were collected by centrifugation (2000 × *g* for 10 min, at 20 °C) and were washed by dehydrated alcohol for three times and then were freezing dried.

2.5. Molecular weight distribution

The molecular weight of polysaccharides was determined using gel permeation chromatography (GPC) on Sephadex G-150 column (60 × 2.5 cm, i.d.). The column was eluted by 0.02 M PBS at a flow rate of 8.0 mL/h. Fraction was collected for every 4 mL. The total carbohydrate of each fraction was determined by using phenol–sulfuric acid method. The molecular weight of polysaccharides was obtained from the regression line of the standard molecular weight compared with fraction number plot. The calibration curve was made with dextran standards of different molecular weighs (Dextran T-500, T-70, T-40, and T-10) (Fu, Chen, Dong, Zhang, & Zhang, 2010).

2.6. Determination of the monosaccharide composition

The composition of neutral monosaccharide of UPS and PS was measured by gas chromatography after converting them into acetylated derivatives (Chaplin & Kennedy, 1994). Briefly, 30 mg of different samples were hydrolyzed in a sealed glass tube with 2 M trifluoroacetic acid (TFA) at 120 °C for 4 h. The hydrolysate was evaporated to dryness. The acid was removed under reduced pressure by repeated coevaporations with methanol. The hydrolysate was then converted into alditol acetates according to conventional procedures. Gas chromatography was performed on a Shimadzu GC-14B instrument with capillary column (HP-5, 30 m × 0.32 mm × 0.5 μm). The operation was performed in the following conditions: injection temperature: 250 °C; detector temperature: 260 °C; column temperature programmed: 150–210 °C increasing at 10 °C/min for 6 min; then increasing to 255 °C at 15 °C/min for 3 min; and finally increasing to 260 °C at 1 °C/min for 5 min. Nitrogen was used as the carrier gas and maintained at 1.0 mL/min. Arabinose, xylose, galactose, glucose, rhamnose, mannose and fructose were used as the standards.

2.7. Morphological analysis

Scanning electron micrographs were obtained with an environmental scanning electron microscope (ESEM, Philips XL-30, Philips-FEI Co., Eindhoven, The Netherlands). The polysaccharide samples of were placed on a specimen holder with the help double-sided adhesive tapes and coated with gold powder (Yu, Wang, Jin, Sun, & Yu, 2009). Each sample was observed with 5000-fold magnification at an accelerating potential of 20 kV during micrography.

2.8. Beverage formulation

2.8.1. Preparation of hawthorn juice

The hawthorn berry extract was prepared in accordance with conventional method by using water. In brief, fresh fruit was washed, cleaned, and crushed into particles of 90 mesh, and infiltrated with hot water (90 °C, hawthorn berry-water (g/mL) ratio 1:6) for 30 min. The particles were further ground by a gum machine into a size less than 15 μm. After enough amount of water was added (based on the amount of the solid content in fresh hawthorn) and uniformly stirred at a temperature lower than 50 °C. The mixture was kept at a temperature ranging from 30 to 70 °C for a period ranging from 1 to 6 h and was then hydrolyzed for 6 h and filtered. The filtrate was ultra-filtered and sterilized with ultrasonication (25 kHz, 1500 W, power density 100 W/cm^2, and flowing speed 4 m/s). The resulting extract was filtrated and concentrated to a formation of a concentrated hawthorn berry extract.

2.8.2. Preparation of apple juice

Good-quality apple juice is made from a blend of apple varieties. Briefly, after selected and washed, fresh apples were directly