

Optimization of enrichment and purification of polyphenols from blueberries (*Vaccinium* spp.) by macroporous resins XAD-7HP

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ABSTRACT

Blueberries are rich sources of phenolic compounds and exhibit strong antioxidant activities and a wide variety of biological activities. Polyphenols were extracted from blueberry by solvent extraction method using ethanol solution. The purification of polyphenols from blueberries (*Vaccinium* spp.) by macroporous resin XAD-7HP was optimized by adsorption and desorption tests. The cellular antioxidant activities of the purified polyphenols were investigated. The adsorption data indicated that the process was best explained by the Langmuir isotherm model. The best conditions were determined as: blueberry polyphenols concentration, 2.00 mg/g; pH 2.0; ethanol desorption solution concentration, 70 % (v/v); flow rates for feeding and elution, 1 and 2 bed volume (BV)/h, respectively; and a water wash volume of 3 BV. Under these conditions, the purity of the blueberry polyphenols was improved from 46.3 to 86.48 %. The EC₅₀ (concentration for 50 % of maximal effect) and CAA (cellular antioxidant activity) values of the blueberry polyphenols were 151.58 mg/mL and 6.39 μ mol QE/100 g sample, respectively. The results demonstrate that purification of polyphenols by macroporous resins XAD-7HP will provide foundation for the development of large-scale separation of blueberry polyphenols in industry.

Keywords: Blueberry (*Vaccinium* spp.); Polyphenol; Macroporous resin XAD-7HP; Purification; Cellular antioxidant activity (CAA)

INTRODUCTION

Polyphenols are secondary plant metabolites that are widely distributed in plant tissues and commonly accumulate in peels (Marti et al., 2015). Blueberry polyphenols, in particular, exhibit a wide variety of biological activities, including anti-inflammatory (Cheng et al., 2014), anti-aging (Afzal et al., 2015), anti-diabetes (Margina et al., 2013), antihypertensive, antibacterial (Zhang et al., 2013), and anti-cancer (Kim et al., 2004), and have been found to lower cholesterol and prevent osteoporosis (Sofidiya et al., 2014). In view of these attractive pharmacological properties, blueberry polyphenols have the potential for use as food additives or in nutraceutical products. Therefore, a method to obtain pure polyphenols from blueberries is need.

Macroporous resins have been used to improve the purity of bioactive compounds prior to their identification and separation by techniques such as high-speed

counter-current chromatography (Li et al., 2014), high-performance liquid chromatography (Liu et al., 2008), and mass spectrometry (Brito et al., 2014). However, there is only limited information on the purification of polyphenols from blueberries by macroporous resins.

In this study, the purification of polyphenols was optimized by static and dynamic adsorption and desorption tests. The cellular antioxidant activity of the purified products was also analyzed. This method can serve as a reference for the purification of polyphenols from other materials.

MATERIALS AND METHODS

Materials and chemicals

Ten kilogram of very ripe blueberries (*Northland*) were collected from Shenyang Qipan mountain (Shenyang, China) on 10th July 2014. Folin-Ciocalteu reagent, gallic acid standards and macroporous resins XAD-7HP were

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purchased from Sigma (U.S.). Pure ethanol, 95 % ethanol, hydrochloric acid, sodium hydroxide, and sodium carbonate were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

Extraction of polyphenols from blueberries

The extraction method was used with some modification as reported previously (Feng et al., 2015).

Determination of the mass concentration of the polyphenols

The total phenolic content (TPC) was measured using the Folin–Ciocalteu method based on Islam et al. (2002), with slight modification. The TPC of the blueberries was expressed as milligram gallic acid equivalent per milliliter of sample solution (mg GAE/mL).

Determination of polyphenol purity (Yao et al., 2015)

After purification, the blueberry polyphenol extract was dried to obtain a powder under vacuum using an LG 0.2 vacuum freeze drier (Xinyang Quick-freezing Instruments Manufacturing Co., Ltd., Henan, China). Then, the powder was dissolved in distilled water and the polyphenol concentration of the solution was determined. The purity of the polyphenols P (%) was calculated as:

$$P \% = \frac{\rho_0 \times V}{m} \times 100 \quad (1)$$

Where ρ_0 is the TPC of the extract (mg GAE/mL), m is the weight of the dried powder (g), and V is the volume of the polyphenols solution (mL).

Pretreatment of macroporous resins (Sun et al., 2013)

Resins (10 g) were loaded separately in a glass column (I.D. \times L = 20 mm \times 600 mm). The resins were soaked with 95 % ethanol (50 mL) for 24 h, and then washed with distilled water until the eluents were clear. The resins were then washed with 5 % HCl (100 mL) followed by distilled water until the pH of the eluent became neutral. The column was then washed with 3 % NaOH (100 mL), followed by distilled water until the eluent reached pH 7.0. Samples of the pre-treated resins were kept in a DHG-9070A heating and drying oven (Shanghai Jing Hong Laboratory Instrument Co., Ltd., Shanghai, China) at 60°C for 24 h.

Static adsorption and desorption tests

Pre-treated XAD-7HP resin (3.0 g) was mixed with blueberry extract (50 mL) in a flask and placed in an oscillator at 150 rpm and 25°C for 12 h. An aliquot of the supernatant (1 mL) was obtained every 2 h. The adsorption capacity was calculated using equation (2). After the adsorption equilibrium had been reached, the resin was washed with distilled water, and the surface of the resin was dried with filter paper. Then, 95 % ethanol (50 mL) was

added in the flask. The desorption process was conducted under the same conditions. An aliquot of the supernatant (1 mL) was obtained every 2 h. The desorption ratio was calculated using equation (3).

Adsorption and desorption ratios and capacities were calculated as follows:

$$Q = \frac{(\rho_0 - \rho_1) \times V_1}{m} \quad (2)$$

$$D = \frac{\rho_2 \times V_2}{m \times Q} \times 100 \quad (3)$$

Where Q is the adsorption capacity (mg/g dry resin), and D is the desorption ratio (%) at equilibrium; ρ_0 and ρ_1 are the initial and equilibrium concentrations of the polyphenols in the blueberry extract solutions (mg/mL), respectively; and m is the dry weight of the resin (g). V_1 and V_2 are the initial and equilibrium volumes of the polyphenols solutions (mL).

The pretreated XAD-7HP resin (3.0 g) and polyphenol extracts (50 mL) with different concentrations (0.25, 0.50, 0.75, 1.00, 1.50, 2.00, 3.00 mg/mL) and pH values (pH 1, 2, 3, 4, 5, 6, 7, 8) were added into 250 mL Erlenmeyer flasks. The pH value was determined by pH-meter (PHSJ-3F, Shanghai Precision and Scientific Instrument Corporation). The flasks were shaken in an oscillator at 150 rpm and at 25°C for 2 h. Then, the resins were washed with distilled water, and the surface of the resins was dried with filter paper. Finally, ethanol (50 mL) was added in the flasks, respectively.

Adsorption isotherms

XAD-7HP resin was selected for the adsorption isotherm testing. Pretreated resin (3.0 g) was added to the polyphenol extracts (50 mL) at different concentrations. Adsorption was conducted at three different temperatures (25, 35, and 45 °C) in an oscillator at 150 rpm. The equilibrium adsorption isotherms for the total polyphenols were determined using the Langmuir and Freundlich equations. The Langmuir equation describes the adsorption behavior of a monomolecular layer, whereas the Freundlich equation can be used to describe the adsorption behavior of a monomolecular layer as well as that of a multi-molecular layer (Gao et al., 2007).

Langmuir equation:

$$\frac{C_e}{Q_e} = \frac{1}{Q_m K_L} + \frac{C_e}{Q_m} \quad (4)$$

Where Q_e is the equilibrium adsorption capacity of the resin (mg/g); C_e is the equilibrium concentration of the total polyphenols in solution (mg/mL); Q_m is the theoretical

maximum adsorption capacity (mg/g); and K_L is a constant for the adsorption equilibrium.

$$\text{Freundlich equation: } Q_e = K_F C_e^{1/n} \quad (5)$$

Where the constant K_F and the exponent $1/n$ can be calculated by plotting $\log Q_e$ versus $\log C_e$.

Dynamic adsorption and desorption testing

A glass column (I.D. \times L = 20 mm \times 600 mm) was loaded with XAD-7HP resin, producing a resin bed volume (BV) of 60 mL. The blueberry polyphenolic extract was loaded onto the column at different flow rates. The column was then washed with water (5 BV) to remove sugars and other compounds that did not absorb on the resin. The resin was desorbed using 70 % ethanol at different flow rates. The eluent was collected and analyzed for TPC.

Cellular antioxidant activity (CAA) assay

The CAA test was carried out as previously described (WOLFE and LIU, 2007). The CAA value of a sample was calculated based on method of Boaventura et al., (2015).

Statistical analysis

All of the data are expressed as the mean \pm standard deviation (SD) of three parallel tests. Statistical analysis was performed with SPSS 18.0 and Origin 8.0 software.

RESULTS AND DISCUSSION

Adsorption and desorption kinetics

The adsorption and desorption kinetics of the polyphenolics on XAD-7HP are shown in Fig. 1 (A). XAD-7HP reached adsorption and desorption equilibrium in approximately 2 h. Over the first 2 h, the adsorption and desorption capacities increased quickly; thereafter, the increases were gradual. Lin et al. (Lin et al., 2012) reported that the interaction of boundary-layer diffusion and intraparticle diffusion kinetics has an effect on the adsorption process of phenolics on XAD-7 resin. Yi et al. (Yi et al., 2015) also found that the adsorption and desorption ratio of polyphenols increased rapidly within 2 h to reach the maximum value.

Adsorption isotherms

The Langmuir and Freundlich isotherm models were used to assess the adsorption data for the polyphenols. The equations' constants and correlation coefficients are listed in Table 1. The correlation coefficients for the Langmuir model are higher than those of the Freundlich model, showing that the former better fits the data, and also indicating that the adsorption of polyphenols by XAD-7HP occurs via monomolecular layer formation. Buran et al. (Buran et al., 2014) and Sun et al. (Sun et al.,

Table 1: Langmuir and Freundlich parameters of blueberry polyphenols adsorption on the XAD-7HP resin

Temperature (°C)	Langmuir equation			Freundlich equation		
	Q_m	K_L	R^2	K_F	$1/n$	R^2
25	90.9090	0.4188	0.9934	65.7471	0.0365	0.1118
35	76.9231	0.0226	0.9969	53.7671	0.2647	0.7976
45	66.6667	0.0165	0.9942	42.8176	0.4732	0.7800

2015) also found that the adsorption equilibrium was best described by the Langmuir model. In the Langmuir model, the theoretical maximum adsorption capacity (Q_m) decreases as the temperature increases, indicating that adsorption is an exothermic process. K_L is an indicator of the stability of the interaction between the adsorbate and adsorbent surface (Zhang et al., 2008). The K_L values decrease with rising temperature, also implying that the adsorption of the polyphenols is reduced as the temperature rises. In the Freundlich model, a value of $1/n$ that is less than 1 indicates the favorability of the sorbent to adsorb the solute (Xi et al., 2015). According to Table 1, the $1/n$ values at all temperatures were less than 1, and thus, the XAD-7HP resin was appropriate for adsorbing blueberry polyphenols. The Langmuir adsorption isotherms of XAD-7HP at 25, 35, and 45°C are shown in Fig. 1 (B).

Static adsorption and desorption on XAD-7HP

The concentration of the polyphenols sample can have an effect on the purity and adsorption capacity (Yi et al., 2015). As shown in Fig. 2 (A), the adsorption capacity of the resin increases as the concentration of the polyphenols increases in the range 0.3 – 2.00 mg/g, whereas it decreases slightly thereafter. This phenomenon may be due to the concomitant increase in the amount of other impurities with further increases in the concentration of the sample polyphenols, which may compete with the blueberry polyphenols for active sites on the resins, thus, the adsorption of the polyphenols is reduced (Liu et al., 2014). Moreover, greater concentration of blueberry polyphenols would create higher driving force for mass transfer (Ding et al., 2012). The adsorption capacity reaches a maximum at a concentration of 2.00 mg/g.

The pH can have a significant effect on adsorption by the resin, because it can affect the interaction between the polyphenols and the adsorption sites of the adsorbent (Wang et al., 2013). The adsorption capacity of the resin increases initially and then decreases as the pH increases (Fig. 2 (B)), and is highest at pH 2. This result indicates that hydrogen bonding may play a significant role in the adsorption process of the XAD-7HP resin. According to Wan et al., at higher pH values, the phenolic hydroxyl groups in the polyphenols are dissociated, which resulting in the reduction of hydrogen bonding interactions and therefore,

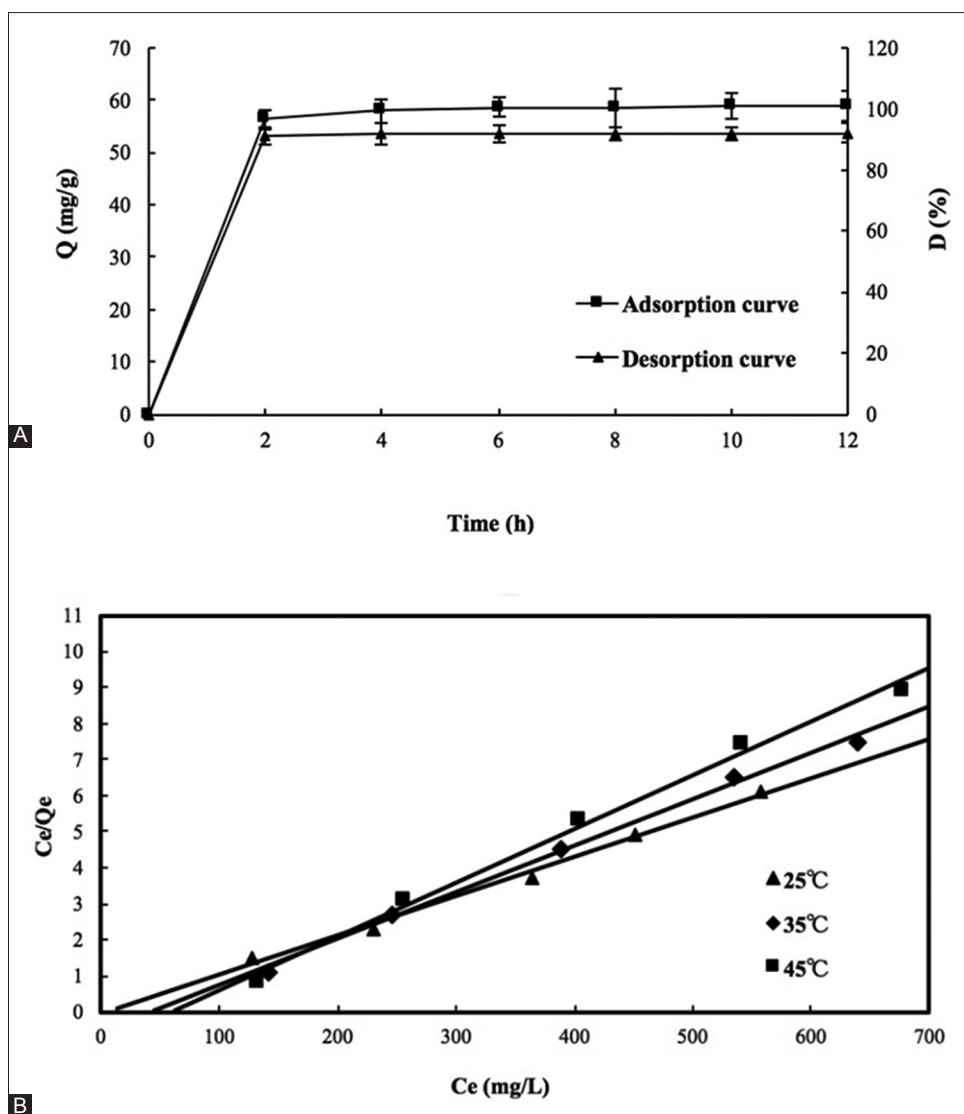


Fig 1. (A) Kinetic adsorption and desorption curves of macroporous resin XAD-7HP. (n = 3) (B) Adsorption isotherms based on the Langmuir equation for the polyphenols. (n = 3).

leading to lower adsorption capacity of polyphenols (Wan et al., 2014). The same result was reported by Wang et al. (Wang et al., 2013), who found that pH 2.0 was optimal for the adsorption sample solution. Datta et al. (Datta et al., 2011) similarly found that pH 5.5 was the most suitable for the adsorption of polyphenols from ginger rhizomes; the difference in optimal pH may be due to the different phenolic profiles of the two species.

As shown in Fig. 2 (C), the concentration of the ethanol solution has a significant effect on desorption from the resin. The desorption ratio increases sharply at first and then decreases, and reaches a maximum at 70 % ethanol. Therefore, 70 % ethanol was chosen for further testing. Similar results were reported by Monsanto et al. and Lin et al., who also found that 70 % ethanol was the best eluent (Monsanto et al., 2015; Lin et al., 2012).

Dynamic adsorption and desorption on XAD-7HP

The breakthrough volume is defined as the volume of the extract loaded on the column at which the concentration of the polyphenols in the eluent is 10 % that of the initial extract. From Fig. 3 (A), at flow rates of 0.5, 1, 1.5, 2, 2.5, and 3 BV/h the breakthrough volumes are 120, 100, 70, 60, and 50 mL, respectively. The breakthrough point decreases as the loading flow rate increases (Mi et al., 2012). The breakthrough point appears latest at the 0.5 BV/h flow rate; however, the slower flow rate would lead to a longer production cycle. Therefore, the polyphenolic sample was loaded at a flow rate of 1 BV/h, and the corresponding loading sample amount was 100 mL.

Fig. 3. Dynamic adsorption and desorption on XAD-7HP resin. (n = 3) (A) The effect of the loading flow rate on kinetic adsorption. (B) The effect of the desorption flow rate

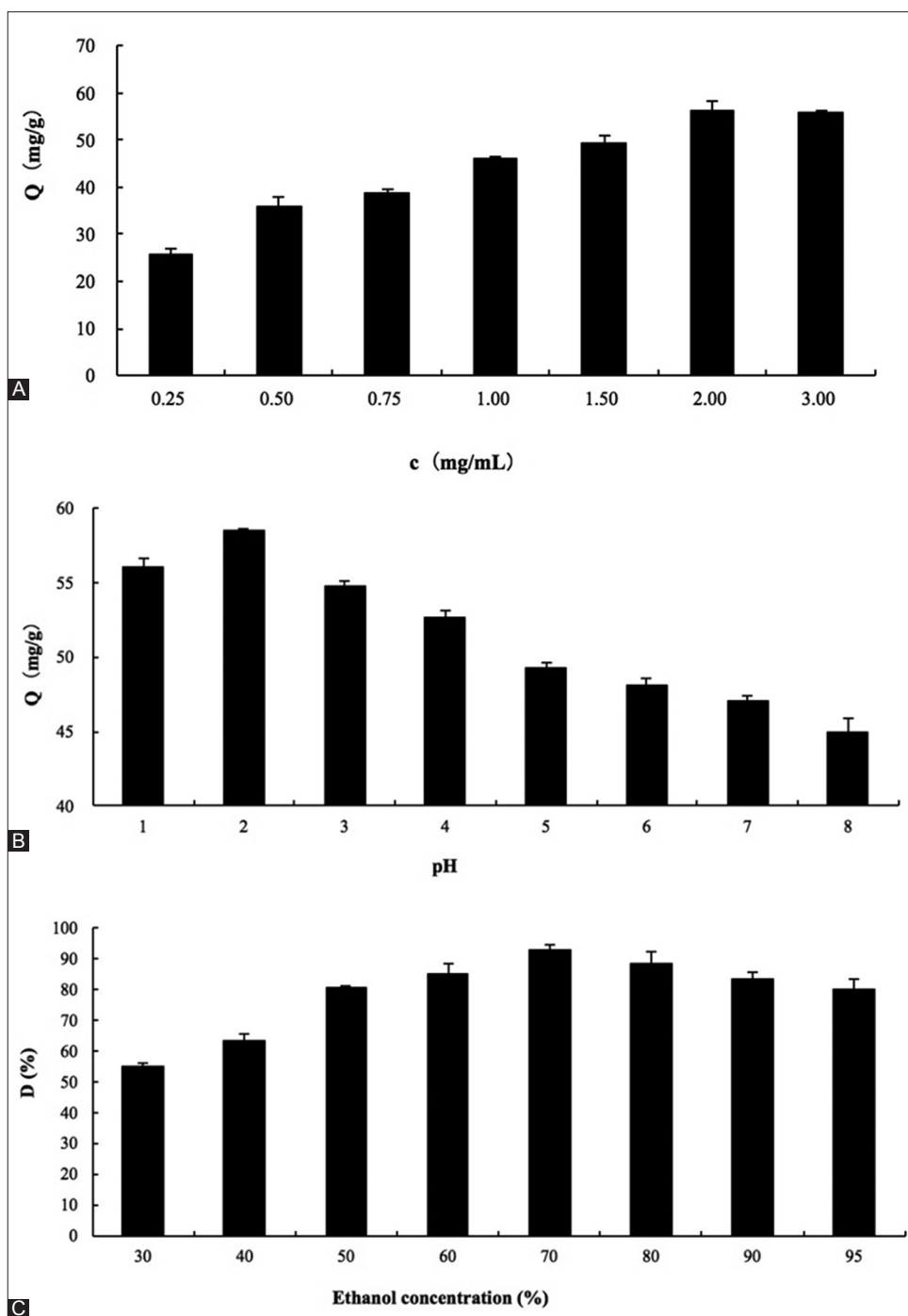


Fig 2. Static adsorption and desorption on XAD-7HP resin. (n = 3) (A) The effect of sample concentration on the adsorption capacity of the polyphenols. (B) The effect of sample solution pH on the adsorption capacity of the polyphenols. (C) The effect of ethanol concentration on the desorption rate.

on kinetic desorption. (C) The effect of the desorption flow rate on the desorption ratio. (D) The effect of the amount of water on the polysaccharide concentration in the eluent.

The polyphenols were eluted with 70 % ethanol at a flow rate of 1, 2, 3, or 4 BV/h, respectively. The peaks of the curves obtained by elution at flow rates of 1 and 2 BV/h are narrow and have no obvious tailing, whereas those at

a flow rate of 3 or 4 BV/h are wider and have obvious tailing (Fig. 3 (B)). The elution rate also has an effect on the desorption rate. The desorption rate decreases as the elution speed increases, and reaches a maximum at the 1 BV/h flow rate. A decline in the desorption rate was not obvious when the elution speed was increased to 2 BV/h; therefore, to shorten the production cycle, 2 BV/h was selected as the elution rate (Fig. 3 (C)).

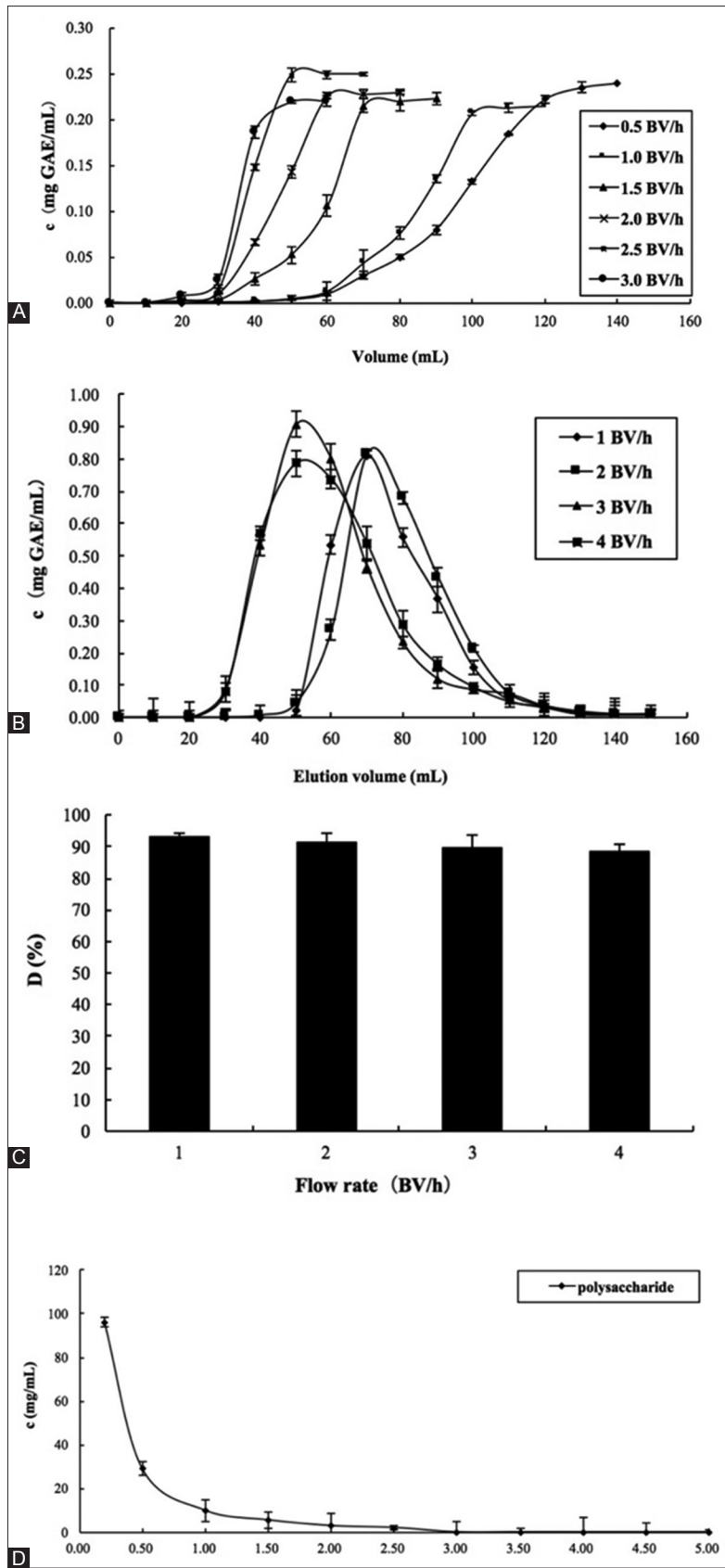


Fig 3. Dynamic adsorption and desorption on XAD-7HP resin. (n = 3) (A) The effect of the loading flow rate on kinetic adsorption. (B) The effect of the desorption flow rate on kinetic desorption. (C) The effect of the desorption flow rate on the desorption ratio. (D) The effect of the amount of water on the polysaccharide concentration in the eluent.

Finally, in order to remove the non-polyphenols and the unadsorbed polyphenols from the samples, the column was washed with distilled water. As shown in Fig. 3 (D), the polysaccharides in the samples can be effectively removed using a water volume of 3 BV.

Cellular antioxidant activity of blueberry polyphenols

According to the CAA assay, there was no cytotoxicity when the concentration of blueberry polyphenols was less than 300 mg/mL. The EC₅₀ and CAA values of the blueberry polyphenols were 151.58 mg/mL and 6.39 μmol QE/100 g samples. WOLFE et al. (WOLFE et al., 2008) have reported that the EC₅₀ and CAA values of the blueberry were 27.0 mg/mL and 19.0 μmol QE/100 g, respectively.

CONCLUSIONS

From the static and dynamic tests, the optimum purification conditions by macroporous resin XAD-7HP were determined as: blueberry polyphenols concentration, 2.00 mg/g; pH 2.0; ethanol desorption solution concentration, 70 % (v/v); flow rates for feeding and elution, 1 and 2 BV/h, respectively; and a water wash volume of 3 BV. Under these conditions, the purity of the blueberry polyphenols was improved from 46.3 to 86.48 %. The cellular antioxidant activities of the purified polyphenols were investigated. The EC₅₀ and CAA values of the blueberry polyphenols were 151.58 mg/mL and 6.39 μmol QE/100 g sample. The results obtained from this study could be used for further applications of polyphenols from plant materials.

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Authors' contributions

Conceived and designed the experiments: Xianjun Meng, Bin Li and Xinyao Jiao. Performed the experiments: Xinyao Jiao. Analyzed the data: Xiuyan Zhang. Contributed reagents/materials/analysis tools: Qi Zhang and Xiuyan Zhang. Wrote the paper: Xinyao Jiao.

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