Isolation of Water Soluble Polysaccharides from *Morinda officinalis* How*

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Abstract: The water soluble polysaccharides were extracted with hot water and subsequently precipitated with ethanol from *Morinda officinalis* How (MOP). And the crude polysaccharides were then isolated by anion-exchange chromatography. Effect of different chromatography conditions on adsorption and elution of MOP were discussed, and the results showed that, the adsorbent exhibited better adsorption performance for MOP under higher pH and lower ion strength of buffer solutionn. Suitable column chromatography condition obtained was: Tris concentration of buffer 0.02 mol/L, pH of buffer 8.0 ~ 8.5 and flow rate 1.5 mL/min. Four polysaccharide fractions, named MOHP-Ⅰ, MOHP-Ⅱ, MOHP-Ⅲ and MOHP-Ⅳ respectively were obtained using step-wise elution manner.

Key words: *Morinda officinalis* How; polysaccharides; isolation; effect
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巴戟天水溶性多糖分离纯化的研究

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摘要: 采取水提醇沉工艺获得了巴戟天粗多糖MOP,用离子交换柱层析方法对粗多糖进行了分级纯化,考察了不同层析缓冲液条件下,巴戟天多糖的动态吸附情况。结果表明:较高pH值和低离子强度的缓冲液有利于巴戟天多糖的吸附,确定层析用缓冲液pH值为8.0~8.5,Tris浓度为0.02 mol/L,流速1.5 mL/min;在此层析条件下,采用阶段梯度洗脱方式,在NaCl浓度为0, 0.1和0.5 mol/L时分别获得巴戟天多糖组分MOHP-Ⅰ, MOHP-Ⅱ, MOHP-Ⅲ,用NaOH洗脱得到了MOHP-Ⅳ组分。

关键词: 巴戟天; 多糖; 分离; 影响

Polysaccharide is another important macromolecular compound apart from nucleic acid and protein. Within the last few years, tremendous advances have been made in the research of polysaccharides. Later work has shown that polysaccharides are involved in a number of biochemically important functions, such as cell-cell interaction and communication, attachment for infectious bacteria, viruses, toxins and hormones, to mention just a few. Recent research of polysaccharides mainly focus on the isolation, structure, pharmacology and structure-activity relation, among of which, isolation of polysaccharides is a prerequisite for other research.

Morinda officinalis How (family Rubiaceae) is a

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plant extensively used as a Yang-tonic agent for about 2 000 years in China. The plant grows mainly in humid areas of southeast China, such as Guangdong, Fujian, Guangxi Province. Previous research showed that[1], the water or ethanolic extracts of *Morinda officinalis* exhibited anti-aging, antidepressant and immunocompetence promotion action. Owing to being abundant in carbohydrates, anthraquinone, amino acid, organic acid and vitamin, *Morinda officinalis* is a valuable plant suitable for both medicinal and food use. Carbohydrates, accounting for 49.79% ~ 58.25%, is a significant constituent of *Morinda officinalis*. CAI et al[2]. researched the water extracts of *Morinda officinalis*. Using chemical and spectroscopic methods, they obtained four inulin-type oligosaccharides-nystose, 1F-fructofuranosylnystose (O-β-D- fructofuranosyl-[(2→1)- O-β-D-fructofuransyl]$_3$-α-D-glucopyranoside), inulin-type hexasaccharide (O-β-D-fructofuranosyl-[(2→1)- O-β-D-fructofuransyl]$_4$-α-D-glucopyranoside) and inulin-type heptasaccharide (O-β-D-fructofuranosyl-[(2→1)- O-β-D-fructofuransyl]$_5$-α-D-glucopyranoside) respectively. Through animal experiments, ZHANG et al[3]. drew the conclusion that, the water extracts of *Morinda officinalis* mainly including these oligosaccharides possessed antidepressant effect.

This study is concerned with the isolation of water soluble polysaccharides present in *Morinda officinalis*, which has not been reported previously. Effects of various chromatography conditions such as pH, ion strength and velocity of mobile phase on polysaccharides adsorption are investigated in detail. The optimization of protocol for the isolation will help to prepare well-defined polysaccharides fractions in sufficient amount to ensure further research in structure and pharmacology.

1　Materlal and Methods

1.1　Materials

The *Morinda officinalis* were purchased from the planter in Deqing County, Guangdong Province, China. DEAE Sepharose CL-6B was obtained from Pharmacia Biotech (Uppsala, Sweden). All other reagents were of analytical grade.

1.2　Methods

1.2.1　Extraction of polysaccharides from *Morinda officinalis*

The ground dried roots of *Morinda officinalis* (200 g) were defatted with acetone. The defatted meal was extracted with distilled water (3 L) at 60 ℃ stirring for 3 h. The extractive was centrifugated at 8 000 r/min for 20 min in a high-speed centrifuge (model 3K3D, Sigma, Germany). The pellet was resuspended, and this procedure was repeated again. All the supernatants were collected, and concentrated to 1/5 volume under vacuum subsequently. The residue was extracted with Sevag reagent ($CHCl_3$:"BuOH =4∶1) five times to deproteinize. After removing the Sevag reagent, nine volumes of absolute ethanol was added to the extract and kept at 4 ℃ overnight in refrigerator to precipitate polysaccharides. The precipitate was redissolved in distilled water, then added to absolute ethanol. This procedure must be repeated three times to obtain polysaccharides as more as possible. Lastly, the precipitate was frozen at −80 ℃ overnight and lyophilized in vacuum freeze dryer (model ALPHA 2 − 4, Christ, Germany) to obtain 10.6 g brown polysaccharides powder, named MOP.

1.2.2　Fractional isolation of MOP

Solutions of MOP were fractionated on a column (50 cm × 2.6 cm) of DEAE Sepharose CL-6B[4,5] previously equilibrated with the start buffer. Elution was carried out under different velocity using Tris-HCl buffer solution with different Tris concentration and pH as eluent. Fractions were collected (5 mL) and analysed for total carbohydrate content and protein by the phenol-sulfuric acid method and spectrophotometric. The breakthrough curve of MOP was drawn according with the total carbohydrate content of each fraction, as well as the adsorption capacity of MOP on the anion- exchange adsorbent was calculated.

1.2.3　Determination of total carbohydrate content

Total carbohydrate content was determined by the modified phenol-sulfuric acid method as follows. 2 mL of appropriate dilute sample (concentrations between 20 − 100 μg/mL) was mixed with 1 mL of 80%

(W/W) phenol solution[6], then incubated with 5 mL sulfuric acid for 20 min at room temperature. The absorbance of the solution was measured at 490 nm against the water blank. A calibration curve using glucose as standard was established.

1.2.4 Determination of protein

The fractions were determined the protein content using spectrophotometric analysis measured at 280 nm against the water blank.

2 Results and analyse

2.1 Effect of Tris concentration of eluent on MOP adsorption

The water extracts of *Morinda officinalis*, MOP were dissolved in the Tris-HCl buffer of pH = 8.0 which Tris concentration was 0.02, 0.05, 0.1 and 0.2 mol/L respectively, and continuously pumped onto the column packed with DEAE Sepharose CL-6B at a flow rate of 1.5 mL/min. The breakthrough curve was drawn according with the total carbohydrate content change of each fraction, and shown as Fig. 1. And the adsorption capacity under each Tris concentration was 0.11, 0.053, 0.044 and 0.047 mg MOP/mL adsorbent.

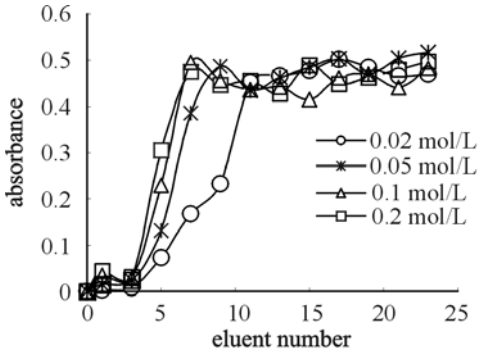

图1 不同Tris浓度下巴戟天多糖的穿透曲线

Fig. 1 Breakthrough curves of MOP with different Tris concentration of eluent

2.2 Effect of pH of eluent on MOP adsorption

Fig. 2 presented the changes in breakthrough curve with different pH (7.0, 7.5, 8.0, 8.5 and 9.0) of eluent buffer, and other conditions were: 0.02 mol/L of Tris concentration, 1.5 mL/min of flow rate. Corresponding adsorption capacity was 0.12, 0.11, 0.14 and 0.17 mg MOP/mL adsorbent.

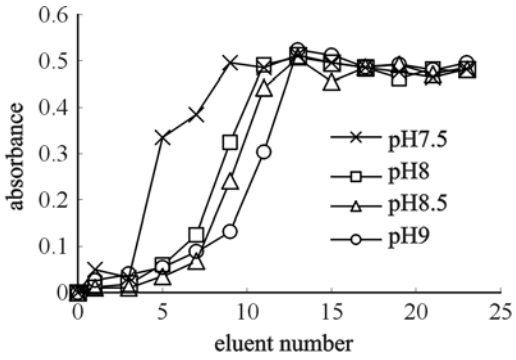

图2 不同pH下巴戟天多糖的穿透曲线

Fig. 2 Breakthrough curves of MOP with different pH of eluent

2.3 Effect of velocity of eluent on MOP adsorption

The influence of different velocity (0.5, 1.0, 1.5 and 2.0 mL/min) of eluent on MOP adsorption was illuminated by the breakthrough curves in Fig. 3, and used eluent was Tris-HCl solution of pH = 8.0 with Tris concentration 0.02 mol/L. The calculated adsorption capacity data was 0.13, 0.12, 0.12 and 0.096 mg MOP/mL adsorbent respectively.

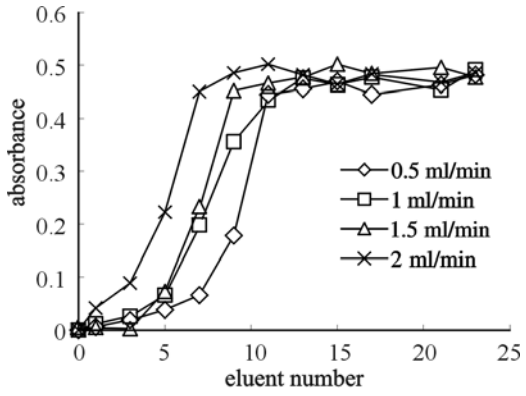

图3 不同流速下巴戟天多糖的穿透曲线

Fig. 3 Breakthrough curves of MOP under different velocity of eluent

2.4 Elution profile for the isolation of MOP

On the basis of the above adsorption experiments, we discussed the elution conditions of the MOP. Solutions (5 mL) of MOP (50 mg) in 0.02 mol/L Tris-HCl buffer of pH = 8.0 were loaded onto the column. Elution was carried out with the same buffer containing 0, 0.1, 0.2, 0.5, 1.0, 2.0 mol/L NaCl successively and finally with 1.0 mol/L NaOH at a flow rate of 1.5 mL/min. The column fractions (5 mL) were monitored for total carbohydrates content and protein using the phenol-sulfuric acid and

UV-spectrophotometric. The elution profile was shown in Fig. 4.

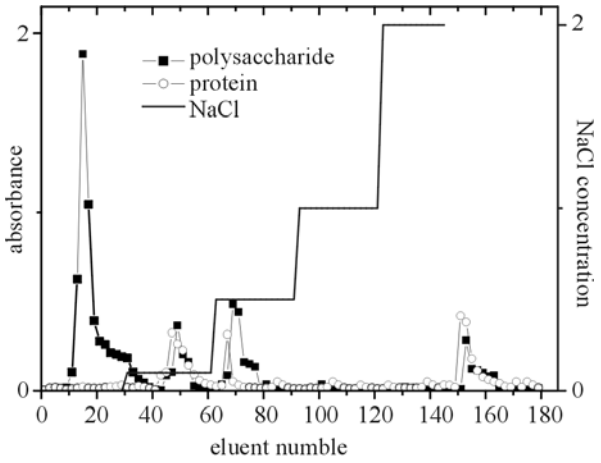

图 4　巴戟天多糖在DEAE Sepharose CL-6B柱上的洗脱曲线

Fig. 4　Elution profile of MOP on DEAE Sepharose CL-6B column

3　Discussion

It is known to us all that the primary principle of ion-exchange chromatography is the ion exchange of adsorbent and the isolated sample, so the presence of ion is prerequisite. Polysaccharides are often weakly ionized in alkaline solution[7], and the ionization will weaken when the quantity of free ion in solution increase, which is disadvantageous to the adsorption of polysaccharides on adsorbent. So there should be less free ion in buffer as possible as can in order to benefit for the adsorption of polysaccharides. The data of adsorption capacity under different Tris concentration also verified that, the adsorption capacity had a downtrend with the increase of Tris concentration, and had a highest value when the Tris concentration was 0.02 mol/L. But the breakthrough curves in Fig. 1 displayed that, with the decrease of Tris concentration, the time of reaching adsorption equilibrium had a slight increase. Besides, a certain numble of free ion was essential to maintain the buffer capacity of solution. Therefore, 0.02 mol/L of Tris concentration was appropriate to adsorption of MOP.

pH of buffer solution had great effect on the adsorption of MOP. The adsorption capacity of MOP increased with the increment of pH, while the breakthrough curves in Fig. 2 right moved obviously, predicating the prolong of adsorption equilibrium time. This is due to the alkaline environment is advantage to ionize of polysaccharide, which will reinforce the ion exchange, resulting in more polysaccharides being adsorbed onto adsorbent. On the other hand, exorbitant pH will bring too firm attraction between polysaccharides and adsorbent, therefore, resulting in difficulty for the sequent elution. We determined the recovery polysaccharides after chromatography only 50% of the initial polysaccharides. So, there was a balanced pH to guarantee both adsorption capacity of MOP and facility of elution operation. In this experiment, 8.0 ~ 8.5 was the exact pH range for adsorption of MOP.

Velocity of eluent adopted in chromatography also influences adsorption of polysaccharides. High velocity will enhance the radial and axial diffusion in chromatography column, hence, broaden flow unit and increase the height equivalent to one theoretical plate, which result in the reduce of the adsorption of polysaccharides on adsorbent. Low velocity can improve adsorption capacity, but will increase the molecular diffusion and prolong elution time. In the H-u curve of Van Deemter equation, there is a optimal velocity for chromatography. The curves in Fig. 3 illuminated obvious prolong of time for adsorption equilibrium, and data of adsorption capacity changed slightly. Taken together, we choose 1.5 mL/min as proper velocity.

Following the above adsorption experiments, the elution conditions were researched. Using step-wise elution manner, we respectively obtained MOHP-Ⅰ (21.8 mg), MOHP-Ⅱ (6.9 mg), MOHP-Ⅲ (12.2 mg) with 0, 0.1, 0.5 mol/L of NaCl concentration and MOHP-Ⅳ (3.7) with NaOH. Elution profile Fig. 4 showed that, there were no fractions under high NaCl concentrations such as 1.0 and 2.0 mol/L. Absorbance curve at 280 nm revealed that there contained protein in all fractions except for MOHP-Ⅰ. Experiments data also showed that the DEAE Sepharose CL-6B was reused up to five cycles without decrease in its capacity.

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In conclusion, a simple, easy and cheap protocol for the isolation of polysaccharides from *Morinda officinalis* using anion-exchange chromatography was achieved. The acquirement of purified polysaccharide could assure the demand for next research in structure and pharmacology of *Morinda officinalis*.

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